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Poster

282. Fate Specification and Generation of Neuronal Diversity

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 282.01/A1

Topic: A.01. Neurogenesis and Gliogenesis

Support: Citizens United for Research in Epilepsy (CURE)

Title: Local environment shapes phenotypic variations of hippocampal and neocortical chandelier cells

Authors: *H. TANIGUCHI, Y. ISHINO, M. J. YETMAN, S. M. SOSSI, A. STEINECKE, Y. HAYANO

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Abstract: Different cortical regions processing distinct information, such as the hippocampus and the neocortex, share common cellular components and circuit motifs but form unique networks by modifying these cardinal units. Cortical circuits include diverse types of GABAergic interneurons (INs) that shape activity of excitatory principal neurons (PNs). Canonical IN types conserved across distinct cortical regions have been defined by their morphological, electrophysiological, and neurochemical properties. However, it remains largely unknown whether canonical IN types undergo specific modifications in distinct cortical regions and display "regional variants". It is also poorly understood whether such phenotypic variations are shaped by early specification or regional cellular environment. The chandelier cell (ChC) is a highly stereotyped IN type, which innervates axon initial segments of PNs, and thus serves as a good model to address this issue. Here we show that Cadherin-6 (Cdh6), a homophilic cell adhesion molecule, is a reliable marker of ChCs and Cdh6-CreER mice provide genetic access to hippocampal ChCs (h-ChCs). We demonstrate that compared to neocortical ChCs (nc-ChCs), h-ChCs cover twice as much area and innervate twice as many PNs. Interestingly, a subclass of h-ChCs exhibits calretinin (CR) expression, which is not found in nc-ChCs. Furthermore, we find that h-ChCs appear to be born earlier than nc-ChCs. Surprisingly, despite the difference in temporal origins, ChCs display host region-dependent axonal/synaptic organization and CR expression when heterotopically transplanted. These results suggest that local cellular environment plays a critical role in shaping terminal phenotypes of regional IN variants in the hippocampus and the neocortex.

Disclosures: H. Taniguchi: None. Y. Ishino: None. M.J. Yetman: None. S.M. Sossi: None. A. Steinecke: None. Y. Hayano: None.

Poster

282. Fate Specification and Generation of Neuronal Diversity

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 282.02/A2

Topic: A.01. Neurogenesis and Gliogenesis

Support: CAPES

Title: Cell lineage progression in the adult hippocampus revisited: Evidence of progenitor transdifferentiation

Authors: *D. S. MOURA¹, C. M. QUEIROZ², M. R. COSTA²

¹Brain Institute-Ufrn, Natal, Brazil; ²Brain Inst., Univ. Federal do Rio Grande do Norte, Natal, Brazil

Abstract: Cell lineage in the adult hippocampus comprises multipotent (Type 1 and 2a) and neuron-determined (Type 2b and 3) progenitors. The current model assumes that multipotent stem cells generate neuron-determined intermediate progenitors that become post-mitotic neurons in a unidirectional way. In the present work, we fate-mapped neuronal progenitors using Dcx-CreERT2 and CAG-CAT-EGFP double-transgenic mice, hereafter referred to as cDCX/EGFP. We show that three days after tamoxifen-mediated recombination in cDCX/EGFP adult mice, virtually all GFP+ cells in the dentate gyrus co-expresses DCX. However, within 30 days, about 15% of GFP+ cells become GFAP+ astrocytes. These data suggest that Type 2b/3 DCX+ progenitors either retain the capacity to generate astrocytes or can regress to more primitive stages (Type 2a or 1). To evaluate these possibilities, we boosted neurogenesis in the dentate gyrus by local administration of epileptogenic drugs. Intrahippocampal injection of kainic acid (KA) led to a significant increase in the number of GFP+ cells in both ipsi and contralateral dentate gyrus. Remarkably, in the ipsilateral dentate gyrus, most GFP+ cells adopted glial morphologies and expressed the astrocytic protein GFAP. Conversely, on the contralateral side, GFP+ cells differentiated mainly into granular neurons. Intriguingly, at early time-points after recombination, we observed a small number of unexpected labeled cells displaying radial-glia morphology, a hallmark of Type 1 multipotent progenitors, suggesting that some type2b progenitors transdifferentiated. Intrahippocampal injection of pilocarpine also leads to an important increase in the number of GFP+ cells in both ipsi and contralateral dentate gyrus, but the vast majority of cells differentiate into neurons. Importantly, both drugs were able to bilaterally elicit paroxysms, high-amplitude discharges and seizures. Altogether, these results indicate that Type 2b/3 progenitors are not restricted to the generation of neurons, but rather retain bi-potency and/or the ability to return to more primitive stages in the lineage and generate astrocytes. Under basal conditions, generation of astrocytes represents a small fraction of newly generated cells in the adult hippocampus, but under pathological conditions, this proportion can

be massively altered. Finally, these observations also suggest that the drug used to model temporal lobe epilepsy differentially affects cell lineage progression in the adult hippocampus.

Disclosures: C.M. Queiroz: None. M.R. Costa: None.

Poster

282. Fate Specification and Generation of Neuronal Diversity

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 282.03/A3

Topic: A.01. Neurogenesis and Gliogenesis

Title: Role of mitochondrial fusion dynamics in adult hippocampal NSCs lineage progression and new neuron development

Authors: *S. WENDLER¹, G. WANI¹, J. GÖBEL¹, M. BERGAMI^{1,2} ¹Univ. Hosp. of Cologne, CECAD Inst., Koeln, Germany; ²Ctr. for Mol. Med., Koeln, Germany

Abstract: The dentate gyrus (DG) of the hippocampus is one of the very few restricted regions in the adult mammalian brain, where neurogenesis persists lifelong. Here, radial glia-like neural stem cells (NSCs) can enter cell division and give rise to fast-dividing progenitor cells, which in turn generate new neurons. Once born, the new neurons undergo a step-wise maturation process that lasts several weeks and during which they integrate into the pre-existing DG network. Recent work suggests that along this lineage, and in particular from the moment NSCs become activated, substantial changes in their energy metabolism take place, with genes involved in mitochondrial function becoming increasingly up-regulated, once the cell divides and progresses towards a post-mitotic (neuronal) stage. On the contrary, little is known on the physiological relevance of mitochondrial dynamics during this lineage progression, and in particular, whether changes in fusion and fission events (known to dynamically shape and maintain the mitochondrial network in cells) may alter the fate of NSCs or development of new neurons. Here, we utilized a set of Cre-inducible mouse lines to conditionally manipulate the proteins Mfn1 or Mfn2, which are the main drivers of mitochondrial outer membrane fusion, specifically in adult NSCs and their neuronal progeny in vivo. Genetic deletion of Mfn1 or Mfn2 disrupted the structure of the mitochondrial network in adult NSCs, as revealed by crossings with a mitoYFP reporter line. Analysis of proliferation in neural stem and progenitor cells revealed that these genetic manipulations did not affect the early steps along the lineage, as similar numbers of new neurons were generated in presence or absence of Mfn1 and 2. To assess the maturation process and survival of newly-born neurons upon loss of mitochondrial fusion, we used a mouse line expressing the cytosolic marker tdTomato, allowing for a detailed analysis of cellular morphology in labeled cells. In Mfn2 conditional knock-out mice we found a selective depletion of new neurons, which became manifest from the moment they started to incorporate into the hippocampal network, that is, when they switched from a DCX+ to a NeuN+ stage. This

indicates, that mitochondrial fusion is dispensable for earlier stages along the neurogenic lineage, while it becomes essential for the survival of new DG neurons during a period critical for their integration into the pre-existing circuit.

Disclosures: S. Wendler: None. G. Wani: None. J. Göbel: None. M. Bergami: None.

Poster

282. Fate Specification and Generation of Neuronal Diversity

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 282.04/A4

Topic: A.01. Neurogenesis and Gliogenesis

Support: Israel Science Foundation 1051/15 ISF

Title: Uncovering floor plate descendants in the ependyma of adult mouse CNS by mapping of Nato3-expressing cells

Authors: *N. BEN-ARIE

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Abstract: During embryonic development of the central nervous system (CNS), the expression of the bHLH transcription factor Nato3 (Ferd3l) is unique and restricted to the floor plate of the neural tube. In mice lacking Nato3 the floor plate cells of the spinal cord do not fully maturate, whereas in the midbrain floor plate, progenitors lose some neurogenic activity, giving rise to a reduced population of dopaminergic neurons. Since the floor plate is considered to be disintegrated at the time of birth, Nato3 expression was never tested postnatally and in adult mice. Here, we utilized a Nato3 knockout mouse model in which a LacZ reporter precisely replaced the coding region under the endogenous regulatory elements, such as its expression recapitulates the spatiotemporal pattern of Nato3 expression. Nato3 was found to be expressed in the CNS throughout life in a highly-restricted manner along the medial cavities: in subpopulations of cells in the third ventricle, the cerebral aqueduct, the fourth ventricle, the central canal of the spinal cord, and the subcommissural organ, a gland located in the midbrain. A few unifying themes are shared among all Nato3-positive cells: all are positioned in the midline, are of an ependymal type, and contact the cerebrospinal fluid (CSF) similarly to the embryonic position of the floor plate bordering the lumen of the neural tube. Taken together, Nato3 defines an unrecognized subpopulation of medial cells positioned at only one side of circular ependymal structures, and it may affect their regulatory activities and neuronal stem cell function.

Disclosures: N. Ben-Arie: None.

Poster

282. Fate Specification and Generation of Neuronal Diversity

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 282.05/A5

Topic: A.01. Neurogenesis and Gliogenesis

Support: MRC grant MRK/022377/1

Title: Capture Hi-C reveals the chromatin interactome and long-range gene regulatory landscape of cerebellar granule neuron progenitors

Authors: *M. BASSON¹, K. RIEGMAN^{1,2}, C. GEORGE², C. MOHAN¹, D. WHITTAKER^{3,4}, B. HUNTLY⁴, D. SIMS², C. OSBORNE¹

¹King's Col. London, London, United Kingdom; ²Univ. of Oxford, Oxford, United Kingdom; ³Craniofacial development and stem cell biology, King's College, London, London, United Kingdom; ⁴Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Cellular differentiation and cell type-specific gene expression are under the control of distal regulatory elements in the genome. These elements, which include elements with enhancer or repressor activities, can be located at some distance from the gene being regulated. Chromatin conformation capture techniques provide information on genome organization by capturing regions in the genome that are in close proximity to each other. Here we applied such an approach to define the chromatin interactome of mouse cerebellar granule neuron progenitors (GNPs). GNPs are transit amplifying, lineage committed neural progenitors that are responsible for the postnatal growth and the formation of the characteristic foliated structure of the cerebellum. Using a Capture Hi-C approach, we identified over 75,000 possible interactions with over 20,000 gene promoters throughout the genome. The promoter-interacting regions were characterized further by a combination of ATACseq and ChIP-seq analysis to identify putative enhancer elements. The chromatin remodeling factor CHD7 (chromodomain helicase DNA binding factor 7) that controls GNP proliferation and differentiation has been shown to primarily localize to distal regulatory regions where it affects DNA accessibility. A comparison of DNA accessibility at these putative distal regulatory elements in control and CHD7-deficient GNPs defined the CHD7-regulated enhanceosome of GNps. These findings provide fundamental insights into the long-range gene regulatory landscape of cerebellar GNPs and will form the basis of future studies aimed at elucidating chromatin reorganization during GNP differentiation and the regulation of cerebellar growth and development by factors that coordinate these interactions.

Disclosures: M. Basson: None. K. Riegman: None. C. George: None. C. Mohan: None. D. Whittaker: None. B. Huntly: None. D. Sims: None. C. Osborne: None.

Poster

282. Fate Specification and Generation of Neuronal Diversity

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Program#/Poster#: 282.06/A6

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R03NS097887

NIH Grant R01NS094144

NIH Grant R01CA163493

Title: Autophagy sustains hyper-activation of mTORC1 in TSC1-deficient neural stem cells to promote tuberous sclerosis complex-associated defects

Authors: *C. WANG, M. HAAS, S. YEO, S. CHEN, J. WEN, J.-L. GUAN Univ. of Cincinnati Dept. of Cancer Biol, Cincinnati, OH

Abstract: Tuberous Sclerosis Complex (TSC) is a neurodevelopmental disorder characterized by hyperactivation of mechanistic target of rapamycin (mTOR) complex 1, causing abnormal cell growth, proliferation, and differentiation in affected brains. We found that conditional knock out of Tsc1 with hGFAP-Cre in mouse (designated as TSC1 cKO mice) resulted in hyperactivation of mTORC1 in postnatal neural stem cells (NSCs), which impaired self-renewal and neurogenesis in the subventricular zone. Moreover, TSC1 cKO mice developed subependymal nodules (SEN) with a mixed neuronal and glial lineage as found in human subependymal giant astrocytoma. The mTORC1 inhibitor rapamycin suppressed mTORC1 hyperactivation and rescued the maintenance and differentiation defects in TSC1-null NSCs. The tumorigenesis of SEN was also suppressed by rapamycin in mutant mice. Interestingly, although mTORC1 hyperactivation is known to inhibit autophagy through phosphorylating ULK1 in many cells, the autophagy activity is not completely diminished in TSC1-deficient NSCs. To study the functions and mechanisms of autophagy in regulating mTORC1 activation, we generated a double conditional knockout mouse (designated as 2cKO) with deletion of TSC1 and FIP200 in postnatal NSCs. We found that high mTORC1 activity in NSCs of TSC1 cKO mice was restored in 2cKO mice to comparable level as in wild type mice. Moreover, the impaired self-renewal, neurogenesis, and tumorigenesis of TSC1-null NSCs were also rescued in 2cKO mice. Metabolic analysis of NSCs in various mice showed an increased glycolysis and mitochondrial oxidative phosphorylation in TSC1-null NSCs. The rewired metabolism was driven by hyperactivated mTORC1 and required intact autophagy in TSC1-deficient NSCs. Lastly, we found that the combination of a glycolysis inhibitor 2-Deoxy-D-glucose and an autophagy inhibitor chloroquine, but not either compound alone, significantly reduced high mTORC1 activity and

pathological defects in TSC1-deficient NSCs. Together, these results suggest a novel metabolic mechanism for autophagy to regulate mTORC1 activity in a neurodevelopmental disease model.

Disclosures: C. Wang: None. M. Haas: None. S. Yeo: None. S. Chen: None. J. Wen: None. J. Guan: None.

Poster

282. Fate Specification and Generation of Neuronal Diversity

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 282.07/A7

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

Title: Hox genes are essential for the diversification and connectivity of spinocerebellar tract neurons

Authors: *M. BAEK¹, A. W. HANTMAN², T. M. JESSELL³, J. S. DASEN⁴ ¹Neurosci., NYU Sch. of Med., New York, NY; ²Janelia Res. Campus, Howard Hughes Med. Inst., Ashburn, VA; ³Departments of Neurosci. and Biochem. and Mol. Biophysics, HHMI, Columbia Univ., New York, NY; ⁴Neurosci., NYU school of medicine, New York, NY

Abstract: Motor control relies on the relay of muscle-derived sensory information to the central nervous system. Proprioceptive sensory information is processed locally within spinal circuits and is delivered to higher brain centers through spinal projection neurons. Spinocerebellar tract neurons (SCTNs) relay proprioceptive sensory information to the cerebellum; the central cervical nucleus (CCN), caudal cervical SCTNs, and Clarke's column neurons (CC) relay sensory information from the neck, forelimb, and hindlimb muscles, respectively. Although the anatomical organization of diverse SCTN populations has been well characterized, their specification is poorly understood. To define the molecular profile of SCTNs, we performed transcriptional profiling of retrogradely labeled SCTNs. RNA sequencing analysis identified novel molecular markers for CC and CCN. We also found that during embryonic development, expression of specific Hox transcription factors distinguish these and additional subsets of SCTNs. By combining immunostaining with retrograde tracing from the cerebellum, we determined the expression pattern of Hox genes in the SCTNs; Hox4-8 paralogous define subtypes of cervical SCTNs, while Hox9 and Hox10 genes are specifically expressed in the thoracic SCTNs. We tested whether Hox genes establish SCTNs identity and connectivity through genetic manipulation of their activities. We found that Hoxc9 is required for the development of thoracic SCTNs, including CC neurons. In Hoxc9 mutants, markers of CC neurons were absent in thoracic segments of the spinal cord. Strikingly, in Hoxc9 mutants CC neurons appear to be transformed to the identity of SCTN populations normally present at cervical levels of the spinal cord; in Hoxc9 mutants thoracic SCTNs show caudal cervical SCTNs characteristic cell body positions, Hox gene expression, and sensory input sources. In

contrast to caudal cervical SCTNs, CCN neurons in the rostral cervical region are not expanded in Hoxc9 mutants, which is reminiscent of the Hoxc9 mutant phenotype in motor neurons. Collectively these studies indicate that, similar to spinal motor neurons, the subtype diversity and connectivity of spinal interneurons relies on Hox-dependent genetic programs.

Disclosures: M. Baek: None. A.W. Hantman: None. T.M. Jessell: None. J.S. Dasen: None.

Poster

282. Fate Specification and Generation of Neuronal Diversity

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 282.08/A8

Topic: A.01. Neurogenesis and Gliogenesis

Support: Canadian NSERC Discovery grant

NCE NeuroDevNet/KidsBrainHealth Network award

Title: Identification of active enhancers during cerebellum development through co-regulation temporal analysis of enhancer RNAs

Authors: *M. RAMIREZ¹, R. ROBERT², P. ZHANG¹, F. CONSORTIUM³, J. SONG⁴, D. GOLDOWITZ¹

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Abstract: The developing cerebellum requires intricately controlled expression of specific gene regulatory networks across time. Due to advancement in genomic technology, the known mechanisms responsible for the regulation of gene expression during development have expanded to incorporate non-coding cis- and trans-acting elements. Among these elements are enhancers, which are distal regions to transcript start sites (TSSs) that have previously been known to selectively activate genes in a tissue-specific and temporal-specific manner under various conditions. Recently, bi-directional transcription of non-coding RNAs at enhancer domains, known as enhancer RNAs (eRNAs), has been positively correlated with the expression of both nearby genes and their regulatory target genes. eRNA transcription has been defined as a signal of active enhancers during various biological processes.

The identification of active enhancers during brain development, specifically for the cerebellum, has yet to be elucidated. The goal of our work is to use eRNA expression to identify active enhancers contributing to the fine control of gene expression throughout cerebellar development. Transcriptomic data produced by the FANTOM5 consortium through Cap Analysis Gene Expression (CAGE) followed by high throughput sequencing were utilized to quantify eRNA

expression for a catalogue of predicted enhancers. This was performed for embryonic and early postnatal stages of murine cerebellum development (from embryonic day 11 to postnatal day 9). Previously defined enhancers were curated based on dynamic eRNA expression throughout the time course. The analytical pipeline defined 238 eRNA-enhancers that are active during cerebellum development. Comparison with other developing murine tissues was performed and revealed that these eRNA-expressing enhancers are highly specific to cerebellar development. Finally, co-expression networks including 10,316 expressed TSSs were defined to identify predicted regulatory target genes. The identified co-expression modules revealed enhancer elements activated at different stages in development. Validation by ChIP-seq for enhancer associated chromatin modifications and other experimental approaches will be conducted to produce a set of robust active enhancers.

Disclosures: M. Ramirez: None. R. Robert: None. P. Zhang: None. F. Consortium: None. J. Song: None. D. Goldowitz: None.

Poster

282. Fate Specification and Generation of Neuronal Diversity

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Topic: A.01. Neurogenesis and Gliogenesis

Support: ARCS Foundational Grant; Global Impact Award

NIH T32 Training Grant: 4T32GM007103-41

NINDS Grant: R01NS096176-02

Title: Lin28-mediated mRNA translation promotion is critical for neural progenitor cell fate and brain development

Authors: *S. A. HERRLINGER

Univ. of Georgia, Athens, GA

Abstract: The development of the brain is a dynamic and complex process that requires precise timing of neural precursor cell (NPC) behaviors. While many transcriptional and epigenetic regulators of neurodevelopment are known, the roles that translational regulators play are poorly understood. Lin28 is an RNA-binding protein that is highly expressed in NPCs at the early stage of brain development. Two homologs for Lin28 exist in mammals (Lin28a/b). Our previously published work showed that Lin28a single-knockout leads to reduced NPC self-renewal and microcephaly (smaller brains) in mice (Yang et al., Development, 2015). Using Nestin-Cre, we found that NPC-specific overexpression of Lin28a causes macrocephaly (enlarged brains) by promoting NPC self-renewal. These studies show that Lin28a promotes NPC expansion and

brain development. Because there exists 76% homology between both homologs of Lin28, it is likely that redundant function is shared between them. We found that double knockout mutants of Lin28a/b exhibit an open neural tube defect (NTD) predominantly in the hindbrain. In vivo NPC behavior analyses confirm a decrease in proliferation of hindbrain NPCs in Lin28a/b double mutant mice both prior to neural tube closure and sustained after neural tube closure failure. Precocious differentiation can also be observed immediately after neural tube closure failure in the double mutant hindbrain region, while there is no notable change in apoptosis. These results suggest that Lin28a/b are required for NPC fate and brain development. Lin28a has been reported to promote or inhibit mRNA translation in a cell type dependent manner. To address the mechanism of action of Lin28 in the developing brain, we used the Rpl24 (encoding a ribosomal subunit) hypomorphic allele mouse, a well-established genetic tool to reduce mRNA translation. Whereas Lin28a depletion alone results in smaller brains, Lin28a knockout in the background of Rpl24 hypomorphic allele results in NTDs, which recapitulate the NTDs in Lin28a/b double knockouts. Furthermore, Rpl24^{Bst/}-mediated decrease of mRNA translation completely rescued the macrocephaly defect from NPC-specific Lin28a overexpression. Together, these results suggest that reduced mRNA translation in NPCs is sufficient to cause microcephaly, Lin28 promotes mRNA translation in the developing brain, and Lin28-mediated translation promotion is required for NPC cell fate control and brain development.

Disclosures: S.A. Herrlinger: None.

Poster

282. Fate Specification and Generation of Neuronal Diversity

Location: Halls A-C

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Program#/Poster#: 282.10/A10

Topic: A.10. Development and Evolution

Support: European Research Council

Marie Curie

NARSAD

FPI Ministerio de economia y competitividad España

Title: Deep homology of the genetic program controlling dopaminergic neuron differentiation in nematodes & mammals

Authors: *L. R. GOMEZ¹, M. MAICAS¹, A. ESCRICHE¹, C. CUCARELLA¹, R. RUIZ¹, Á. JIMENO¹, L. CHIRIVELLA¹, A. PEREZ², I. FARIÑAS², N. FLAMES¹ ¹Neurobiologia del Desarrollo, Insituto De Biomedicina De Valencia CSIC, Valencia, Spain; ²Biología celular, Univ. de Valencia, Valencia, Spain **Abstract:** Dopamine (DA) is one of the main neurotransmitters in the brain and regulates a variety of complex behaviors. Understanding the regulatory logic of DA terminal differentiation is important for both basic as well as translational research. Using the model organism *C. elegans* we found that a combination of three transcription factors (TFs) from three different families (ETS, DLL and PBX) directly regulate terminal differentiation of DA cells (Doistsidou et al. 2013).

The most ancestral mammalian dopaminergic population are the olfactory bulb (OB) dopaminergic neurons. Interestingly mouse orthologs for both ETS (Etv1/Er81) and DLL (Dlx2) TFs are already known to be required for mouse OB DA differentiation. Pbx1 and Pbx2 have been recently shown to control OB neurogenesis, however a specific role on dopaminergic terminal differentiation has not been assessed.

Here we show that PBX1 and PBX2 are expressed in the adult OB DA neurons, however, only specific DA lineage conditional *Pbx1* mutant mice exhibit a dramatic decrease in the number of DA neurons of the OB suggesting that PBX1 but not PBX2 is required for the correct DA specification. Moreover, lineage tracing experiments indicate that *Pbx1* mutant cells remain in the OB but they are unable to properly differentiate. We find that *Pbx1* is required for the expression of other transcription factors that are known to be important for the DA differentiation such as COUP-TF1 and MEIS2. In addition, overexpression of *Pbx1* in the mouse SVZ progenitors is sufficient to induce DA differentiation in the OB. Considering the evolutionary conservation of PBX, ETS and DLX TF on dopaminergic neuron differentiation we further analyzed to what extent the regulatory programs are homologous. Surprisingly, we find that worm orthologs of COUP-TF1, MEIS2 and PAX6 are also required in nematode DA specification. Our findings demonstrate a remarkable extent of homology in the specification of a critically important neuronal cell type, conserved over a billion years of

evolution.

Disclosures: L.R. Gomez: None. M. Maicas: None. A. Escriche: None. C. Cucarella: None. R. Ruiz: None. Á. Jimeno: None. L. Chirivella: None. A. Perez: None. I. Fariñas: None. N. Flames: None.

Poster

282. Fate Specification and Generation of Neuronal Diversity

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 282.11/B1

Topic: A.01. Neurogenesis and Gliogenesis

Title: Mechanisms of fate specification in Cux2-expressing neural progenitors

Authors: *S. P. FREGOSO¹, B. DWYER¹, S. J. FRANCO²

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Abstract: During neocortical development, neurons are produced by a diverse pool of neural progenitors. A subset of these progenitors express the Cux2 gene and predominantly produce late-born excitatory neurons, but the upstream pathways that specify this subset of progenitors remain unknown. Elucidating how Cux2 expression is activated in forebrain progenitors could provide insights into mechanisms of fate specification. To uncover the transcriptional network that regulates Cux2 expression, we are characterizing an ultraconserved Cux2 enhancer that we find recapitulates endogenous Cux2 expression in the developing forebrain. Using bioinformatic analysis of this enhancer to identify putative transcription factor binding sites, we found several potential binding sites for well-known forebrain patterning genes, including Emx1/2, Lhx2/6/8, and Dlx1/2. We are currently testing the role of these candidate transcription factors in regulating expression from the Cux2 enhancer using a combination of in vitro cell culture and in vivo studies. Additionally, as many putative *Cux2* regulators identified in our analysis are directly downstream of BMP signaling, we reasoned that BMPs could regulate Cux2 expression in the developing forebrain, as has been reported in other embryonic tissues. We demonstrate that BMP4 can upregulate Cux2 expression from both the Cux2 enhancer and the endogenous Cux2 locus in cell culture and in neocortical explants, respectively. While BMP signaling is known to be important for patterning forebrain progenitor domains, our results suggest that BMPs may also be an important contributing factor for activating the transcriptional network that directs $Cux2^+$ progenitor fate.

Disclosures: S.P. Fregoso: None. B. Dwyer: None. S.J. Franco: None.

Poster

282. Fate Specification and Generation of Neuronal Diversity

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant GM076430-09

NIH Grant EY022543

Title: Overturning the role of Math5 (Atoh7) in retinal ganglion cell specification

Authors: *J. BRODIE-KOMMIT¹, H. SHI², F. WU³, T. GLASER⁴, T. M. SCHMIDT⁵, X. MU³, J. H. SINGER², S. HATTAR^{6,7}

¹Biol., John Hopkins Univ., Baltimore, MD; ²Biol., Univ. of Maryland, College Park, MD; ³Univ. at Buffalo - Downtown Campus, Buffalo, NY; ⁴Dept. of Cell Biol. and Human Anat., Univ. of California Davis, David, CA; ⁵Neurobio., Northwestern Univ., Evanston, IL; ⁶Biol. and Neurosci, Johns Hopkins Univ., Baltimore, MD; ⁷Section on Light and Circadian Rhythms (SLCR), Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Retinal ganglion cells (RGCs) are the sole conduits of light information to the brain for both image and non-image forming functions. RGC differentiation occurs by retinal progenitor cells expressing transcription factors in a developmentally temporal fashion, including Math5 (Atoh7). Math5, a bHLH transcription factor, is thought to be necessary for RGC specification; since Math5 null mice lack 95% of RGCs, lack an optic nerve, lack adult retinal vasculature, yet contain an overgrown version of the developmental hyaloid vasculature. A recent publication used lineage tracing to show that a subset of RGCs do not express Math5. Therefore, we re-examined the Math5 null mouse line on a background that prevents RGC death by knocking out the proapoptotic gene, Bax. Surprisingly, we found that even in the absence of Math5, substantial numbers of retinal progenitor cells differentiate into RGCs. These rescued RGCs predominantly phenocopy wildtype RGCs gene expression (including Brn3a, Brn3b, & Isl1). However, despite restoring a large numbers of RGCs, the optic nerve still failed to develop in these animals, similar to the Math5 null mice. Since RGCs are classically defined as output neurons, we wanted to investigate whether these rescued RGCs receive rod/cone light input. Indeed, using multielectrode array recordings, RGCs from Math5/Bax double knockout animals displayed ON, OFF and ON/OFF rod/cone light responses. These data show there are two populations of RGCs, those that require Math5 for specification and those that do not, however all RGCs require Math5 for survival. Surprisingly, even though RGCs are rescued, in the double null retina, the hyaloid vasculature fails to regress, suggesting that communication between the Math5 dependent RGCs and the hyaloid vasculature is required for regression. Overall, these data suggest that Math5 is not required for proper specification of all RGCs, but that the RGCs that require Math5 for specification, or downstream Math5 effectors, are required for the survival of all RGCs, proper optic nerve development, and hyaloid vasculature regression.

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Poster

282. Fate Specification and Generation of Neuronal Diversity

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 282.13/B3

Topic: A.01. Neurogenesis and Gliogenesis

Support: T32MH015174

Title: Non-canonical Wnt signaling through Ryk drives SST cortical interneuron fate

Authors: *M. MCKENZIE¹, L. COBBS², G. J. FISHELL³, E. AU⁴ ¹Pathology and Cell Biol., Columbia Univ. Med. Ctr., New York, NY; ²NYULMC, New York, NY; ³Harvard Univ., Cambridge, MA; ⁴Columbia Univ., New York, NY

Abstract: Cortical GABAergic interneurons exhibit remarkable diversity in their intrinsic firing properties, subtype marker expression, layer organization, synaptic connectivity and morphology. The mechanisms underlying the generation of this diversity are largely unknown. We have identified a rostral-caudal Wnt gradient within the medial ganglionic eminence (MGE) that delineates the specification of the two main interneuron subtype classes. Caudally-situated MGE progenitors receive high levels of Wnt signaling and give rise to somatostatin (SST)expressing cortical interneurons. Parvalbumin-expressing basket cells, by contrast, originate mostly from the rostral MGE where Wnt signaling is attenuated. Interestingly, canonical Wnt signaling through b-catenin is not required for this process. Wnt signals transmitted via nuclear translocation of the intracellular domain of the non-canonical receptor Ryk, however, are sufficient to drive interneuron progenitors to a SST fate. Inhibition of Ryk signaling by a function blocking antibody conversely decreases the production of SST positive interneurons. Graded Ryk gain of function experiments performed in mouse ES-derived cortical interneurons reveal a dose-dependent effect, suggesting Ryk signaling acts in a gradient. These data, in combination with in vivo genetic loss of Ryk signaling in MGE derived lineages reveal a complex and important role for non-canonical Wnt signaling in establishing the correct ratios of mature cortical interneuron subtypes.

Disclosures: M. McKenzie: None. L. Cobbs: None. G.J. Fishell: None. E. Au: None.

Poster

282. Fate Specification and Generation of Neuronal Diversity

Location: Halls A-C

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Topic: A.01. Neurogenesis and Gliogenesis

Support: F32NS074742

K99MH104595 MH071679 MH111529 NS074972 NS081297

Title: Heterotypic transplantations reveal how the environment directs interneuron diversity and maturation

Authors: *T. J. PETROS¹, G. QUATTROCOLO², G. FISHELL² ¹Developmental Neurosci. Br., NIH/NICHD, Bethesda, MD; ²Neurosci., NYU, New York, NY

Abstract: Neural progenitors in the ganglionic eminences give rise to diverse GABAergic interneuron subtypes that populate all forebrain regions. Medial ganglionic eminence-derived progenitors are initially fated towards parvalbumin- or somatostatin-expressing classes during embryogenesis, a process that is guided by spatial and temporal gradients, and the mode of neurogenic division. The extent to which these cells are genetically predefined into specific interneuron subtypes, or rather their mature characteristics are obtained upon interaction with environmental cues, remains unknown. To address this question, we performed homo- and heterotopic transplantation of early postnatal MGE-derived cortical and hippocampal interneurons. Grafted cells migrated and displayed neurochemical, electrophysiological and morphological profiles similar to endogenous interneurons. Our findings indicate that the proportion of interneuron classes was largely determined by the host region. However, some transplanted cells retained characteristics indicative of their donor region, indicating that they may be more 'hard-wired' and not influenced by environmental signals. Our approach enables us to study how the environment regulates interneuron differentiation and maturation, which is critical for understanding normal interneuron development and the burgeoning therapeutic application of this transplantation approach.

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Poster

282. Fate Specification and Generation of Neuronal Diversity

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Muscular Dystrophy Association

New Your Stem Cell Foundation

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Title: Role of de novo DNA methyltransferases in the development and function of human motor neurons

Authors: *J. ORTEGA¹, M. ZILLER², D. SANTOS³, A. MEISSNER⁴, E. KISKINIS³ ¹Dept. of Neurol., Northwestern University, Feinberg Sch. of Medici, Chicago, IL; ²Max Planck Inst. of Psychiatry, Munich, Germany; ³Northwestern Univ., Chicago, IL; ⁴Broad Inst. of MIT and Harvard, Cambridge, MA

Abstract: DNA methylation is an epigenetic modification that is essential for gene regulation and development in mammals. DNA methylation patterns are exerted by three distinct DNA methyltransferase (DNMT) enzymes; DNMT1 is mainly responsible for genome wide maintenance of methylation patterns during replication, while DNMT3A and DNMT3B establish de novo DNA methylation. DNMT3A is known to be widely expressed in neural progenitors along the central nervous system (CNS) and to be critical for cell fate choice. However, the targets for *de novo* methylation mediated by DNMT3A, as well as the functional redundancy with DNMT3B during CNS development, remain unclear. Here, we utilized a powerful human embryonic stem cell (ESC) differentiation model for the generation of spinal motor neurons (MNs) in combination with genetic mutations in the *de novo* DNA methyltransferase machinery to assess the specific role of DNMT3A and 3B on: (1) neural lineage fate decisions and, (2) the physiological properties of postmitotic neurons. Over the MN differentiation, the two de novo DNMTs displayed opposite expression patterns. While the expression levels of DNMT3B rapidly decreased in the initial stages of differentiation, DNMT3A exhibited a dynamic expression pattern with sustained high levels at later stages. Systematic characterization of DNMT3A and DNMT3B KO and wild type cell lines over the MN differentiation protocol by different immunolabeling techniques showed that DNMT3A KO ESCs exhibited a reduced capacity to generate neurons and MNs, compared to isogenic control cells. RNA-sequencing and whole-genome bisulfite sequencing (WGBS) analysis demonstrated that this effect is in part due to a modification in the expression pattern of an important group of transcription factors. During MN differentiation, DNMT3A KO cultures displayed silenced expression of pro-neuronal transcription factors and up-regulation of neural floor plate transcription factors compared to the isogenic control cell line, while DNMT3B KO cultures remained unaffected. Strikingly, we also discovered that DNMT3A KO MNs displayed abnormal morphological, biochemical and electrophysiological properties. Our study underscores the importance of *de novo* DNA methylation during human neurogenesis, dissecting the regulatory link between DNA methylation dynamics and MN specification and functional maturation.

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Poster

282. Fate Specification and Generation of Neuronal Diversity

Location: Halls A-C

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Project NORTE-01-0145-FEDER-000008-Porto Neurosciences and Neurologic Disease Research initiative at I3S supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through FEDER.

Title: A role for Tlx3 and Hipk2 in Prrx11 phosphorylation during the development of nociceptive neurons

Authors: *C. M. REGUENGA^{1,3}, A. F. DIAS^{3,2}, F. MONTEIRO^{3,2}, D. LIMA^{3,2} ¹Biomedicine - Exptl. Biol. Unit, ²IBMC- Inst. De Biologia Mol. E Celular, Porto, Portugal; ³Faculdade de Medicina da Univ. do Porto, Porto, Portugal

Abstract: Proper balance in the number of excitatory glutamatergic and inhibitory GABAergic neurons in dorsal spinal cord is essential for the correct functioning of the neuronal circuitry integrating sensory information from the periphery to the brain. It is known that the homeodomain transcription factors Tlx3 and Prrx11 are essential for the determination of the excitatory phenotype. Noteworthy, Prrx11 phosphorylation appears to play an important role in the spinal cord glutamatergic population development, changing from very high levels at early stages, where neural fate commitment takes place, to progressively low levels from E15.5 onwards. Multiple phosphorylation sites were mapped in the Prrx11 primary structure and four correspond to conserved phospho-S/T-P sites, which suggest a role for the prolyl isomerase Pin1 in regulating Prrxl1 function. These S/T-P sites give rise to distinct phosphorylation profiles, which are key determinants of Prrx11 conformation and thereby protein functioning. The phosphorylation states acquired by Prrxl1 during different developmental phases promote the association of Prrx11 with distinct molecular partners. We showed, by 2D electrophoresis and reporter assays, that both Tlx3 and Hipk2 (homeodomain-interacting protein kinase 2) modulate Prrxl1 activity by controlling its phosphorylation status. In addition, Hipk2 is not transcriptionally regulated by Tlx3, as revealed by qPCR, which suggests that Tlx3 promotes Prrx11 phosphorylation via Hipk2 recruitment to the transcriptional protein complex. Finally, we also implicated the Hipk2/Pin1 protein complex in the positive modulation of Tlx3 transcriptional activity, further implicating phosphorylation events in the molecular interplay between Tlx3 and Prrx11. The role of post-translational mechanisms in embryonic development is now beginning to be explored, and ours are the first data pointing to their importance in the differentiation of the nociceptive neurons.

Disclosures: C.M. Reguenga: None. A.F. Dias: None. F. Monteiro: None. D. Lima: None.

Poster

282. Fate Specification and Generation of Neuronal Diversity

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 282.17/B7

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH KO8

AOSpine North America

Title: microRNA controls over corticospinal motor neuron development

Authors: *J. L. DIAZ¹, V. B. SITHTHANANDAN¹, V. LU¹, J. L. MACDONALD³, N. GONZALEZ-NAVA⁴, L. PASQUINA⁵, A. WHEELER⁵, P. SARNOW², T. PALMER¹, J. D. MACKLIS⁶, S. A. THARIN¹

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Abstract: Corticospinal motor neurons (CSMN), residing in layer V of the neocortex, are the brain component of the multi-neuron circuitry controlling voluntary movement in mammals. CSMN are closely related to deep layer callosal projection neurons (CPN) and have the same birthdates, however very different projection targets and functions. CPN are similarly glutamatergic cortical projection neurons, but are involved in associative cognitive function and project to the contralateral cortex. While several transcription factors important for both CSMN and CPN have been identified, much of the molecular regulation of this process remains incompletely understood. MicroRNAs (miRNAs) are post-transcriptional gene regulators that act by promoting degradation or repressing translation of target mRNAs, including those encoding transcription factors. miRNAs are ideally suited to control developmental programs, as multiple miRNAs can converge on multiple targets to repress translation. While miRNAs have been shown to play a role in the earliest stages of cortical development, their role in cortical projection neuron development and diversity is unknown. A differential miRNA expression analysis of CSMN vs. CPN revealed 20 miRNAs that are more highly expressed by CSMN vs. CPN at postnatal day 1 (P1) mouse. Several of these miRNAs are encoded on a single genomic miRNA cluster. One cluster-encoded miRNA, miR-CSMN1, is enriched in CSMN compared to CPN at P1 but not at P4. Predicted gene targets of miR-CSMN1 include CITED2 and LMO4, two transcription factors important for CPN and associative projection neuron development. Gainand loss-of function studies demonstrate that miR-CSMN1 can control cortical projection neuron fate in embryonic cortical cultures. Similar functional studies on other cluster-encoded miRNAs are ongoing. Characterizing cluster-encoded miRNA controls on CSMN development may

inform stem cell-based regenerative therapies for paralysis in clinically relevant conditions, including spinal cord injury and ALS.

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Poster

282. Fate Specification and Generation of Neuronal Diversity

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Support: NIMH R01 MH081880

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UCSF CTSI, 1111111

Predoctoral Training in Neurobiology, T32 GM07449-40

Title: Mafb and c-Maf control the balanced neurogenesis of MGE-derived PV⁺ and SST⁺ GABAergic cortical interneurons

Authors: *L.-L. PAI¹, D. VOGT², A. C. PEREZ³, M. WIMER³, G. MCKINSEY⁵, J. S. HU⁶, M. SANDBERG⁶, R. PLA⁶, L. V. GOODRICH⁸, J. T. PAZ⁴, J. L. RUBENSTEIN⁷ ¹Neurosci., Univ. of California, San Francisco, San Francisco, CA; ²Dept. of Pediatrics and Human Develop., Michigan State Univ., Grand Rapids, MI; ³Dept. of Neurol., ⁴Neurol., Gladstone Inst. of Neurolog. Dis., San Francisco, CA; ⁵Dept. of Psychiatry, ⁶Psychiatry, ⁷Nina Ireland Lab. Dev Neurobiol, Univ. of California San Francisco, San Francisco, CA; ⁸Harvard Med. Sch., Boston, MA

Abstract: GABAergic cortical interneurons (CINs), primarily generated by medial and caudal ganglionic eminences (MGE & CGE), control circuit excitability by acting as a breaking system in the brain. CIN malfunctions are implicated in neuropsychiatric diseases including autism spectrum disorder, schizophrenia and epilepsy. Understanding CIN generation and maturation are crucial in order to better understand interneuronopathy-related brain diseases. MGE-derived CINs are composed of two broad subgroups: parvalbumin- (PV; late-born) and somatostatin- (SST; early-born) expressing. While MGE interneuron progenitor fate is initially determined by the Nkx2.1 and Lhx6 transcription factors (TFs), factors regulating the control over CIN fate and generation are still being uncovered. Here, we characterized two TFs, Mafb and c-Maf, which are genetically downstream of Nkx2.1, Lhx6 and Dlx, as critical for generating the correct balance of early and late born MGE CIN progenitors.

Combined deletion of Mafb and c-Maf from MGE progenitors resulted in ~70% reduction of MGE-derived CINs at P35, with preferential loss of the PV subgroup. Surviving CINs reside in deeper cortical layers and are more likely to be early-born SST interneurons. At embryonic ages, EdU birthdating experiments showed no change of MGE CIN progenitor numbers with deletion of Mafb and c-Maf but an increase of precocious neurogenesis. Notably, we also found a robust increase of SST CINs in the neocortex of these mutants. Together, these results suggest Mafb and c-Maf control the balance of neurogenesis in late progenitors of the MGE, and these early disruptions cause a shift in the balance of PV^+ and SST^+ CINs.

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Poster

282. Fate Specification and Generation of Neuronal Diversity

Location: Halls A-C

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

Support: European Research Council-Advanced Grant "Locomotor Integration"

The Swedish Research Council

Title: Transcriptome analysis of spinal excitatory neurons

Authors: *L. BORGIUS¹, V. R. CALDEIRA¹, E. PROUX-WÉRA², M. RASING¹, P. LÖW¹, O. KIEHN¹

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Abstract: For neuronal circuits in the spinal cord that control locomotion, developmentally expressed transcription factors have served as entry points for functional assignment of left-right and flexor-extensor coordination circuits, as well as for rhythm-generation circuits. It is clear though that functions within each of these circuits most often are represented by molecularly heterogeneous groups of neurons, where further specification by molecular markers is needed to dissect the circuitries. In other cases, known groups of neurons already specified by distinct markers may not completely capture the functionality of the circuits and alternative or supplementary markers will be needed. For rhythm-generation several glutamatergic molecularly

defined groups of cells are thought to be involved without any of them being responsible alone.creating the function alone. In order to capture new markers for this important groups of neurons in the spinal cord we have performed FAC-sorting, RNA-sequencing and differential expression analyses on glutamatergic neurons from the mouse ventral spinal cord. We compared the postnatal transcriptional expression profile of all glutamatergic neurons in the SC, the Vglut2-expressing neurons, to that of non-glutamatergic neurons as well as to one of the glutamatergic subgroups so far linked to rhythm-generation, the Shox2 interneurons (Dougherty et al. 2013). Amongst the transcripts up-regulated in the Vglut2-expressing neurons are wellknown glutamatergic developmental-markers such as *Sim1*, *Evx2*, *Lhx3*, *Chx10*, *Shox2* and *Lbx1*. The analysis also identified more then 200 receptors, transcription factors, ion-channels and other neurotransmitters specifically

expressed in the entire population of Vglut2-positive neurons as compared to non-glutamatergic neurons and Shox2-positive neurons. Our findings identify novel glutamatergic subgroups in the spinal cord and provide tools for further specification of spinal cord functions. In addition, since Vglut2 positive neurons are found in many regions of the nervous system, this work provide new molecular entry points to reveal the diverse function of this group of transmitter-defined neurons.

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Poster

282. Fate Specification and Generation of Neuronal Diversity

Location: Halls A-C

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

Support: MOST 104-2311-B-010-010-MY3

MOST 106-2321-B-010-012

Title: Preferential expression of Foxp2 in striatonigral projection neurons in the striatum of adult mouse brain

Authors: L. FONG, *F.-C. LIU Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: The striatum comprises GABAergic projection neurons which accounts for ~90% of striatal neurons. There are two populations of striatal projection neurons (SPNs), striatonigral SPNs of the direct pathway and striatopallidal SPNs of the indirect pathway. Striatonigral SPNs express dopamine D1-class receptor, whereas striatopallidal SPNs express dopamine D2-class receptor. It has been proposed that activation of striatonigral pathway facilitates movement,

whereas activation of striatopallidal pathway inhibits movement. The antagonistic but balanced activity of these two pathways is important for motor control. Foxp2, a language-associated gene, is expressed at high levels in the striatum. Our recent study suggests that Foxp2 plays an important role in synaptogeneis of corticostriatal circuits and vocal communication (Chen et al., 2016). It is of interest to characterize striatal cell types that express Foxp2. Here, we analyzed the expression pattern of Foxp2 with respect to specific cell types of SPNs and interneurons in the adult mouse striatum. Based on double in situ hybridization and immunostaining, we found that Foxp2 was expressed in SPNs, but not in striatal interneurons. Moreover, Foxp2 was preferentially expressed in striatonigral SPNs. This result is consistent with the previous BAC transgenic mouse study showing that Foxp2 expression is primarily associated with Drd1-EGFPpositive neurons in the striatum (Vernes et al., 2011). We also analyzed the expression pattern of Foxp1, a member in Foxp family. In contrast to the preferential expression of Foxp2 in striatonigral SPNs, Foxp1 was expressed in both populations of striatonigral and striatopallidal SPNs. Foxp1 was not expressed by striatal interneurons, either. In summary, the cell-type preference of Foxp2 expression in the striatum implicates a unique role of Foxp2 in regulation of striatonigral SPNs in striatal circuits.

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Poster

282. Fate Specification and Generation of Neuronal Diversity

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

Support: NIH R01 NS065054

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Bob Allison Ataxia Research Center

Title: Distribution, morphology, and function of serotonergic neurons in the larval zebrafish spinal cord

Authors: *J. E. MONTGOMERY¹, T. D. WIGGIN¹, B. CORWIN¹, C. LILLESAAR², L. BALLY-CUIF³, M. A. MASINO¹

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Abstract: Vertebrates possess two distinct cellular sources of spinal serotonin. The first source is the raphe nuclei, from which supraspinal projections descend to innervate the spinal cord. The second source is a population of intraspinal serotonergic neurons (ISNs) that are intrinsic to the

spinal cord. ISNs in fish and amphibians are almost exclusively localized to the ventromedial spinal cord, in close proximity to motor neurons and premotor interneurons. Thus, it is hypothesized that the ISNs are involved in modulating locomotor output. Here, we use a combination of *in vivo* optical imaging strategies to investigate the morphology and activity of the ISNs. The tryptophan hydroxylase 2 and pet1 Ets-domain transcription factor promoters were used to drive expression of fluorescent reporters in the ISNs. First, examination of EGFP expression revealed that ISNs somas were ventrally-located and evenly distributed along the rostrocaudal axis of the spinal cord. Next, we photoconverted Kaede in single ISNs between 3 and 10 days post fertilization to quantify and track cell morphology, such as projection distances, neurite lengths, and arborization. Morphological characteristics exhibited the greatest changes between 3 and 4 days post fertilization, which corresponds to the onset of mature beat-and-glide swimming behavior. Finally, genetically-encoded calcium indicators were used to study the relationship of ISN activity to fictive locomotor output. ISNs displayed a variety of activity states: quiescence, tonic activity, and phasic with motor activity. All three of these activity states were observed during both fictive swimming episodes and periods of rest. ISN activity not being phasically coupled with locomotor activity is consistent with the ISNs having a modulatory influence on locomotor output, rather than being an integral part of the pattern generating network.

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Poster

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Title: KAT3 proteins are crucial for the maintenance of neuronal identity

Authors: *M. LIPINSKI, J. M. CARAMES, N. CASCALES-PICÓ, M. T. LÓPEZ-CASCALES, J. FERNÁNDEZ-ALBERT, J. MEDRANO-RELINQUE, A. MEDRANO-FERNÁNDEZ, B. DEL BLANCO, S. CANALS, J. P. LOPEZ-ATALAYA, A. BARCO Inst. De Neurociencias De Alicante UMH-CSIC, San Juan De Alicante, Spain

Abstract: Studies in different organisms have demonstrated that neuronal identity needs to be actively maintained throughout life by the combined action of transcription factors and chromatin modifiers. The paralogue lysine acetyltransferases 3 (KAT3) CBP/KAT3A and p300/KAT3B play an important role in the regulation of transcription and chromatin threedimensional structure. Although both proteins are essential for the normal development of the nervous system, their specific functions in post-mitotic neurons remain unclear. In order to investigate this matter, we produced inducible forebrain-specific knockout (ifKO) mice for either one or for both KAT3 proteins. Interestingly, we observe that only when both proteins are knocked out simultaneously, mice show severe neurological phenotypes and an increased mortality. These deficits are accompanied by a rapid and progressive reduction in the dendritic complexity of forebrain neurons and loss of normal electrophysiological properties. Remarkably, these dramatic changes correlate with a strong and specific downregulation of neuron-specific gene expression whereas housekeeping genes were mostly unaffected. We also observed that the lack of KAT3 caused a robust decrease in histone acetylation at specific residues, such as H2B panacetylation and H3K27ac. Overall, our experiments demonstrate that KAT3 proteins are necessary for maintaining neuronal identity and function during adulthood, most probably through the regulation of acetylation levels at neuronal enhancers and gene bodies.

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Poster

282. Fate Specification and Generation of Neuronal Diversity

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

Support: NSF-BCS 1627993

Title: Neural patterns underlying the development of planning tool use

Authors: *O. OSSMY, B. KAPLAN, M. XU, K. E. ADOLPH Dept. of Psychology and Ctr. of Neural Sci., New York Univ., New York, NY

Abstract: Flexible, purposeful tool use requires action planning. Adults' action plans keep both the initial contact with the tool and the end goal in mind, even when the end goal stretches far into the future. Children, however, show dramatic deficits in planning when the end goal is not immediately accessible to perception. For example, participants of all ages normally reach for the handle of a hammer using an overhand radial grip. But when the handle points away from the

dominant hand, an initially uncomfortable underhand grip is required to ensure the desired final position of the tool. This phenomenon of sacrificing comfort in the initial grip to allow for a comfortable end position is called "end-state comfort." In contrast to adults, young children frequently select a comfortable initial grip, as if planning for *start-state comfort*. As a consequence, their hand is in an awkward end-state position when they use the tool. Indeed, children do not show consistent planning for end-state comfort until 10 or 12 years of age. Here, we examined the possible sources of differences in action planning between young children and adults. We innovated a novel method for obtaining electroencephalography (EEG), headmounted eye tracking, motion tracking, and video simultaneously in an end-state comfort hammering task. First, we replicated previous work showing deficits in 4-year-old children's action planning. Then we compared EEG and visual attention for each age group for trials requiring planning for end-state comfort (handle pointing away from dominant hand) and trials that required planning only for start-state comfort (handle pointing toward dominant hand). At the neural level, we found differences in readiness potential over sensory-motor sites preceding initial end-state comfort grips in adults compared with the readiness potential preceding similar grips in young children. We used support vector machine (SVM) and random forest (RF) algorithms to describe preparatory neural patterns underlying differences in planning between the age groups. We also show that participants' fixation location and motion kinematics are correlated with their grip. These results indicate that young children's deficits in planning for end-state comfort stem from differences in neural activity and visual attention prior to moving the hand.

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Poster

283. Autism Genetic Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 283.01/B14

Topic: A.07. Developmental Disorders

Support: NSERC

Title: Abnormal prostaglandin E_2 signalling results in autism-associated behaviours in novel mouse models

Authors: *C. T. WONG^{1,2}, I. BESTARD LORIGADOS^{2,3}, R. RAI-BHOGAL^{2,3}, D. A. CRAWFORD^{1,2,3} ¹Kinesiology and Hlth. Sci., ²Neurosci. Grad. Diploma Program, ³Dept. of Biol., York Univ., North York, ON, Canada **Abstract: Background**: Prostaglandin E2 (PGE₂) is an endogenous lipid-derived signalling molecule produced by Cyclooxygenase-2 (COX2). PGE₂ is important in brain development and function. We have previously shown that PGE₂ affects calcium regulation in growth cones, migration, proliferation, and differentiation in neural stem cells *in vitro*. Moreover, PGE₂/COX2 signalling can be disrupted through genetic changes along the pathway or exogenous factors, such as diet and exposure to infections, inflammation, and chemicals; all which have been associated with autism. Behavioural outcomes that manifest from abnormal PGE₂/COX2 signalling during prenatal development *in vivo* remain unknown.

Objective: The major goal of this study is to determine if mice exposed to PGE_2 during the critical time in prenatal development or mice lacking the PGE_2 producing enzyme (COX2) exhibit behaviours typically seen in autism. We also aim to determine whether there are age or sex differences in these animal models.

Methods: In this study, we examined offspring from a minimum of three independent litters for PGE₂-exposed and homozygous COX2^{-/-} groups. In the PGE₂-exposed model, a single subcutaneous injection of 16,16-dimethyl-PGE₂ ($0.2\mu g/g$) was administered to pregnant C57bl/6 wildtype mice on embryonic day 11, corresponding to neurogenesis. 129S6 wildtype mice served as the controls for the COX2^{-/-} mice. Subjects were split into young (4-6 weeks old) and adult (8-12 weeks old) groups, and underwent the following behavioural tests on separate days: marble test, 3-chamber test, open field test, and inverted screen test.

Results: We showed that PGE₂-exposed and COX2^{-/-} mice buried more marbles than control mice, indicating both models display greater repetitive and anxiety-related behaviour compared to respective controls. In the 3-chamber test, PGE₂-exposed and COX2^{-/-} mice spent less time in the novel mouse chamber and more time in the novel object chamber compared to controls, demonstrating lower sociability in both groups. In the open field test, PGE₂-exposed and COX2^{-/-} mice travelled a greater pathlength than controls, suggesting increased hyperactivity in both groups. In the inverted screen test, no differences in fall percentage were seen in PGE₂-exposed mice, while COX2^{-/-} mice fell more than their controls. Age and sex differences were also observed in both models for all behavioural tests.

Conclusions: Our findings determined that disruption in PGE₂/COX2 signalling during prenatal development can result in autism-like behaviours. We provide new evidence supporting the usage of these novel model systems for studying neurodevelopmental disorders such as autism.

Disclosures: C.T. Wong: None. I. Bestard Lorigados: None. R. Rai-Bhogal: None. D.A. Crawford: None.

Poster

283. Autism Genetic Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 283.02/B15

Topic: A.07. Developmental Disorders

Support: Hussman Foundation # HIAS15006

Title: Relationship between cadherin 11 and autism traits

Authors: *Y. WANG¹, *Y. WANG¹, C. WANG², X. YUAN² ¹Hussman Inst. For Autism, Baltimore, MD; ²Hussman Inst. for Autism, Baltimore, MD

Abstract: Genome-wide association studies and whole exome sequencing have suggested that genetic variants of type II cadherins, including Cdh8, Cdh9, Cdh10, Cdh11, Cdh12, Cdh13, are associated with autism. However, whether there is a causal relationship between cadherin alterations and autism traits remains to be clarified. Based on genome-wide gene co-expression analysis, we found that in the developing human brain, a group of well-established autism risk genes exhibits convergent expression profiles. Interestingly, the expression of one type II cadherin family member Cdh11, but not Cdh9, is highly correlated with the expression of several well-established autism risk genes, suggesting greater relevance of Cdh11 than Cdh9 in the development and function of autism-related brain circuits. The functional linkage of Cdh11 and Cdh9 with autism was further evaluated by behavioral analysis of Cdh11 and Cdh9 gene knockout (KO) mice. Cdh11 KO mice exhibited behavioral abnormalities that are very similar to core behavioral traits and comorbidities of people with autism, including higher explorative activity, reduced anxiety level, increased repetitive behavior, reduced social interactions, and impaired motor coordination. In contrast, Cdh9 KO mice only exhibited very mild abnormalities in the same behavioral tests. In cerebellar tissues from Cdh9 and Cdh11 KO mice, activities of the PI3K signaling pathway, a pathway implicated into autism pathology, were also affected differently. These findings establish a potential relationship between Cdh11 rather that Cdh9 and autism traits, and identified Cdh11 KO mice as an important animal model for autism. Our findings also highlight the effectiveness of the genome-wide gene co-expression analysis in the prediction of the functional impact of novel gene variants in autism studies.

Disclosures: Y. Wang: None. C. Wang: None. X. Yuan: None.

Poster

283. Autism Genetic Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 283.03/B16

Topic: A.07. Developmental Disorders

Title: An altered neurodevelopmental profile in mice deficient for autism associated Neurexin1 gene communicative and motor aspects at an early stage

Authors: *M. L. SCATTONI¹, A. CARUSO¹, S. DELLA NOTTE¹, C. FERNANDES² ¹Inst. Superiore di Sanita, Roma, Italy; ²King's Col. London, London, United Kingdom

Abstract: Autism Spectrum Disorders (ASD) are a group of neurodevelopmental disorders with early onset of symptoms, characterized by socio-communicative deficits and patterns of stereotyped behaviours. Although the causes of ASD remain unclear, evidence strongly supports the role of genetic factors in their aetiology, including mutations in Neurexin (NRXN) family genes. Our study aimed the behavioural phenotyping of mice with deletion of Nrxn1 α gene encoding for a neuronal presynaptic cell-adhesion molecule in order to identify autistic-like features, as soon as possible during the early developmental period. We evaluated the ontogentic profile of the vocal response during the first weeks of life through a detailed analysis of the ultrasonic vocalizations. Ultrasonic vocalizations are emitted by mouse pups in response to isolation from the mother and littermates and considered a suitable tool for the identification of early communication deficits in autism mouse models. Moreover, since motor dysfunctions can predict the onset of the other symptoms in ASD, we performed a fine-grain characterization of spontaneous motor behaviours, recorded simultaneously with the ultrasonic vocalizations. This is the first vocal and motor evaluation of Nrxn1a mutant pups that allows identification of autisticlike phenotypes at an early developmental stage. Our results indicate that an altered profile is detectable in the emission of ultrasonic vocalizations and acquisition of specific motor patterns in $Nrxn1\alpha$ mutant pups, in line with behavioural phenotypes of ASD children. Our findings suggest that the $Nrxn1\alpha$ gene has an important neurodevelopmental function and its deletion causes specific early behavioural abnormalities.

Disclosures: M.L. Scattoni: None. **A. Caruso:** None. **S. Della Notte:** None. **C. Fernandes:** None.

Poster

283. Autism Genetic Models

Location: Halls A-C

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Program#/Poster#: 283.04/B17

Topic: A.07. Developmental Disorders

Title: NRF2 deficient mice exhibit regression following P14 exposure to valproic acid

Authors: *J. GIFFORD, S. A. NORTON, A. W. KUSNECOV, G. C. WAGNER Psychology, Rutgers Univ., Piscataway, NJ

Abstract: Autism is associated with high levels of oxidative stress biomarkers, increased body burdens of environmental toxicants that induce oxidative stress, and decreased levels of antioxidants. The objective of the present study was to advance our model of autistic regression using mice with a deletion of the NRF2 gene (nuclear factor E2-related factor 2), a master regulator for downstream enzymes associated with management of toxicant-generated reactive oxygen species. On postnatal day 14 KO and WT animals were exposed to sodium valproate (VPA; 400 mg/kg; s.c.) or saline and evaluated on a behavioral battery assessing maturation of

normal social and motor skills to classify toxicant-induced deficits along a developmental timeline. Prior studies revealed this treatment induces functional and pathological changes akin to autistic regression. Results of mid air righting testing provide evidence of regression of normal motor development in that saline-treated animals show similar development across genotypes but animals exposed to VPA exhibited a loss of the recently acquired ability to mid-air right with the NRF2 KO mice more affected than WT mice. We operationally define this loss of an acquired skill as *regression*. Additionally, in the rotarod test, KO-treated animals spent significantly less time than WT and saline-treated counterparts on the rotarod. These results indicate a critical importance of NRF2 during development as it might relate to autism and, more generally, the deleterious effects of oxidative stress during early development.

Disclosures: J. Gifford: None. **S.A. Norton:** None. **A.W. Kusnecov:** None. **G.C. Wagner:** None.

Poster

283. Autism Genetic Models

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Topic: A.07. Developmental Disorders

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Title: Studies using Crmp4-KO mice show the functional relevance of Crmp4-deficiency to some symptoms of ASD

Authors: *Y. NAKANO¹, A. TSUTIYA², E. HANSEN-KISS³, B. J. KELLY³, M. NISHIHARA⁴, Y. GOSHIMA⁵, D. CORSMEIER³, P. WHITE³, G. E. HERMAN³, R. OHTANI-KANEKO¹

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Children's Hosp. and Dept. of Pediatrics, The Ohio State Univ., Columbus, OH; ⁴Vet. Med. Sci., Tokyo, Japan; ⁵Yokohama City Univ. Sch. Med., Yokohama, Japan

Abstract: It is known that males have greater risk of developing neurodevelopmental disorders, such as autism spectrum disorders (ASD), than females. Our previous proteomics study identified collapsin response mediator protein (CRMP) 4 as a protein exhibiting sex-different expression during sexual differentiation of the sexually dimorphic nucleus of the hypothalamus (Iwakura et al., 2013). Recently, we found a *de novo* mutation of *CRMP4* in a male patient with ASD from whole exome sequencing. ASD is characterized by impaired social interaction, verbal and non-verbal communication, restricted range of interest, and abnormal sensory perception. The aim of the present study is to investigate the relationship between Crmp4-deficiency in mice and ASD-like behaviors as well as sex different effects of Crmp4-deficiency by comparing social interaction behaviors and sensory perception abilities between Crmp4-KO and wild type (WT) mice of both sexes. Furthermore, in order to obtain the information for mechanisms underlying Crmp4-deficiency-induced ASD-like behaviors, altered gene expressions in brain areas including the cortex, hippocampus, and olfactory bulb were examined in Crmp4-KO and WT mice of both sexes. In addition, we examined the effect of Crmp4-deficiency or de novo mutation of Crmp4 on dendritic morphology of cultured hippocampal neurons. First, eight types of behavioral tests including social interaction test and three-chambered social approach task were performed in young adult (4-11 weeks old) WT and Crmp4-KO mice of both sexes. Crmp4-KO mice exhibited decreased social interaction activity, compared to WTs. It was more severely changed in male Crmp4-KO mice than in females. Furthermore, altered thermal perception was observed only in male Crmp4-KO mice, compared to male WTs. The expression levels of some genes including AMPA receptors (GluR1 and 2) and a dopamine receptor (D1R) were changed mostly in a gender-dependent manner in the brain of Crmp4-KO mice, compared to WTs. Furthermore, Crmp4-deficiency enhanced dendritic branching of cultured hippocampal neurons, compared to WTs. Expression of mutated mouse Crmp4, which is homologous to human mutated CRMP4 found in ASD patient, in cultured hippocampal neurons derived from Crmp4-KO mice also increased their dendritic branching, compared to those derived from Crmp4-KO mice and transfected with WT Crmp4. These studies indicate the functional relevance of a case-specific rare-variant of one molecule, Crmp4, to some symptoms of ASD and suggest its possible mechanisms.

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Poster

283. Autism Genetic Models

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Topic: A.07. Developmental Disorders

Support: LouLou Foundation Grant CDKL5-16-108-01

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NIH Grant 1 R56 N100738-01

Title: Loss of CDKL5 impairs hippocampus-dependent memory and hippocampal LTP in a mouse model of CDKL5 disorder

Authors: *S. HAO^{1,2}, Z. WU^{1,2}, B. TANG^{1,2}, Y. HUANG^{1,2}, H. Y. ZOGHBI^{1,2,3,4,5}, J. TANG^{1,2} ¹Dept. of Pediatrics, Baylor Col. of Medici, Houston, TX; ²Jan and Dan Duncan Neurolog. Res. Institute, Texas Children's Hosp., Houston, TX; ³Dept. of Mol. and Human Genetics, Baylor Col. of Medici, Houston, TX; ⁴Dept. of Neuroscience, Baylor Col. of Medici, Houston, TX; ⁵Howard Hughes Med. Institute, Baylor Col. of Medici, Houston, TX

Abstract: Mutations in the X-linked gene cyclin-dependent kinase-like 5 (CDKL5) have been identified to induce CDKL5 disorder, a neurodevelopmental disease characterized by early-onset seizures, severe intellectual disability, motor impairment, and autistic features. A few studies showed that CDKL5 animal models recapitulate some of the CDKL5 symptoms including cognitive deficits. Although long-term potentiation (LTP) serves as a neural substrate of learning and memory, the synaptic plasticity in CDKL5 mutant mice has not been investigated. Here, we assessed the hippocampus-dependent learning and memory function in a unique CDKL5 knockout mouse model with exon 6 deletion (B6.129(FVB)-Cdkl5^{tm1.1Joez}/J). In fear conditioning task, the male CDKL5 knockout mice ($Cdkl5^{-/y}$) froze significantly less than wide type (WT, $Cdkl5^{+/y}$) littermates in both contextual and cued fear memory test, consistent with the previous report. Importantly, we uncovered that the female heterozygous $Cdkl5^{+/-}$ mice were impaired in contextual fear memory as well, compared to WT controls. In another hippocampus-dependent task, passive avoidance, both male and female CDKL5 mutant mice had shorter latencies to enter the dark compartment during memory retention test in comparison with WT littermates. Guided by these behavioral data, we examined hippocampal LTP in awake, free moving $Cdkl5^{-/y}$ mice vs. WT controls. Field recordings from the hilar region of the dentate were measured in response to electrical stimulation in the medial part of the perforant path. The recording and stimulating electrodes were implanted 2-3 weeks before the tests. Theta burst stimulation induced 174% potentiation in mutant mice compared to 239% in WT controls 1 h after induction, indicating the impairment of synaptic plasticity in Cdkl5^{-/y} mice. These results demonstrate that CDKL5 mediates hippocampal learning and memory likely through the regulation of hippocampal synaptic plasticity.

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Poster

283. Autism Genetic Models

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Topic: A.07. Developmental Disorders

Support: FAPESP Grant 2013-01610-7

Capes Grant

Title: Zinc as a therapy in an experimental model of autism prenatally induced by valproic acid

Authors: *L. C. CEZAR¹, T. B. KIRSTEN², C. C. N. FONSECA³, A. P. N. LIMA¹, M. M. BERNARDI², L. F. FELICIO¹

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Abstract: Autism is a complex developmental disorder characterized by numerous behavioral impairments, such as communication, socialization and cognitive inflexibility. Valproic acid (VPA) is an anti-epileptic drug with teratogenic activity that has been related to autism. In rodents, exposure to VPA during the pregnancy induced symptoms similar to those found in the human condition of that disorder. However, there are no effective treatments for autism. Particularly, exposure to VPA may alter zinc metabolism resulting in a transient deficiency of zinc. Therefore, we selected zinc as prenatal treatment to prevent VPA-induced deficiencies. In a rat model of VPA-induced autism, we tested the treatment with zinc supplementation. Wistar female rats received either saline solution or VPA (400 mg/kg, i.p) on gestation day (GD) 12.5. In order to test the zinc supplementation effect, after 1 h of treatment with saline or VPA, a dose of zinc (2 mg / kg) was injected. The offspring was tested for abnormal communication behavior by ultrasound vocalization task on post-natal day 11 (PND), repetitive behavior and cognitive ability was tested by T-maze task on 29 PND and social interaction was tested by play behavior task on 30 PND. We also evaluated tyrosine hydroxylase protein (TH) expression, in the striatum. VPA treatment induced alterations in various behavioral parameters. VPA group showed decreased ultrasonic vocalization, repetitive/restricted behavior, cognitive inflexibility, impairment of socialization, characterized by decreased play behavior and reduction in striatal TH levels as compared with saline control group. The zinc treatment reduced VPA-induced autism-like behavioral changes. Zinc supplementation generated behavioral improvement in the cognitive inflexibility, attenuated the VPA-induced deficit on social play behavior, and restored the vocalization pattern. However, we found no evidence of zinc effect on the VPA-induced reduction in the TH striatum levels. The persistence of low TH expression in VPA-Zn group suggests that Zn-induced behavioral improvement in autistic rats may not depend on TH activity.

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Poster

283. Autism Genetic Models

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Topic: A.07. Developmental Disorders

Support: AMED Grant

Grant-in-Aid for Scientific Research on Innovative Areas Research

Title: Analysis of empathic neural circuits regulated by oxytocin

Authors: *S. YADA, K. HORIE, K. NISHIMORI

Tohoku Univ., Sendai-Shi, Japan

Abstract: Human has highly sociality. Social behavior is essential in human society, in particular, compassionate and cooperative behaviors which help others without expecting external compensation are important, It is said that motivation for causing such social behaviors is "empathy" [Decety et al. 2016]. Empathy is impaired in many neuropsychiatric disorders including autism spectrum disorder (ASD). Although ASD has a high prevalence rate (~1%), its etiology and treatment are still unknown. ASD is a congenital neurodevelopmental disorder that is impaired in sociality and communication skill. Patients show perseveration and avoid interpersonal relationships. It is thought that neuroscientific analysis of empathy broadly lead to the treatment of social disorders of many psychiatric disorders including ASD. In recent years, Oxytocin (Oxt) and its receptor (Oxtr) are focused on ASD study because it has been reported that administration of Oxt to ASD patients and model mice has restored social disorders [Brian et al. 2015, Watanabe et al. 2015]. Oxt is a peptide synthesized in the paraventricular nucleus and supraoptic nucleus in the brain and is known to be involved in social behaviors such as maternal behavior and affinity. There are few reports on the association between empathy and Oxt / Oxtr and it has not been comprehensively understood yet. In this study, we aim to clarify how the Oxt / Oxtr system controls empathy. We performed empathic behavioral tests using Oxt / Oxtr knockout mice and prairie vole (Microtus ochrogaster) to identify brain regions involved in empathy. Prairie voles are known to show strong monogamous system and used as a model animal in social behavior research, since there is a report that wild-type prairie voles show empathetic behavior between monogamous pair [Burkett et al. 2016]. In the behavioral test, after pair bonding, we provide a fear stimulus to female mouse or prairie vole. We measured empathy as allogrooming behavior from paired male to female which showed fear response. It resulted that wild-type mice and prairie voles showed empathic behavior. However, empathic behaviors

were reduced in Oxt / Oxtr knockout mice compared with wild type ones. In addition, immediately early gene activations were detected in anterior cingulate cortex and olfactory nucleus during empathic behavior.

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Poster

283. Autism Genetic Models

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Topic: A.07. Developmental Disorders

Support: King's Health Partners

Title: Transient early gestational antithyroid treatment in mice results in behavioral deficits in adult offspring

Authors: M. PITSIANI¹, L. J. WILSON¹, A. RAJIC⁴, D. BELL⁴, C. FERNANDES², *R. J. WINGATE³

¹MRC Neurodev Disorders, ²MRC SGDP, ³King's Col. London, London, United Kingdom; ⁴Fac. of Health, Sci. and Technol., Univ. of Suffolk, Ipswich, United Kingdom

Abstract: Hypothyroidism within a critical window of brain development leads to severe mental retardation later in life associated with maldevelopment of the cortex and cerebellum. However, a variety of sources of evidence suggest that transient hypothyroidism in early gestation, prior to the critical window for "cretinism", is associated with cognitive deficits in offspring in later life including Autistic Spectrum Disorder. We have previously examined the affects of transient depletion of thyroid hormone levels on the early development of the avian cerebellum. In chick, early hypothyroidism disrupts the formation of the nuclei that comprise the output of the cerebellum well before the thyroid hormone- dependent events in cortical and cerebellar development. We therefore hypothesised that thyroid-dependent cerebellar disconnection may underlie cognitive deficits in late life. Hence, transient early gestational hypothyroidism might result in discrete cerebellar functional and anatomical consequences that are distinct from cortical phenotypes associated with late embryonic and early postnatal hypothyroidism. To test this, we used a mouse model and treated pregnant dams with either Methimazole (0.1%)in the drinking water) or control treatment from 0 to 14 days gestation. ELISA analysis showed that Methimazole (MMI) treatment results in a significant drop in T3 and T4 levels at 14 days gestation. Litters (n = 16) were then raised through to P64 and challenged with a battery of behavioural and motor tests (juvenile social play, adult social investigation, three chamber test, olfactory habituation/dishabituation test, open field, light/dark, elevated plus maze, grooming, marble burying, Y maze spatial discrimination, rotarod, muscle grip).

The brains of adult offspring in experimental and control groups were analysed by X-ray micro-CT scan following immersion in Iohexol (150-200 mg/mL) for 14 days. Gross morphology of both groups was broadly similar, suggesting that the behavioural phenotype result from relatively subtle changes in cell number, physiology or connectivity.

The only significant differences in behaviour were detected in open-field test of anxiety and a three-chamber test for sociability. These phenotypes are sex-specific. Male offspring show heightened anxiety (open-field). Female mice show reduced social interaction (three-chamber test). Both results are consistent with features of mouse models of human autistic spectrum disorder. This suggests that the findings in clinical and epidemiological studies of human cohorts can be simulated in a simple model of transient gestational hypothyroidism in mice.

Disclosures: M. Pitsiani: None. L.J. Wilson: None. A. Rajic: None. D. Bell: None. C. Fernandes: None. R.J. Wingate: None.

Poster

283. Autism Genetic Models

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Topic: A.07. Developmental Disorders

Support: NIH 1P50MH096891

Title: Sociability development in w=mice with cell-specific deletion of the NMDA receptor GluN1 (NR1) subunit gene

Authors: *S. L. FERRI¹, A. A. PALLATHRA², H. KIM², H. C. DOW², W. B. BILKER³, T. ABEL¹, S. J. SIEGEL⁴, E. S. BRODKIN²

¹Physiol., The Univ. of Iowa, Iowa City, IA; ²Psychiatry, ³Biostatistics, Univ. of Pennsylvania, Philadelphia, PA; ⁴Psychiatry, USC, Los Angeles, CA

Abstract: N-methyl-D-aspartate receptors (NMDARs) are important for brain development, synaptic plasticity, and learning and memory. NMDAR dysfunction has been linked to a number of neurodevelopmental disorders including autism and schizophrenia. While loss of NMDAR has been previously associated with social deficits, age and sex specificity has been understudied. We used the Cre-Lox system to ablate the the NMDAR subunit GluN1 (NR1) specifically in either excitatory or a subset of inhibitory neurons with a CaMKII or parvalbumin (PV) promoter, respectively, and measured social approach behavior of 30-day-old and 70-day-old male and female mice in a three-chambered apparatus. We found that deletion of GluN1 in either cell type had no effect on distance traveled in the arena. Mice lacking GluN1 in PV neurons showed no change in time spent sniffing the cylinder containing the social target compared to PV-Cre littermate controls, regardless of age or sex. However, both male and female mice lacking GluN1
in forebrain excitatory neurons showed a significant increase in time spent sniffing the cylinder containing the social target mouse compared to CaMKII-Cre littermate controls, at both 30d and 70d of age. These findings may shed light upon cell types and brain areas involved in autism, schizophrenia, and other disorders of sociability.

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Poster

283. Autism Genetic Models

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Topic: A.07. Developmental Disorders

Support: NIH Grant R15S088776

Title: Characterizing ultrasonic vocalizations in NS-Pten knockout pups: Implications for autism

Authors: *M. BINDER, J. LUGO

Psychology & Neurosci., Baylor Univ., Waco, TX

Abstract: A signaling cascade that plays a crucial role in the development of an autistic-like phenotype is the PI3K/AKT/mTOR pathway. Mouse models that illustrate this connection include Fmr1, Tsc1, and Neuron-subset specific (NS)-Pten knockout mice. While numerous studies have investigated ultrasonic vocalizations (USVs) in Fmr1 and Tsc1 knockout mice, none have investigated USVs in NS-Pten knockout mice using the Avisoft recording system. In light of this, the present study utilized a maternal separation paradigm to characterize neonatal vocalizations in NS-Pten knockout male and females on postnatal days 8 and 11. We found that knockout pups emitted fewer vocalizations for both sexes (p < .05). Knockout males had calls of a shorter duration and lower peak amplitude on day 8, while showing a shorter duration, lower peak amplitude, and higher peak and fundamental frequency on day 11 (p < .001). Knockout females vocalized at a lower peak amplitude and fundamental frequency, and a higher peak frequency on day 8, while showing a shorter duration and higher peak and fundamental frequency on day 11 (p < .001). Spectrographic analyses also revealed significant differences in call type for both genotypes and sexes (p < .05). These findings demonstrate that deletion of NS-Pten results in significant decreases in vocalizations across both sexes. Additionally, our findings indicate that the aberrant vocalizations and increased call duration seen in other mTOR models are also present in NS-Pten knockout mice. Our study provides evidence of a connection between hyperactive mTOR signaling and ultrasonic vocalizations. The results may provide new insights into the impact of mTOR signaling and an autistic-like phenotype.

Disclosures: M. Binder: None. J. Lugo: None.

Poster

283. Autism Genetic Models

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Topic: A.07. Developmental Disorders

Support: NIH GRANT R01 MH090127

CAPES

Morrison Trust

Title: Pathogenic role for kynurenine pathway in maternal immune activation-induced autismlike behavior in mice

Authors: *D. S. COELHO¹, A. M. GARRISON², K. KIMENEZ², L. REDUS², J. VALDERAS², J. C. O'CONNOR³

¹Pharmacol., Univ. of Texas Hlth. Sci. Ctr. At San A, San Antonio, TX; ²Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX; ³Pharmacol., UTHSCSA, San Antonio, TX

Abstract: Prenatal inflammation is a risk factor for neurodevelopmental disorders. In rodent models, maternal immune activation (MIA) causes phenotypes in offspring that resemble many of the features of these human disorders, but the pathogenic mechanisms remain unclear. Immune activation upregulates the tryptophan metabolizing enzyme indoleamine 2,3dioxygenase (IDO) which mediates many of the behavioral consequences of inflammation in adults, and experimentally increasing kynurenine levels of pregnant dams causes developmental consequences in the offspring. Therefore, we hypothesized that MIA-induced upregulation of IDO during gestation is a pathogenic mechanism by which fetal neurodevelopment is disrupted and autism-like phenotypes develop. Pregnant female IDO-/- mice or control C57BL/6J mice were administered 20 kg/mg polyinosinic:polycytidylic acid (poly I:C) or saline i.p. on gestational day 12.5. At various ages, autism-like behaviors were measured. Repetitive selfgrooming behavior was significantly increased in WT-MIA male offspring compared to WTsaline controls. Self-grooming behavior in IDO-/- mice was not affected by MIA. MIA reduced preference for social novelty, measured in the 3-chamber test, in male offspring independent of IDO, while it increased social novelty preference in WT female mice. In mice with targeted deletion of kynurenine monooxygensase (KMO), endogenous kynurenine levels are markedly increased, and offspring of KMO KO dams exhibit several autism-like behaviors. Our data suggest that IDO may play an important pathogenic role in the development of specific autismlike behaviors caused by MIA.

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Poster

283. Autism Genetic Models

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Topic: A.07. Developmental Disorders

Support: CIHR to LAMG MOP-142308

Title: Preadolescent oxytocin ameliorate effects of maternal corticosterone and SSRI exposure on male and female offspring

Authors: *W. QIU¹, K. GO², L. CASANUEVA², A. R. GOBINATH², S. E. LIEBLICH², P. DUARTE-GUTERMAN², L. A. M. GALEA³

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Abstract: Postpartum depression can affect up to 15% of women. Fluoxetine is a common antidepressant prescribed to treat postpartum depression. Although the data are conflicting, perhaps to do timing and nature of exposure, developmental exposure to selective serotonin reuptake inhibitors (SSRIs), like fluoxetine, during the peri-partum and postpartum period may increase the risk of Autism Spectrum Disorder in children. Oxytocin can enhance social interactions in rodents, and intranasal oxytocin is currently being investigated in clinical trials as a treatment for children with Autism Spectrum Disorder. The aim of this study is to investigate the effects of maternal postpartum corticosterone (to induce depressive-like behavior in the dam) and maternal fluoxetine administration on male and female offspring. Oxytocin is a peptide hormone that does not readily cross the blood-brain barrier. Here we used a nanoparticle drug delivery system TriozanTM to facilitate oxytocin entering the central nervous system in preadolescent offspring. We hypothesized that the use of Fluoxetine during the postpartum period to dams will decrease social behaviour in both male and female offspring. Administration of high corticosterone to the dam increases maternal depressive-like behavior, reduces maternal care and increases anxiety-like behavior in male, but not female, offspring. We expect that oxytocin, given to preadolescent offspring, will reverse the adverse effect of maternal fluoxetine and normalize social behaviour, microbiome composition, proinflammatory cytokines, and adult neurogenesis compared to controls. Dams will be given corticosterone (40mg/kg) to simulate postpartum depression and fluoxetine (10mg/kg) for 23 days. Administration of oxytocin (0.5mg/kg) and TriozanTM (0.25mg/mL) in offspring will occur for 10 days from postnatal day 25 to day 34. Offspring will be tested for social, anxiety and locomotor behaviour during adolescence (postnatal day 35-37) and again in adulthood (postnatal day 70-73). Preliminary

results indicated in adult rats showed that in both males and females, 0.5mg/kg of oxytocin increased social investigation. The results of the current study will determine whether preadolescent exposure to oxytocin mitigates any adverse effects of maternal corticosterone and concurrently fluoxetine on offspring behavior, microbiome, and neuroinflammatory profile. Funding from CIHR to LAMG (MOP-142308).

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Poster

283. Autism Genetic Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 283.14/B27

Topic: A.07. Developmental Disorders

Support: NSERC

Title: Sensory processing deficits in a prenatal immune activation model of altered neurodevelopment

Authors: F. HADDAD, L. LU, C. DEOLIVEIRA, *S. SCHMID Univ. of Western Ontario, London, ON, Canada

Abstract: Altered brain development is associated with neuropsychiatric disorders like autism spectrum disorder (ASD) and schizophrenia. Maternal infection during pregnancy has been linked with an increased risk of offspring developing these disorders, implicating maternal infection in abnormal neurodevelopment. Further epidemiological and preclinical research identified the immune response as a major mediator of these detrimental effects on neurodevelopment. To isolate effects of the innate maternal immune response on fetal brain growth, the viral marker polyinosinic:polycytidylic acid (poly I:C) is commonly used to elicit a pathogen-free immune response. Maternal injection of poly I:C during gestation produces offspring with structural and behavioral changes relevant to core symptoms of ASD and schizophrenia. We investigated the effect of early and mid-gestation (Gestation Days 9 and 14) poly I:C treatment in rats on offspring social behaviour, spontaneous open field activity and sensory processing. Sensory processing was measured through habituation and multisensory prepulse inhibition of the acoustic startle response, while social behavior was investigated using a 3-chamber apparatus measuring sociability and social recognition. All tests were done in adolescence (6 weeks) and adulthood (4 months) to investigate the change in phenotype across age in the offspring. Our results show that mid-gestation immune activation impaired visual but not auditory prepulse inhibition (PPI) in both adolescence and adulthood, and this effect was sexdependent in adolescence. In contrast, spontaneous short and long-term habituation of startle as

well as spontaneous open field activity were unaffected. Additionally, adolescent GD-14 poly I:C offspring showed decreased sociability with familiar animals but normal social recognition. Social behaviour in adulthood was unaffected. Preliminary results from GD-9 immune activation show similar deficits in multisensory PPI with a decrease in visual PPI compared to the controls. However, no effects of early immune activation were found in social behavior or spontaneous open field activity. The observed visual PPI and sociability impairments can be linked to neurodevelopmental disease phenotypes, particularly those involving sensory processing and multisensory integration deficits. Future studies will attempt to study the association of these phenotypes with anatomical and molecular findings, as well as whether they correlate with higher order cognitive function.

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Poster

283. Autism Genetic Models

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Patrick Wild Crentre

Department of Biotechnology India

Title: Behavioural characterisation of a rat lacking the GAP domain of SynGAP

Authors: *D. KATSANEVAKI^{1,2,3}, S. TILL^{1,2,3}, S. NAWAZ^{4,2}, O. DANDO^{1,2,3}, L. MIZEN¹, G. HARDINGHAM^{1,2,3}, S. CHATTARJI^{4,2,3}, E. WOOD^{1,2,3}, O. HARDT^{5,2,3}, D. WYLLIE^{1,2,3}, P. KIND^{1,2,3}

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Abstract: SYNGAP1 haploinsufficiency is one of the most common causes of nonsyndromic intellectual disability and autism. De novo mutations in the SYNGAP1 gene lead to the loss of the encoded protein SynGAP, one of the most abundant of postsynaptic proteins. SynGAP acts as a regulator of signaling pathways linked to AMPA- and NMDA-receptor plasticity at the post synaptic density. The *Syngap* heterozygous mouse model has been widely used to understand the pathophysiology underlying abnormal SynGAP-mediated signaling. Recent advances in techniques for genome manipulation allow for the generation of rat models of neurodevelopmental disorders; enabling phenotypes to be validated across species and addressing cognitive and social dysfunction, using paradigms that are more difficult to assess in mice. In this study, we examine the pathophysiology associated with a heterozygous deletion of the C2 and catalytic GAP domain of the protein, in rats (HET). Importantly, this smaller form of SynGAP is produced and localises to synapses at the same levels as wild type SynGAP. As was found with a homozygous null deletion of SynGAP in mice, homozygous loss of the C2 and GAP domains results in perinatal death in rats. However, in contrast with HET mice, HET rats do not present with hyperactivity and can be habituated to an open field environment. To examine associative recognition memory, we tested the rats in five spontaneous exploration tasks for short-term memory, object-recognition (OR), object-location (OL), object-place (OP), objectcontext (OC) and object-place-context (OPC). We also tested long-term memory in OR and OL. Both groups were able to perform short-term memory tasks, but only wild type rats performed above chance in OL with a 24h delay, suggesting deficits in long-term spatial memory. We then tested if partial loss of the GAP domain in SynGAP affects social behaviour in rats and we found that HET rats exhibited impaired short-term social memory, with no signs of social isolation. One consistent observation through all the tasks was decreased exploratory activity of the HET rats which was due to increased immobility. To examine this further we tested rats in a cued-fear conditioning paradigm, where we found that HET rats had generalised fear and abnormal fear extinction. These findings do not fully recapitulate abnormalities reported in the mouse model of Syngap haploinsufficiency, suggesting that some key behavioural phenotypes may be speciesspecific. We are currently examining the effect of the full protein deletion in rats, to complement existing data and provide greater insight into cognitive dysfunction associated with dysregulation in SynGAP-mediated signaling.

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Poster

283. Autism Genetic Models

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Topic: A.07. Developmental Disorders

Support: R00 (4R00MH099243-03)

IDDRC, Children's Hospital of Pennsylvania

Title: Assessing the differential integration of reward benefits and costs in neurexin1a mutant mice

Authors: *O. O. ALABI, M. FORTUNATO, M. FUCCILLO Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Abnormalities in how reward shapes behavior are present in a number of neuropsychiatric disorders including autism spectrum disorders and schizophrenia. While there are extensive genetic resources available to study these disorders in mice, few goal-directed tasks have been designed that reliably characterize how mice weigh differential reward benefits and cost in their selection of actions. In this study, we designed a forced-choice two-alternative serial reversal task in which each lever was associated with specific reward outcomes and required operant contingencies. Furthermore, to simultaneously probe flexibility of choice, we used instantaneous performance measures to trigger contingency switches over the course of a session. We developed global and trial-by-trial measures to assess the choice patterns and behavioral flexibility of mice in response to differing "choice benefits" (modeled as reward magnitude ratios) and different types of "choice cost" (modeled as either increasing repetitive motor effort or increased delay to reward delivery). We have made the following observations: (1) mouse choice is highly sensitive to both relative reward ratio and the density of feedback information; (2) choice costs are heavily discounted in environments with large disparities in reward volume; (3) mice differentially altered their response pattern in response to the introduction of delay and effort costs on the higher reward lever. Delay decreased the likelihood that mice would choose the same alternative on subsequent trials (effectively decreasing the positive reinforcing effects of that alternative), while increasing effort thresholds on high-reward alternatives paradoxically increased the reinforcing effects of the non-modified alternative. We are currently employing this behavioral paradigm in Neurexin1 α mutants, a mouse genetic model of neuropsychiatric disease, where preliminary data suggests deficits in both value-based reinforcement and flexible adaptation of choice.

Disclosures: O.O. Alabi: None. M. Fortunato: None. M. Fuccillo: None.

Poster

283. Autism Genetic Models

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Topic: A.07. Developmental Disorders

Support: AMED

Title: Prenatal minocycline treatment alters synaptic protein expression, in oxytocin receptorknockout mice

Authors: *S. MIYAZAKI, S. HIDEMA, K. NISHIMORI

Tohoku Univ., Sendai-Shi, Japan

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impaired communication, difficulty in companionship, repetitive behaviors and restricted interests. Recent studies have shown amelioration of ASD symptoms by intranasal administration of oxytocin and demonstrated the association of polymorphisms in the oxytocin receptor (Oxtr) gene with ASD patients. Deficient pruning of synapses by microglial cells in the brain has been proposed as potential mechanism of ASD. Other researchers have shown specific activation of microglial cells in brain regions related to sociality in patients with ASD. Although the roles of Oxtr and microglia in ASD are in the spotlight, the relationship between them remains to be elucidated. In this study, we found abnormal activation of microglial cells and a reduction of postsynaptic density protein PSD95 expression in the Oxtr-deficient brain. Moreover, pharmacological inhibition of microglia during development can alter the expression of PSD95 and improve abnormal mother-infant communication in Oxtr-deficient mice. Our results suggest that microglial abnormality is a potential mechanism of the development of ASD-like phenotypes.

Disclosures: S. Miyazaki: None. S. Hidema: None. K. Nishimori: None.

Poster

283. Autism Genetic Models

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Topic: A.07. Developmental Disorders

Support: George Washington University

Title: Novel paradigms to analyze digging motivation and social interaction in mouse models of neuropsychiatric disease

Authors: *H. POND¹, O. MCKISSICK¹, M. WILKINSON¹, P. PARLANTI³, M. MANZINI² ²Pharmacol. and Physiol., ¹The George Washington Univ., Washington, DC; ³NEST - Natl. Enterprise for nanoScience and nanoTechnology, Scuola Normale Superiore, Pisa, Italy

Abstract: Digging behavior is often used to test anxiety and repetitive behaviors in mice. However, digging is also a normal mouse behavior, that can be focused toward different goals, i.e. foraging for food, burrowing for shelter, removing noxious stimulus, or even for recreation as has been seen in animals like dogs and ferrets. The marble burying test, where digging is measured as related to the number of marbles buried in deep bedding has become a popular assay for anxiety, obsessive compulsive disorder and repetitive behaviors in autism spectrum disorder (ASD) in mouse models. However, this test can have ambiguous results and does not discriminate between anxiety due to presence of the novel objects, the marbles, and compulsivity using intensity of digging.

We developed the Discriminatory Digging Behavior test a test to make clear determinations between different types of digging behavior in mice based on the driving motivation, distinguishing between foraging (food-seeking) and burrowing (shelter-seeking). Mice are given a choice between emptying a tube filled with paper bedding (the burrow) and digging in deep corncob bedding. By testing mice which were fed ad libitum and then food-deprived, we showed that mice will increase foraging outside the burrow when hungry. To show that this difference was not due to acclimatization to the new environment, we performed the test again on the food deprived mice two weeks after their food intake returned to ad libitum showing that they revert to the previous behavior. When comparing male and female mice, we also found that females do not switch between burrowing and foraging when food deprived as males do. Females have shown different physiological response to food-deprivation than males and they greatly reduce energy expenditure. This sex difference appears to be captured by our test. This novel test can be used to probe brain circuits involved in food seeking, shelter/safety seeking and anxiety and to test animal models of neuropsychiatric disorders. We started by using a mouse model of intellectual disability (ID) and ASD with reduced marble burying performance, mice deficient for the ASD/ID gene Cc2d1a. We found that in fact their burrowing index and burrowing behavior in general was reduced in males, and not altered in females. Because burrowing improves with experience and is also socially facilitated we are now developing variants on this test for assessing learning and social deficits. This cooperative burrowing test can provide a novel social test that is not focused on olfaction as a measure, using mice that are already part of the subject's social structure.

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Poster

283. Autism Genetic Models

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Title: Development of an operant task to quantify social motivation in autism mouse models

Authors: *C. T. WEICHSELBAUM¹, K. PARKER², K. KNIEPKAMP³, M. R. BRUCHAS⁴, J. D. DOUGHERTY⁵

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Abstract: The social motivation theory of autism posits that social interaction may be less rewarding for individuals with autism spectrum disorders (ASD) than typically-developing individuals. However, it has also been suggested that reward processing may be affected more globally in ASD. Mouse models present a promising opportunity to explore these competing hypotheses across a variety of ASD etiologies, such as ASD-associated genetic mutations; however, standard sociality tasks do not directly quantify social motivation. Thus we developed an operant conditioning task to measure the rewarding value of a social stimulus, which may be compared with non-social stimuli to assess whether deficits in reward processing are global or specific to the social domain. We modified a standard mouse operant chamber (Med Associates Inc.) to house a stimulus animal accessible to the test mouse via a guillotine-style door. Bars in the doorway prevent the test mouse from exiting the operant chamber, but allow visual, auditory, olfactory, and tactile contact between mice when the door is raised. Thus the test mouse may be rewarded with social interaction rather than the typical food or drug reward. A progressive ratio (PR) schedule of reinforcement determines the breakpoint for the social reward, that is, how much effort the mouse is willing to exert to obtain that social contact. When rewarded with access to a novel age- and sex-matched conspecific, the majority of wildtype C57Bl/6j mice successfully condition within 5 days on a fixed ratio (FR)-1 schedule, and demonstrate a PR breakpoint comparable to that of non-social rewards (i.e. sucrose pellets). Testing of several ASD genetic mouse models is ongoing. This social operant task represents a valuable addition to the repertoire of sociality tasks used to assess mouse models of ASD, and will help to elucidate the role of social-specific vs. global reward processing deficits in this disorder.

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Poster

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Topic: A.07. Developmental Disorders

Support: NIH IRACDA/INSPIRE Grant: K12GM093854 (JWL)

Title: An autism mouse model exhibits enhanced stress responses associated with increased norepinephrine system activity

Authors: *J. W. LUNDEN¹, C. PENG¹, M. GENESTINE SCHMITT¹, M. DURENS¹, S. PREM¹, V. R. MIRABELLA², A. MARKOV¹, J. H. MILLONIG³, E. M. DICICCO-BLOOM⁴ ¹Neurosci. and Cell Biol., Rutgers - Robert Wood Johnson Med. Sch., Piscataway, NJ; ²Neurosci. and Cell Biol., Rutgers - Robert Wood Johnson Med. Sch., New Brunswick, NJ; ³Neurosci. and Cell Biol., Rutgers Univ., Piscataway, NJ; ⁴Dept Neurosci & Cell Biol/ Pediatrics (Child Neurol. & Neurodevelopmental Disa, Rutgers-Robert Wood Johnson Med. Sch., Piscataway, NJ

Abstract: Autism spectrum disorder (ASD) is a pervasive neurodevelopmental disorder with prevalent psychiatric comorbidities including anxiety and depression. Monoamine neurotransmitters, including norepinephrine (NE) and serotonin, play important roles in modulating anxiety and mood. Significantly, the neural patterning transcription factor Engrailed-2 (En2) is involved in the development of the embryonic mid-hindbrain region, where monoamine neurons emerge, and our studies, as well as others, suggest EN2 is associated with ASD. The present study tested the hypothesis that En2 knockout mice (En2-KO) would have defects in regulating the hypothalamic-pituitary-adrenal (HPA) axis stress system, which is controlled in part by innervating NE fibers and activity. The amygdala and ventral hippocampus (vHipp) exert stimulatory and inhibitory inputs to the paraventricular nucleus of the hypothalamus (PVN) to regulate glucocorticoid release. Multiple regions were characterized structurally (NE fibers) and functionally (c-fos) before and after swim stress. The En2-KO amygdala exhibited increased NE innervation, including increased TH (1.7-fold; p<0.02) and NET (1.5-fold; p<0.0017) proteins and NET fibers (2.3-fold; p<0.0007) though CRF mRNA levels were reduced by 85% (p<0.0019). Similarly, the PVN had increased NET fibers (1.7-fold; p<0.016) yet reduced CRF (50%; p<0.004). Based on these results we speculated that altered NE innervation may produce parallel changes in neural activity, which we assessed by defining nuclear c-fos immunostaining. Swim stress is known to produce increased brain regional c-fos+ cells 2h later. Indeed, in amygdala swim stress induced 30% (p=0.03) greater increase in BLA cfos+ neurons in En2-KO vs WT, and 2-fold greater c-fos+ cells in PVN (p=0.001) suggesting correlations between innervation and activation. As one source of NE neurons we examined

locus coeruleus and found swim stress induced a 6-fold increase in c-fos+ neurons in En2-KO (p<0.0001) compared to only 2-fold increase in WT (p<0.0001), potentially identifying a mechanism for increased NE fiber activity. On other hand, the En2-KO vHipp exhibited 30% lower c-fos response to swim stress compared WT (p=0.0074). Our observations indicate that NE fiber innervation in the *En2*-KO mice is markedly increased in the PVN and BLA, whereas it is reduced in the hippocampus. These results support a model of NE fiber innervation regulating neural activity. Moreover, this pattern of enhanced excitatory signaling (amygdala, PVN) accompanied by reduced inhibitory activity (vHipp) will likely lead to excessive activation of the HPA axis, an outcome under current investigation.

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Poster

283. Autism Genetic Models

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Topic: A.07. Developmental Disorders

Support: Brain and Behavior Research Foundation

Title: The role of STEP in a prenatal valproic acid exposure model of autism

Authors: *M. CHATTERJEE, J. XU, P. SINGH, P. J. LOMBROSO, P. K. KURUP Child Study Ctr., Yale Univ., New Haven, CT

Abstract: Autism Spectrum Disorder (ASD) is characterized by social communication deficits, repetitive and restrictive behaviors. Genetic and environmental factors which interfere with the early stages of neuronal development contribute to an increased risk for ASD. Valproic acid (VPA) is a commonly used antiepileptic drug and mood stabilizer. Maternal exposure of VPA is associated with a significant increase in the occurrence of ASD in humans as well as in rodent models. Prenatal exposure of VPA interferes with the normal synaptic signaling pathways during brain development which eventually contributes to behavioral abnormalities seen in ASD. STEP (STriatal-Enriched Protein tyrosine phosphatase) is a CNS-specific enzyme, which opposes the development of synaptic plasticity. STEP downregulates the surface expression of glutamate receptors as well as several kinases involved in synaptic strengthening. High levels of STEP activity is implicated in several neurological disorders where it contributes to synaptic and cognitive deficits. In this context, reducing STEP activity by genetic ablation or pharmacologic inhibition significantly attenuates the biochemical, behavioral and cognitive deficits in these disorders.

Here, we investigated the role of STEP in a prenatal VPA model of autism. We have found that STEP protein levels are upregulated in the prefrontal cortex of prenatal VPA-exposed mice, which show characteristic autistic symptoms. The increase in STEP level is correlated with a decrease in the phosphorylation status of its substrates (ERK, GluN2B) suggesting a relationship between STEP activity and behavioral abnormalities in these models. We hypothesize that abnormal STEP expression and downregulation of its substrates during critical stages of development contributes to synaptic and behavioral deficits, which could be attenuated by pharmacological reduction of STEP activity. STEP Inhibitor, TC-2153 rescues the behavioral abnormalities like hyperactivity, stereotypy and social deficits in the VPA exposed wildtype offsprings. These pre-clinical experiments will serve as a proof of concept to understand the role of STEP in an environmental model of autism.

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Poster

283. Autism Genetic Models

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Cuerpo Académico de Neurociencias (UV-CA-28)

Cuerpo Academico de Neuroquimica (UV-CA-304)

Title: Modification of GABA receptors in the cerebellum of autistic rats subjected to an enriched environment

Authors: *J. A. PEREZ¹, O. E. CRUZ¹, M. R. TOLEDO-CARDENAS², L. I. GARCIA², G. CORIA-AVILA², M. E. HERNÁNDEZ², J. MANZO² ¹Doctorado En Investigaciones Cerebrales ,UV, Xalapa, Mexico; ²Ctr. en Investigaciones Cerebrales, UV, Xalapa, Mexico

Abstract: Autism is a neurodevelopmental disorder that causes a spectrum of heterogeneous behaviors, having as common denominator problems in social interaction and alterations in both communication and movement. Also, another common denominator is the alteration of the cerebellum, which presents a reduction in size and a significant decrease in the GABAergic

neurotransmission. Considering the central role of GABA in the functioning of the cerebellum, in this project we were interested in analyzing the density of its receptors in an autistic rat model, and the impact of the stimulation of the subjects by an enriched environment. Thus, we did an immunohistochemical analysis of GABA receptors (rGABA) as a basis for the study of this neurotransmitter in the spectrum. We used an autistic Wistar rat model obtained after postnatal injection of a daily dose of 150 mg/kg of valproic acid to pups from day P6 to P12. Four groups of males were used, Controls (Ct) in standard (SE) and enriched (EE) environment, and Autistic (At) in SE and EE. Then, rGABA in the vermis of the cerebellum was quantified. The results showed that At subjects presented a significant reduction of rGABA, whose density increased to level Ct after being exposed to an enriched environment. The results show that no matter the reduction of rGABA in autistic subjects, the enriched environment has an optimal impact in its density. Therefore, data shows non-pharmacological benefits that can be applied to subjects within the spectrum, possibly prompting appropriate modifications in their behavioral displays, which will be analyzed in future studies.

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Poster

283. Autism Genetic Models

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Title: Autism-associated 16p11.2 microdeletion impacts prefrontal connectivity in humans and mice

Authors: ***A. BERTERO**^{1,2}, A. LISKA², R. PAROLISI⁴, M. GRITTI³, M. PEDRAZZOLI⁵, D. GERGELY², G. VALETTI⁴, A. GALBUSERA², A. SARICA⁶, A. CERASA⁶, M. BUFFELLI⁵, R. TONINI³, A. BUFFO⁴, M. PASQUALETTI^{1,2}, A. GOZZI²

¹Univ. of Pisa, Pisa, Italy; ²Functional Neuroimaging Laboratory,Center for Neurosci. and Cognitive Systems @UniTn, Inst. Italiano di Tecnologia, Rovereto, Italy; ³Neurosci. and Brain Technologies Dept., Inst. Italiano di Tecnologia, Genova, Italy; ⁴Dept. of Neurosci. Rita Levi-Montalcini, Neurosci. Inst. Cavalieri Ottolenghi (NICO), Univ. of Turin, Torino, Italy; ⁵Neuroscience, Biomedicine and Movement, Univ. of Verona, Verona, Italy; ⁶Consiglio Nazionale delle Ricerche, Catanzaro, Italy

Abstract: Human chromosome 16p11.2 microdeletion (16p11.2 del), a genetic alteration associated with impaired development and mild intellectual disability, is one of the most

common gene copy number variation in autism, accounting for approximately 0.5-1% of all autism spectrum disorder (ASD) cases. Recent studies suggest that ASD symptoms may be partly mediated by deficiencies in brain synchronization and connectivity arising during development. This study tests the hypothesis that genetic vulnerability to ASD conferred by 16p11.2 might involve dysfunctional communication amongst brain regions. To this purpose, we first analysed resting-state fMRI (rsfMRI) scans in 16p11.2 del and control subjects from the Simons VIP repository. By using motion-related volume scrubbing we were able to retain a sufficient number of control and 16p11.2 del subjects for inter-group statistical mapping (n=28 and n=19, respectively). Inter-group rsfMRI connectivity mapping revealed reduced global connectivity in lateral temporal regions of 16p11.2 del carriers, plus a focal involvement of medial prefrontal areas. To corroborate these findings, we probed prefrontal rsfMRI connectivity in a mouse model of 16p11.2 deletion and found that 16p11.2^{+/-} mice exhibit reduced prefrontal connectivity within the default and salience networks. Importantly, retrograde axonal labelling in 16p11.2^{+/-} mutants revealed altered neuronal density in prefrontal-projecting thalamic-nuclei, suggesting a contribution of thalamo-frontal miswiring to the observed connectivity impairment. We also probed microstructural white matter integrity in $16p1.2^{+/-}$ mice using diffusion tensor imaging and show that 16p11.2 mutants exhibit widespread increase in fractional anisotropy, a finding recapitulating previous observation in children with 16p11.2 deletion. By using electron microscopy, we identified increased axonal diameter in callosal fibers as a possible cellular correlate of these alterations. Taken together, our findings highlight convergent connectivity aberrancies in human 16p11.2 del carriers and in a mouse model of human 16p11.2 deletion, and suggest that ASD-associated 16p11.2 copy number variations can predispose to neurodevelopmental disorders and cognitive disability through a dysregulation of prefrontal functional connectivity.

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Poster

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

Authors: *S. LIN

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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. And gamma oscillation regulated by FS interneurons dysfunction exists in some ASD mouse models. Thus, we hypothesized the gamma oscillation in the mPFC is involved in autism. We found that gamma oscillation dysfunction, decreased Glutamate N-methyl-D-aspartate receptors (NMDARs) function of pyramidal neurons and declined excitability of fast-spiking (FS) interneurons. Together, our findings suggest that the mPFC microcircuit dysfunction may contribute to the ASD-like phenotypes in NL3 R451C KI mice.

Disclosures: S. Lin: None.

Poster

283. Autism Genetic Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 283.25/B38

Topic: A.07. Developmental Disorders

Support: KAKENHI 25117006

KAKENHI 25460345 KAKENHI 26250014 KAKENHI 26461554 KAKENHI 16H06316 KAKENHI 16H06463

Title: Serotonin rebalances cortical tuning and behavior linked to autism symptoms in 15q11-13 duplication mice

Authors: *N. NAKAI¹, M. NAGANO², F. SAITOW², Y. WATANABE³, Y. KAWAMURA⁴, K. TAMADA¹, H. MONAI¹, H. HIRASE¹, H. MIZUMA⁵, H. ONOE⁵, T. MIYAZAKI⁶, M. WATANABE⁶, S. OKABE⁷, M. KANO⁷, K. HASHIMOTO⁴, H. SUZUKI², T. TAKUMI¹ ¹RIKEN BSI, Wako, Japan; ²Nippon Med. Sch., Tokyo, Japan; ³Univ. Hosp. German Cancer Res. Ctr., Heidelberg, Germany; ⁴Hiroshima Univ., Hiroshima, Japan; ⁵RIKEN CLST, Kobe, Japan; ⁶Hokkaido Univ. Sch. Med., Sapporo, Japan; ⁷Univ. Tokyo, Tokyo, Japan

Abstract: Copy number variation (CNV) is one of the most prevalent genomic abnormalities in ASD. We previously generated a 15q11-13 duplication model mouse corresponding to the most cytogenetically frequent CNV in ASD and showed that paternal duplication (15q dup) mice displayed ASD-like behaviors including poor social communication and interaction, behavioral inflexibility and abnormalities of cortical spines and cerebellar functions. We also found a reduction in the level of serotonin (5-HT) and in the volume of the dorsal raphe nucleus (DRN), which contains a large proportion of 5-HT neurons providing serotonergic projections to cortical forebrain regions. In the central nervous system of ASD patients, a decreased rate of 5-HT synthesis in developmental stages was reported, suggesting a hyposerotonergic state. The 5-HT system also has abnormal modulatory effects on cognitive function in ASD, and behavioral abnormalities are thought to result from 5-HT-impaired neural networks although direct evidence is lacking. Excitation/inhibition (E/I) balance is suggested to be disrupted in ASD and enhancement of the GABAergic system rescues physiological and behavioral deficits in ASD model mice. However, the hyposerotonergic state and its physiological and behavioral consequences, particularly for cortical E/I balance and social behavior, remain unexamined in ASD model mice. Here, we show that normal 5-HT levels are essential for the proper maintenance of neocortical E/I balance, correct sensory stimulus tuning, and social behavior. Conversely, low 5-HT levels in 15q dup mice result in impairment of the same phenotypes. Restoration of normal 5-HT levels by early 5-HT intervention with selective serotonin reuptake inhibitor (SSRI) revealed the reversibility of the ASD-related symptoms of 15q dup mice. These findings suggest that 5-HT enhancement may have therapeutic potential for discrete symptoms in ASD.



Disclosures: N. Nakai: None. M. Nagano: None. F. Saitow: None. Y. Watanabe: None. Y. Kawamura: None. K. Tamada: None. H. Monai: None. H. Hirase: None. H. Mizuma:

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Poster

283. Autism Genetic Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 283.26/B39

Topic: A.07. Developmental Disorders

Title: Dysregulation of brain serotonergic systems in BALB/c mice: Relevance for autism and anxiety-like behavior

Authors: *M. W. HALE¹, A. M. RUSSO¹, A. J. LAWTHER¹, B. M. PRIOR¹, L. ISBEL¹, G. SOMERS¹, J. A. LESKU¹, A. RICHDALE², C. DISSANAYAKE², S. KENT¹, C. A. LOWRY³ ¹La Trobe Univ., Melbourne (Bundoora), Australia; ²Olga Tennison Autism Res. Ctr., La Trobe Univ., Melbourne, Australia; ³Dept. of Integrative Physiol. and Ctr. for Neurosci., Univ. of Colorado Boulder, Boulder, CO

Abstract: Although the biological mechanisms underlying autism spectrum disorder (ASD) are not fully understood, evidence suggests that dysregulation of serotonergic systems may play a role in ASD psychopathology. Preclinical models using mice with altered serotonergic neurotransmission may provide insight into the role of serotonin in behaviors relevant to clinical features of ASD. For example, BALB/c mice carry a loss-of-function single nucleotide polymorphism (SNP; C1473G) in *Tph2*, which encodes the rate-limiting enzyme for serotonin synthesis, and frequently have been used to model symptoms of ASD. In this study, juvenile male BALB/c and C57BL/6J mice, which carry the wild type variant of the C1473G SNP, were exposed to two behavioral tests: first, the three chamber sociability test, and one week later to the elevated plus-maze (EPM). Immediately following testing in the EPM, all mice received injections of the aromatic amino acid decarboxylase (AADC)-inhibitor, NSD-1015. One hour later, tissue was collected for quantification of concentrations of 5-hydroxytryptophan (5-HTP) in subregions of the dorsal raphe nucleus (DR) as a measure of Tph2 activity. BALB/c mice showed reduced social behavior and increased anxiety-like behavior, as well as decreased Tph2 activity in the rostral and mid-rostrocaudal DR. Tryptophan hydroxylase 2 activity in the midrostrocaudal DR was correlated with anxiety-like behavior in the EPM. In a subsequent experiment, supplementation of brain serotonin synthesis in BALB/c mice using peripheral administration of 5-HTP combined with the AADC-inhibitor, carbidopa, increased the time spent in the social chamber of the three-chamber sociability test compared with mice treated with carbidopa alone. Finally, an experiment using acute or chronic administration of the selective serotonin reuptake inhibitor fluoxetine in BALB/c mice demonstrated that acute administration of fluoxetine decreased social behavior, while chronic administration of fluoxetine increased

social behavior compared with vehicle-treated controls. Taken together, these data are consistent with the hypothesis that dysregulation of serotonergic systems is associated with mouse behaviors that resemble some of the clinical features of ASD.

Disclosures: M.W. Hale: None. A.M. Russo: None. A.J. Lawther: None. B.M. Prior: None. L. Isbel: None. G. Somers: None. J.A. Lesku: None. A. Richdale: None. C. Dissanayake: None. S. Kent: None. C.A. Lowry: None.

Poster

283. Autism Genetic Models

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 283.27/B40

Topic: A.07. Developmental Disorders

Support: Mercer University Seed Grant

Title: Perinatal hyperserotonemia influences dopamine expression in the midbrain

Authors: Z. ZEISLER, S. SMITH, *K. NORTHCUTT Biol. Dept., Mercer Univ., Macon, GA

Abstract: Prenatal and neonatal hyperserotonemia has been the most consistently documented neurochemical finding in children with autism spectrum disorders. Furthermore, pregnant women taking SSRIs prescribed for depression are more likely to give birth to children with autism. A perinatal hyperserotonemia rat model has been developed using the serotonin agonist 5-methoxytryptamine (5-MT). Daily injections of 5-MT to pregnant dams beginning on embryonic day 12 and then into pups upon birth until postnatal day 20 can induce autism-like symptoms, such as social deficits, sensory hyper-responsiveness, and coordination deficits. Corresponding changes in neural development have also been consistently found in this model. Because alterations in dopaminergic networks can produce symptoms similar to those described above, we examined the effects of perinatal hyperserotonemia on the number of dopamine neurons in the midbrain of juvenile male and female rats. We injected 5-MT (1 mg/kg) or a vehicle solution daily to dams (days 12-21 of gestation) and subsequently to their pups (first 20 postnatal days). On postnatal day 30-32, subjects were perfused and their brains were collected. We processed their brains immunohistochemically for tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis. The number of tyrosine hydroxylase-immunoreactive neurons in the substantia nigra and ventral tegmental area were quantified. We have focused on these midbrain areas because of their importance in movement regulation and motivation. Our results indicate that perinatal hyperserotonemia increases the number of dopaminergic neurons in the substantia nigra, but only in males. In contrast, treatment has no effect on dopamine expression in the ventral tegmental area. Increases in dopamine expression in the substantia nigra of juvenile males could relate to the coordination and impulsivity deficits that are commonly observed with this model and with children with autism. Similar sex differences have been reported in other studies examining the effects of perinatal 5-MT on neurochemistry, such as alterations in oxytocin and serotonin receptor expression. Furthermore, these sex differences in juvenile dopamine expression could contribute to sex differences in behaviors, such as juvenile play behavior, that have been observed in this model.

Disclosures: Z. Zeisler: None. S. Smith: None. K. Northcutt: None.

Poster

283. Autism Genetic Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 283.28/B41

Topic: A.07. Developmental Disorders

Title: Altered maturation of auditory system processing and sensory filtering deficits in the CNTNAP2 knockout rat model of autism: Electrophysiology and behaviour

Authors: *K. SCOTT, B. L. ALLMAN, S. SCHMID

Anat. and Cell Biol., Univ. of Western Ontario, London, ON, Canada

Abstract: The mammalian auditory system undergoes considerable development and experience-dependent plasticity in early life. However, this normal maturation is perturbed in individuals with developmental disorders, such as autism spectrum disorder (ASD). These maturational differences may lead to impairments in auditory processing, and ultimately underlie the communication deficits and altered reactivity to sensory stimuli associated with ASD. For example, individuals with mutations in the autism-linked gene, contactin-associated protein-like 2 (CNTNAP2), are known to experience language processing deficits, yet the contribution of CNTNAP2 to the maturation of auditory processing and sensory filtering remains unknown. We addressed this question with a recently-developed rat model, by comparing the developmental timeline of hearing thresholds and temporal processing using the auditory brainstem response (ABR) and assessing sensory filtering via the acoustic startle response (ASR) in male and female juvenile, adolescent, and adult CNTNAP2 homozygous knockout (cntnap2^{-/-}), heterozygous knockout (cntnap2^{+/-}), and wildtype Sprague Dawley rats. The ABR in response to click stimuli (90 to 10 dB SPL in 5dB steps) was recorded at postnatal day 28, 42, and 70, and the hearing threshold as well as the latency and magnitude of waves I-IV in response to the 90 dB click stimulus were compared between genotypes and across age groups. Auditory sensitivity and sensory filtering (habituation and sensorimotor gating) were assessed behaviourally at P44 and P72 using the magnitude and latency of the ASR. Specifically, the level of startle reactivity to 65 dB - 105 dB noise pulses was used as an index of auditory sensitivity, and habituation of the ASR was measured in response to the repeated presentation of a 105 dB noise pulse. Finally, to

assess sensorimotor gating, the level of prepulse inhibition was determined under a variety of stimuli conditions (i.e., prepulses at 75 or 85 dB; 30ms or 100ms inter-stimulus interval). Ultimately, we found that sound-induced neural activity was transmitted slower throughout the early stages of the auditory pathway in juvenile *cntnap2*^{-/-} rats compared to age-matched wildtype controls; however, this CNTNAP2-related delay was no longer present in adulthood. In contrast, although differences in auditory sensitivity and sensory filtering were also observed, the significant sensorimotor gating deficit found in juvenile *cntnap2*^{-/-} rats did not improve with age. Overall, these results provide insight into the altered maturation of the auditory system in a preclinical model of ASD and how it may lead to altered reactivity to acoustic stimuli.

Disclosures: K. Scott: None. B.L. Allman: None. S. Schmid: None.

Poster

283. Autism Genetic Models

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Topic: A.07. Developmental Disorders

Support: Simons Foundation Autism Research Initiative

Title: Bioinformatics study of non-genetic rodent models with ASD-consistent phenotypes

Authors: *M. A. ESTEVEZ, I. DAS, W. PEREANU, A. A. SARKAR, S. BANERJEE-BASU Mindspec, Inc., McLean, VA

Abstract: There is consensus in the autism research community that autism spectrum disorder (ASD) has a complex etiology, combining both genetic and environmental factors. The Animal Model module of AutDB catalogues rodent ASD models, which can be models of genetic or non-genetic factors. Although it is recognized that the environment plays an important role in ASD etiology, most ASD research has focused on genetic factors. Here we want to focus on animal models of non-genetic factors. In AutDB, we have annotated both mouse and rat models of ASD from peer-reviewed publications. Our recent addition of rat data to the Animal Model module of AutDB has highlighted ASD research focused on environmental factors. Together, our animal model data compiles 185 ASD models from 70 non-genetic factors. The objective of our analysis is to assess non-genetic rodent models with ASD-consistent phenotypes. Because ASD is a neurodevelopmental disorder, we want to analyze the developmental time points that are used in the induction of the environmental models. Our hypothesis is there is a correlation between the development of ASD-consistent phenotypes with the trajectory of brain development. Our data show that two developmental time points have an effect in the emergence of ASD-consistent phenotypes: one is mid-gestational and the other early postnatal. We have also classified the non-genetic factors based on environmental etiological models. This

classification allows us to compare different etiological models, which are based on human studies, to the emergence of ASD-consistent phenotypes in rodents. By comparing the non-genetic factors, based on both etiology and phenotypic data, we can score the importance of the underlying pathways. Overall, our study demonstrates that ASD-consistent phenotypes in rodent models are linked to specific neurodevelopmental time points and etiological pathways.

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Poster

284. Neurodevelopmental Disorders: Environmental Exposures

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 284.01/B43

Topic: A.07. Developmental Disorders

Support: The Institute for Biomedical Science, The George Washington University

Title: Placental allopregnanolone loss alters fetal brain development in a novel mouse model

Authors: *J. O'REILLY¹, D. BAKALAR², A. A. PENN³

¹The Inst. for Biomed. Sci., The George Washington Univ., Washington, DC; ²Ctr. for Neurosci., ³Fetal Med. Institute, Dept. Neonatology, Children's Natl. Hlth. Syst., Washington, DC

Abstract: A major consequence of preterm birth is premature loss of the placenta and the support it provides. The placenta is a critical neuroendocrine organ that supplies the developing fetus with essential hormones, including neurosteroids such as allopregnanolone (ALLO). ALLO is a metabolite of progesterone that is produced via a two-step enzymatic conversion process that depends specifically on 3α -hydroxysteroid dehydrogenase (3α HSD; in mouse encoded by the AKR1c14 gene). ALLO is a potent, positive allosteric modulator of GABAA receptors. High levels of ALLO are provided by the placenta, at a time when synapses are predominantly GABAergic and GABA is excitatory, rather than inhibitory. GABA excitation is critical for maturation and integration of neurons into developing circuits. ALLO is implicated in regulation of neurogenesis, neural outgrowth and survival, migration and synapse stabilization. To directly test placental ALLO's role in fetal cortical and hippocampal development, AKR1c14 floxed mice designed by our laboratory were bred with CYP19-CRE mice to create a placental-specific knockout of 3aHSD, confirmed by qPCR and *in situ* hybridization. Here, the long-term consequences of placental AKR1c14 loss are described. Immunohistochemistry, gene expression assays and progenitor labeling techniques were used to assess anatomical changes. Alterations in GABAergic interneurons (somatostatin and parvalbumin subtypes) were detected in hippocampus and cortex. Adult mice underwent a behavioral test battery (open field, Y maze, 3chamber sociability, and novel object recognition). Adult mice not exposed to placental ALLO

exhibited higher anxiety, decreased sociability, and impaired cognitive function compared to litter-mate controls. The behavioral deficits mirror those seen in human preterm survivors; GABA loss has been implicated in these deficits. Placental ALLO may regulate GABAergic interneuron development in key regions, including hippocampus and cortex. Our results suggest that loss of placental ALLO can dysregulate early GABAergic signaling, resulting in long-lasting neurological deficits. Our novel placental knockout model is now allowing direct testing of this mechanism and will allow testing of neurological rescue based on ALLO and other placental hormones.

Disclosures: J. O'Reilly: None. D. Bakalar: None. A.A. Penn: None.

Poster

284. Neurodevelopmental Disorders: Environmental Exposures

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 284.02/B44

Topic: A.07. Developmental Disorders

Support: Research Foundation, Cerebral Palsy Alliance

Board of Visitors, Children's National Medical Center

Title: Long-term alteration of cerebellar white matter following prenatal allopregnanolone loss

Authors: J. SALZBANK¹, C. VACHER¹, *A. A. PENN^{2,1} ¹Ctr. for Neurosci. Res., ²CRI, Fetal Med., Children's Natl. Med. Ctr., Washington, DC

Abstract: Prematurity is a risk factor for a wide array of neurodevelopmental disorders and CP is no exception; nearly 50% of children who develop CP are born before 37 weeks gestation. While CP is classically associated with cortical white matter injury (WMI), recent studies suggest that cerebellar WMI may also contribute to the disease pathology. Peak growth and maturation of the cerebellum, accelerating rapidly from 24 weeks gestation through postnatal life leaves this region vulnerable to developmental abnormalities. Prematurity deprives the fetus of high levels of placental neuroactive steroids, specifically the progesterone derivative, allopregnanolone (ALLO), widely investigated for its neuroprotective properties, notably by promoting myelination. This study focuses on the impact of early loss of placental ALLO on cerebellar development and long-term function. To examine the cerebellum following loss of placental ALLO, we bred a Cyp19-Cre driven transgenic mouse model to specifically knock out AKR1C14, the gene encoding ALLO's synthetic enzyme, in trophoblast cells, providing a targeted decrease of placental ALLO. Pups received EdU at E15.5 to analyze late-term cell proliferation in the cerebellum. Immunohistochemical staining with Olig2/Ki67/NogoA and Myelin Binding Protein was used to track oligodendrogenesis. Preliminary data indicated that

early loss of ALLO is associated with a decrease in cerebellar oligodendrocyte density. In addition, RNA-seq analysis performed in P30 cerebella showed differential expression of genes specific to myelination processes and axonal outgrowth. In parallel, a battery of cerebellar-specific behavioral tests, including the Erasmus ladder, was used to characterize long-term motor abnormalities. These results suggest long-term cerebellar WM and motor function deficits that persist long after prenatal ALLO loss. Understanding the mechanisms underlying cerebellar growth suppression and injury that follows preterm birth will allow for the development of new interventions to protect this vulnerable region.

Disclosures: J. Salzbank: None. C. Vacher: None. A.A. Penn: None.

Poster

284. Neurodevelopmental Disorders: Environmental Exposures

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Topic: A.07. Developmental Disorders

Support: Cerebral Palsy Alliance

BoV Perinatal Neuroprotection Program

Title: Consequences of placental allopregnanolone withdrawal on mouse brain development

Authors: *C.-M. VACHER¹, J. M. SALZBANK¹, A. LAZUTKIN^{3,4,5,6}, S. SHUVAEV^{3,5}, D. BAKALAR¹, G. N. ENIKOLOPOV^{3,5,6}, A. A. PENN²

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Abstract: Allopregnanolone (ALLO), along with its precursor progesterone, is a neuroactive steroid primarily synthesized prenatally by the placenta and postnatally by the brain. ALLO exerts neurodevelopmental and neuroprotective actions through allosteric activation of GABA-A receptor. In fetal brain, ALLO notably promotes neurogenesis and myelination, and protects developing neurons and glial cells from damage. Preterm delivery is associated with premature loss of placental ALLO, potentially contributing to the long-term consequences of prematurity, including learning impairment, attention deficits and cerebral palsy. To test this hypothesis, we generated a transgenic mouse model in which the gene encoding the synthetic enzyme for ALLO (AKR1C14) is deleted in trophoblastic cells expressing Cyp19-Cre transgene to suppress placental ALLO production specifically. We combined two unbiased approaches to assess this prenatal ALLO loss on brain development: (1) 3D analysis of cell proliferation in the whole

brain (whole mount-CLICK) after EdU injection at late gestation, and (2) RNA sequencing in adult brain to characterize the transcriptome of cerebral cortex, hippocampus, hypothalamus and cerebellum, with our without prenatal placental ALLO exposure. RNA sequencing analysis revealed long-term, sex-specific gene expression changes. Differentially expressed genes include those involved in cell proliferation (in agreement with our cell proliferation analysis), survival apoptosis, neurite outgrowth, neurotransmission, myelination and epigenetics, as well as region-specific functions such as energy homeostasis and somatic growth. By providing new evidence on the importance of placental hormones on shaping and programing the developing brain, our data paves the way for further investigation in the emerging neuroplacentology field that can lead to novel therapeutic approaches to prevent adverse neurological outcomes from preterm birth.

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Poster

284. Neurodevelopmental Disorders: Environmental Exposures

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Program#/Poster#: 284.04/DP01/B46 (Dynamic Poster)

Topic: A.07. Developmental Disorders

Support: NIH K08 DA035972-01

Trailblazer Award Department of Anesthesia at Boston Children's Hospital

Title: Correlation study of prolonged sedation and incidental MRI findings in full-term infants

Authors: H. W. KILCOYNE¹, C. R. L. MONGERSON¹, D. B. PIER², S. L. WILCOX³, R. W. JENNINGS⁴, P. E. GRANT⁶, *D. BAJIC⁵

²Neurol., ³Dept. of Anesthesiol., ⁴Surgery, ⁵Dept Anesthesiol., ¹Boston Children's Hosp., Boston, MA; ⁶Div. of Newborn Med., Boston Childrens Hosp., Boston, MA

Abstract: Prolonged administration of opioids and benzodiazepines used for pain and sedation management in the youngest of patients is associated with a high incidence of drug tolerance and dependence. The clinical impact of such treatment on the full-term infants is largely unknown. We hypothesized that prolonged sedation with opioids and benzodiazepines in full-term infants younger than 1 year is associated with (1) increased incidence of brain abnormalities as per brain MRI scan, (2) increased CSF volumes, and (3) decreased brain volumes in comparison to healthy controls. Subjects were compared between two groups as per IRB approval at the Boston Children's Hospital. Subjects underwent research scan at 3T MRI scanner for 3D T-1 and T2-weighted anatomical images, while charts were reviewed for quantification of sedation. Endpoint analyzes included: (1) length of sedation and weaning (days), (2) total treatment doses per

patient (mg/kg/day), (3) average daily doses during sedation and weaning (mg/kg/day \pm SD), (4) number of anesthesia events, (5) number of incidental findings on brain MRI reports, and (6) estimated normalized cerebrospinal and brain volumes using MANTiS segmentation. Pearson's correlation coefficient was used to measure the linear relations between the different variables analyzed. Morphine and midazolam were the two drugs used the most frequently for prolonged sedation and were administered at the highest doses. We report significant positive linear relationships for the average daily dose of received morphine and midazolam with the number of neuroradiological findings (e.g. abnormalities in extra-axial space, ventricular system, parenchyma, and/or white matter structures) that were not present in any of the controls. There was no significant relationship between the average daily dose of morphine or midazolam despite the apparent positive trend with cerebrospinal fluid volume, and negative trend with estimated brain volume. Given the current standard of care using these drugs for prolonged sedation, future investigations of gray and white matter organization in at-risk full-term infants can provide crucial information of how prolonged sedation can affect brain development and potentially lead to long-term neurobehavioral sequelae.

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Poster

284. Neurodevelopmental Disorders: Environmental Exposures

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 284.05/B47

Topic: A.07. Developmental Disorders

Support: R01GM112715

Title: Brain tissue oxygen regulation in neonates under anesthesia

Authors: *D. P. AKSENOV¹, A. DMITRIEV², M. MILLER¹, A. WYRWICZ¹, R. A. LINSENMEIER² ¹NorthShore Univ. HealthSystem, Evanston, IL; ²Northwestern Univ., Evanston, IL

Abstract: Normal brain function depends on keeping the level of oxygen within a relatively narrow range that is sufficiently high to prevent hypoxia and low enough to minimize generation of toxic oxygen species. Stable blood flow is maintained throughout the brain by cerebrovascular autoregulation, which regulates the response of cerebral arteries to fluctuations in arterial pressure, ensuring sufficient oxygen for basal neuronal activity. It is known that anesthesia has a significant effect on neuronal function and cerebral metabolism which can be different in adults and children. The goal of this study was to compare brain tissue oxygen regulation in neonates in the awake state and during anesthesia. Partial oxygen pressure (PO₂) and single unit (SU)

recordings were performed in the somatosensory cortex of neonatal rabbits (9-12 days postnatal) before, during and after anesthesia with 1 MAC of either isoflurane or sevoflurane delivered both in air and in 80% oxygen. Each animal served as its own control. The same electrodes were used for SU and PO₂ recording. The PO₂ electrodes were polarized at -0.7 volts, and connected to an ammeter (Keithley 614) to record a current that was typically 3 to 10 nA before anesthesia. The current was converted to voltage, notch and low-pass filtered (30 or 50 Hz), and amplified. After completion of PO₂ experiments SU recordings were obtained from the same rabbits before, during and after anesthesia. The multiple SU signals from the microwires were fed through a miniature preamplifier to a multichannel differential amplifier system (Neuralynx Inc, Bozeman, Montana, USA). The signals were amplified, band-pass-filtered (300Hz to 3 kHz), and digitized (32 kHz/channel) using a Neuralynx data acquisition system. Unit discrimination was performed offline using threshold detection followed by a cluster analysis of individual action potential wave shapes using Neuralynx analysis software. Our results showed that when delivered in air, only isoflurane increased brain PO₂. Sevoflurane did not significantly change brain PO₂ likely due to severe respiratory depression. During inspiration of 80% O₂, brain PO₂ increased more when the animals were anesthetized with isoflurane or sevoflurane than when they were awake. This increase was large and varied considerably among the subjects. Single unit activity was greatly suppressed by both isoflurane and sevoflurane, indicating lower consumption of tissue oxygen.

Disclosures: D.P. Aksenov: None. **A. Dmitriev:** None. **M. Miller:** None. **A. Wyrwicz:** None. **R.A. Linsenmeier:** None.

Poster

284. Neurodevelopmental Disorders: Environmental Exposures

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Topic: A.07. Developmental Disorders

Support: NIH Grant R01NS096176

NIH Grant R01NS097231

Title: The African Zika virus is more virulent and causes more severe brain damage compared to the Asian lineage and Dengue virus

Authors: *J.-F. CHEN¹, Q. SHAO¹, S. A. HERRLINGER², M. YANG², M. BRINDLEY² ¹Ctr. for Craniofacial Mol. Biol., Univ. of Southern California (USC), Los Angeles, CA; ²Univ. of Georgia, Athens, GA Abstract: Our group is interested in understanding mechanisms underlying neural tube defects (NTDs) and microcephaly. Focusing on a microcephaly disease gene WDR62, we discovered a novel disease mechanism, in which mitotic delay and cell death of neural progenitor cells (NPCs) lead to microcephaly (Nat. Communication, 2014). Zika virus (ZIKV) infection has been linked with fetal brain abnormalities. By developing the first postnatal mouse model associated with ZIKV, we recently established the causative link between ZIKV and microcephaly. Specifically we discovered that Zika virus infection disrupts neurovascular development and results in postnatal microcephaly with brain damage (Development, 2016). The Zika virus (ZIKV) has two lineages: the ZIKV-Asia and ZIKV-Africa isolates. There is no scientific documentation of ZIKV-Africa related brain defects. Dengue virus (DENV), a close family member of ZIKV, is also not linked with brain disorders. Here we performed the intracerebral inoculation of embryonic mouse brains and found that DENV2 is sufficient to cause microcephaly due to increased cell death in neural progenitor cells (NPCs) and neurons. Compared to ZIKV-Asia, DENV2 grows slower, causes less neuronal death, and fails to cause postnatal animal death. Surprisingly, our side-by-side comparison uncovers that ZIKV-Africa is more potent in causing brain damage and postnatal lethality than ZIKV-Asia. In comparison to ZIKV-Asia, ZIKV-Africa grows faster in NPCs and in the developing brain, causes more pronounced cell death in NPCs and neurons, resulting in more severe neuronal loss. Together, these results reveal that DENV2 is sufficient to cause microcephaly but in a less severe manner than ZIKV-Asia. ZIKV-Africa is more virulent and causes more severe brain damage than ZIKV-Asia, highlighting the need to better understand the neurological complications associated with ZIKV-Africa.

Disclosures: J. Chen: None. Q. Shao: None. S.A. Herrlinger: None. M. Yang: None. M. Brindley: None.

Poster

284. Neurodevelopmental Disorders: Environmental Exposures

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 284.07/B49

Topic: A.07. Developmental Disorders

Support: CAPES

CNPq

Title: Folic acid supplementation during prgnancy partially recovers memory deficits caused by neonatal hypoxia-ischemia in rats

Authors: ***B. FERRARY DENIZ**¹, H. DEOLA CONFORTIM¹, P. M. MIGUEL², L. BRONAUTH¹, B. DE OLIVEIRA¹, L. R. CECHINEL³, I. R. SIQUEIRA³, L. O. PEREIRA¹

¹Federal Univ. of Rio Grande Do Sul, Porto Alegre, Brazil; ²Ciências Morfológicas, ³Univ. Federal Do Rio Grande Do Sul, Porto Alegre, Brazil

Abstract: Folic acid (FA) supplementation during the first trimester of pregnancy is recommended to prevent neural tube closure defects. Our previous studies have demonstrated that treatment with FA after a hypoxic-ischemic (HI) event has a dual effect. Considering this, the aim of this study was to investigate the effect of different doses of FA supplementation during pregnancy on the rat's offspring submitted to neonatal HI. Male and Female Wistar rats were obtained from a local breeding colony by Federal University of Rio Grande do Sul (Approval n° 28136). After mating confirmation, female rats were divided into 3 groups, according to FA supplementation: standard diet (SD), supplemented with 2 mg/kg of FA (FA2) and supplemented with 20 mg/kg of FA (FA20). After birth, all animals returned to receive the SD diet. At the 7° post-natal day (PND), puppies were submitted to Levine-Vanucci model of HI (permanent occlusion of the right carotid and hypoxia during 90 min), generating 6 groups: 1) control with SD diet (CTSD), 2) HI with SD diet, 3) CT with FA2 (CTFA2), 4) HIFA2, 5) CT with FA20 (CTFA20) and 6) HIFA20. Starting at 60° PND, it was performed the novel object recognition (NOR) and the inhibitory avoidance tasks (n=9-13/group). Another group of animals were euthanized at 60° PND and had their hippocampi dissected to evaluate the BDNF concentration (n=5-7/group). In NOR, no differences were observed in the exploration time of the two similar objects in the first session. In the second session, HIPD group had lower novelobject preference index than all control groups, indicating a memory deficit by the lesion. HIPD was also different from HIFA20 and HIFA2 was similar to HIPD and control groups. Deficits caused by HI injury were partially prevented by the lower dose of FA and completely prevented by the higher dose of FA. In the second day of inhibitory avoidance task, HIPD had lower latency to step down the platform when compared to CTPD. HI supplemented with FA groups had no differences with CT and HIPD groups, showing that there was an aversive memory deficit caused by the HI lesion and a partially recovery by FA supplementation. Considering the BDNF concentration, no differences were found in the contralateral hippocampus (left hemisphere). All HI groups had higher BDNF concentration in the ipsilateral side, indicating no effect of FA supplementation. In conclusion, FA supplementation during pregnancy was able, at least, to partially recovery memory deficits caused by HI lesion, indicating a fetal programming effect. This recovery is not due to BDNF concentration.

Disclosures: B. Ferrary Deniz: None. **H. Deola Confortim:** None. **P.M. Miguel:** None. **L. Bronauth:** None. **B. de Oliveira:** None. **L.R. Cechinel:** None. **I.R. Siqueira:** None. **L.O. Pereira:** None.

Poster

284. Neurodevelopmental Disorders: Environmental Exposures

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Topic: A.07. Developmental Disorders

Support: UTMB Presidential Scholars Program (CT, KW)

NIH/NIEHS-T32ES007254 (CT) NIEHS Center Grant P30 ES006676 (FL) NIH/NIMH R01 MH095995 (FL) NIH/NIMH R01 MH111107-01A1 (FL)

Sealy Center for Enviornmental Health Medicine (LH, BA)

Title: Developmental effects of the pyrethroid insecticide deltamethrin on medium spiny neurons of the nucleus accumbens

Authors: *C. M. TAPIA¹, K. WINTERS², M. NENOV¹, L. HALLBERG³, B. AMEREDES³, T. GREEN¹, F. LAEZZA¹

¹Pharmacol. and Toxicology, ²Neurosci. and Cell Biol., ³Inhalation Toxicology Core Facility, Univ. of Texas Med. Br. Galveston, Galveston, TX

Abstract: Deltamethrin (DM) is a commonly used pyrethroid insecticide that exerts its effect on insect voltage-gated sodium (Nav) channels by delaying onset of Nav channel inactivation, which is important for the initiation and propagation of action potentials mediating neuronal excitability. Due to high evolutionary conservation of Nav channels and cross reactivity among species, it is imperative to investigate the possible effects of DM in humans. New findings from epidemiological studies correlate the presence of pyrethroid metabolites in urine to an increased risk of attention deficit hyperactivity disorder (ADHD) diagnosis in children. Furthermore, supporting evidence show that rats exposed to DM exhibited ADHD-like behavioral phenotypes, which was attributed to the dopaminergic (DA) reward pathway in the nucleus accumbens (NAc). Similar dysregulation of DA medium spiny neurons (MSNs) has been implicated in multiple neuropsychiatric disorders such as anxiety and depression. In the DA MSN, there is a prevalence of Nav 1.6 channel isoform that is important in synaptic transmission. Nav1.6 heterologous cell studies have shown that prolonged DM exposure significantly potentiated Nav 1.6 mediated persistent and tail currents. Here, we investigate the mechanism of MSN dysfunction due to developmental DM exposure. Pregnant female B6 mice were exposed to 3.0 mg/kg of DM throughout pregnancy and lactation. Then, male mice littermates from post-natal day ~30 were used for subsequent experiments. We employed whole-cell patch-clamp electrophysiology in coronal brain slices to monitor changes in NAc MSNs firing due to developmental DM exposure. At 110 pA of injected current, DM exposure significantly decreased the mean number of action potentials when compared to control (DM 6.8 \pm 2, C 15 \pm 2.4, n= 7-12, p<0.05 with Student t-test). At 190 pA of injected current, DM exposure significantly lowered the instantaneous firing frequency when compared to control (DM 18.4 \pm 4.2 Hz, C 34.7 \pm 5.3 Hz, n= 7-12, p<0.05 with Student t-test). These effects were observed over a series of several current steps. No changes were observed in other active and passive electrical

properties. These studies will advance our knowledge of the toxic activity of DM in the developing brain and help assess risk exposure in the human population.

Disclosures: C.M. Tapia: None. K. Winters: None. M. Nenov: None. L. Hallberg: None. B. Ameredes: None. T. Green: None. F. Laezza: None.

Poster

284. Neurodevelopmental Disorders: Environmental Exposures

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Topic: A.07. Developmental Disorders

Support: CIHR

Title: The effect of vincristine on the developing brain: A longitudinal MRI study in a mouse model

Authors: *L. SPENCER NOAKES¹, C. NICHOLLS², B. J. NIEMAN^{3,4}

¹Mouse Imaging Ctr., The Hosp. For Sick Children, Toronto, ON, Canada; ²Univ. of Waterloo, Waterloo, ON, Canada; ³Hosp. For Sick Children, Toronto, ON, Canada; ⁴Ontario Inst. for Cancer Res., Toronto, ON, Canada

Abstract: Introduction

Acute lymphocytic leukemia (ALL) is the most common form of childhood cancer. Advances in modern medicine have allowed a survival rate of up to 90%. However, children are often left with late effects, which impair cognitive ability, cause cardiac dysfunction, and ultimately limit the quality of life of leukemia survivors. It is widely believed that the combination chemotherapy used in treatment for ALL is responsible for late effects. However, because many chemotherapy agents are administered together in a complex, multi-phase treatment, it is not possible to determine which agents are most toxic. In recent work, we have studied a mouse model of chemotherapy treatment in which we systematically characterized the effects of the most common chemotherapy agents individually. We found that when mice were treated with vincristine at an infant equivalent age, they exhibited significant volume deficits in the brain at early adulthood. In this work, we measured brain development in mice longitudinally to establish how vincristine-induced changes in brain development emerge.

Description of Project

At an infant equivalent age, male and female mice were treated with vincristine intravenously. Mice were imaged using *in vivo* MRI once before treatment, and three times after until they reached nine weeks of age. Treated mice were compared to saline-treated littermates to assess differences in volume across the brain as a function of age.

Results and Conclusion

Changes due to vincristine treatment were widespread, with prominent changes in white matter regions, some areas in the cortex and the cerebellum. Changes began after treatment and emerged progressively over time, with the deficit becoming most significant at the final imaging time point. This work provides an important indication of the progressive—as opposed to acute—impact of vincristine on brain development and implicates vincristine as a toxic component of ALL treatment.

Disclosures: L. Spencer Noakes: None. C. Nicholls: None. B.J. Nieman: None.

Poster

284. Neurodevelopmental Disorders: Environmental Exposures

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Program#/Poster#: 284.10/B52

Topic: A.07. Developmental Disorders

Support: NIEHS P01 ES002848 Project3

USEPA 83543401 Project 3

Title: Long-term effects of perinatal bisphenol A exposure on the number of microglia and synapses in the medial prefrontal cortex of rats

Authors: *L. M. WISE, S. RHOADS, S. WANG, J. M. JURASKA Dept. of Psychology, Univ. of Illinois Dept. of Psychology, Champaign, IL

Abstract: Bisphenol A (BPA) is an environmental endocrine disruptor that has been detected in the vast majority of the United States population. Previous research from our lab has found an increase in the number of neurons and glia in the medial prefrontal cortex (mPFC) of male rats following perinatal exposure. The current study explores the effects of environmentally relevant doses of BPA on the number of microglia and synapses in the mPFC. 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. Brain tissue was collected in adulthood (~P90) and, using immunohistochemistry, adjacent sections were stained for IBA-1 (microglia) or synaptophysin (synapses). The density of microglia and synapses in the mPFC was calculated using the optical disector in StereoInvestigator. Preliminary results do not show a long-term effect of BPA on the density of microglia or synapses in the mPFC. However, the density may not indicate the actual number of cells or synapses. The density will be multiplied by the volume of the mPFC in order to determine the actual number of microglia and synapses. In addition, the morphology of microglia in the mPFC was assessed. There is a trend towards a decrease in the percent of microglia in the ameboid stage with perinatal BPA exposure. No differences were found in the other morphological stages. Disclosures: L.M. Wise: None. S. Rhoads: None. S. Wang: None. J.M. Juraska: None. Poster

284. Neurodevelopmental Disorders: Environmental Exposures

Location: Halls A-C

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Topic: A.07. Developmental Disorders

Support: NIH P01 ES002848-Project 3

USEPA 83543401 Project 3

NIH T32 ES007326

Title: Perinatal exposure to either bisphenol A or phthalates and a high-fat diet minimally affect oxidative stress within the medial prefrontal cortex of both male and female pups

Authors: *D. G. KOUGIAS¹, L. M. WISE², A. P. BELAGODU¹, J. M. JURASKA³ ¹Neurosci. Program, Univ. of Illinois at Urbana-Champaign, Champaign, IL; ²Dept. of Psychology, Univ. of Illinois Dept. of Psychology, Champaign, IL; ³Psychology, Univ. of Illinois, Champaign, IL

Abstract: Since endocrine-disrupting chemicals (EDCs), like phthalates and bisphenol A (BPA), are extensively used as plasticizers and chemical agents in a variety of consumer products, humans are ubiquitously exposed. However, due to environmental contamination, diet is presumed to be the main source of exposure to most of these EDCs, with fatty foods containing the highest concentrations. Given this correspondence and that high-fat diets and these EDCs can separately increase oxidative stress and inflammation systemically, it is important to study them together to examine the potential for interactive effects. Furthermore, since these EDCs can readily cross the placenta and disrupt the actions of hormones that drive the organizational circuitry of the developing brain, the gestational period appears to be a particularly vulnerable window to the effects of EDCs. Unsurprisingly, prenatal EDC exposure in humans is associated with adverse neurodevelopmental and behavioral outcomes. Likewise in rodents, there are perinatal EDC exposure studies corroborating the adverse effects on cognitive behavior; however, these studies have not investigated whether these adverse effects are related to local inflammation. Previously, we have shown that EDC exposure and a high-fat diet perinatally in rats can lead to sex-specific behavioral and cognitive effects in adolescence and adulthood that rely on the medial prefrontal cortex (mPFC).

Here, we examined the effects of perinatal high-fat diet and EDC exposure on oxidative stress markers in the mPFC. Throughout gestation to postnatal day (P) 10, dams were provided a control or high-fat diet. Dams were also orally dosed with either a phthalate mixture or BPA

throughout gestation, but only phthalates can be passed to pups through lactation, so that BPA was directly administered to pups until P10, whereas phthalate consumption continued in dams. Pups were sacrificed on P10 within hours of the last oral EDC administration and their brains were harvested and processed for ELISA. Our results indicate that males may be more vulnerable to brain-specific oxidative stress from perinatal environmental factors, but neither treatment nor diet had consistent or systematic effects on oxidative stress markers within the mPFC.

Disclosures: D.G. Kougias: None. L.M. Wise: None. A.P. Belagodu: None. J.M. Juraska: None.

Poster

284. Neurodevelopmental Disorders: Environmental Exposures

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Topic: A.07. Developmental Disorders

Support: NIH P01 ES002848-Project 3

EPA USEPA 83543401 Project 3

NIH T32 ES007326

Title: The effect of perinatal phthalate exposure on the number of synapses in the medial prefrontal cortex

Authors: *E. SELLINGER¹, D. G. KOUGIAS², J. M. JURASKA³ ¹UIUC, Champaign, IL; ²Neurosci. Program, Univ. of Illinois at Urbana-Champaign, Champaign, IL; ³Psychology and Neurosci. Program, Univ. of Illinois, Champaign, IL

Abstract: The perinatal period is crucial for brain development while at the same time, a period where the brain is particularly prone to environmental insult. One such environmental concern is exposure to phthalates, a class of endocrine-disrupting chemicals used as plasticizers, solvents, and emulsifiers in a variety of products that are known to readily cross the placenta and can be passed to offspring though lactation. Previously, we have shown perinatal phthalate exposure in rats can lead to sex-specific behavioral and cognitive changes in adolescence and adulthood that rely on the medial prefrontal cortex (mPFC). Due to these behavioral findings, in this study we examine the effect of perinatal phthalate exposure on the number of synapses in the adult mPFC. Dams were dosed orally with an environmentally relevant phthalate mixture composed of 35.35% DEP, 21.12% DEHP, 15.12% DiNP, 15.10% DBP, 8.16% DiBP, and 5.15% BBP at 0, 200, or 1000 µg/kg/day. Pups were sacrificed in adulthood and their brains harvested. Immunohistochemistry was performed on coronal slices of the mPFC for synaptophysin, a

presynaptic vesicle marker, and then stereologically analyzed. Our preliminary results indicate that increasing phthalate exposure generally decreases synaptic density, particularly in layer V/VI, in both sexes. However, the addition of mPFC volume measurements will allow for the calculation of total synapse number in the mPFC, which will provide a more complete picture of the lasting impact of perinatal phthalate exposure on mPFC synaptic development.

Disclosures: E. Sellinger: None. D.G. Kougias: None. J.M. Juraska: None.

Poster

284. Neurodevelopmental Disorders: Environmental Exposures

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Topic: A.07. Developmental Disorders

Support: NIH P01 ES002848-Project 3

EPA USEPA 83543401 Project 3

NIH R21 ES026896

NIH T32 ES007326

Title: Effects of a phthalate combination during perinatal development on apoptosis in the mPFC of male and female rats

Authors: *J. M. JURASKA¹, J. WILLING¹, D. G. KOUGIAS²

¹Psychology, Univ. of Illinois, Champaign, IL; ²Neurosci. Program, Univ. of Illinois at Urbana-Champaign, Champaign, IL

Abstract: Phthalates are endocrine disrupting compounds that can affect androgenic and estrogenic systems, and are found ubiquitously throughout the environment in a variety of consumer products. The developing fetus can exposed to phthalates through placental transfer and lactation during critical periods of neural development. Previous work indicates that exposure to the phthalate DEHP during prenatal development can decrease cell number and increase apoptosis in the rodent neocortex. Here we examine the effects of exposure to a combination of phthalates found in pregnant human females on the development of the medial prefrontal cortex (mPFC) in male and female rats. Pregnant and lactating rats were fed a cookie with a dose of 0mg/kg, 1mg/kg or 5mg/kg phthalate solution containing six different phthalates (including DEHP and DBP, which have previously been shown to induce apoptosis) at environmentally relevant levels. Dosing occurred from embryonic day 2 through postnatal day (P)10. Brain and body weights were collected from male and female pups at P10 and P25. We additionally examined markers for apoptosis in the medial mPFC at P10, and total mPFC volume

at P25. Both male and female pups dosed with 5mg/kg phthalates had a higher density of TUNEL positive cells in the mPFC, indicating higher levels of cell death, while the 1mg/kg dose caused a reduction in mPFC volume at P25 in both sexes. Additionally, there was a sex difference in the amount of apoptosis in the 1mg/kg group at P10 with effects in males, but not females. Collectively, these results imply that combinations of common phthalates found in the environment can affect the development of the mPFC in a dose and a sex-dependent manner in a brain region critical for executive function.

Disclosures: J.M. Juraska: None. J. Willing: None. D.G. Kougias: None.

Poster

284. Neurodevelopmental Disorders: Environmental Exposures

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 284.14/B56

Topic: A.07. Developmental Disorders

Support: NIH grant ES022759

Title: Chronic exposure to Bisphenol F, a substitute for Bisphenol A, and anxiety in male mice

Authors: *A. TALAROVICOVA, J. W. IRVIN, H. E. SCHRADER, E. F. RISSMAN North Carolina State Univ., Raleigh, NC

Abstract: In many products, Bisphenol F (BPF) is being used as a substitute for Bisphenol A (BPA). Traces have already been found in canned foods as well as in river sediments. Emerging in vitro data show that BPF may not be a safe alternative to BPA, pointing to similar, or even more potent endocrine disruptive abilities. BPF increases progesterone and upregulates the expression of Cyp2d4 in the brain, the enzyme that converts progesterone to allopregnanolone, a potent GABAa modulator. Given the potential of BPF to affect inhibitory signaling in the brain, we used chronic BPF exposure and examined anxiety in adult CD1 male mice. We selected an oral route of administration and doses that reflect likely human exposure to BPF (0mg, 0.5mg 5mg and 50mg BPF/kg food). After 7 weeks of exposure animals were tested in the Open field and the Elevated plus maze test. Based on toxicity studies with much higher doses of BPF we hypothesized that the chronic exposure to all 3 of the low doses of BPF would decrease activity and increase anxiety-like behavior in male mice. Our initial data from the Open field test so far show no differences in activity between the groups. Additionally, contrary to our expectations the animals exposed to BPF are less anxious as reflected by a tendency to spend more time in the center of the Open field and lower amount of defecation. We will discuss the results further in relation to direct versus parental exposure to BPF.
Disclosures: A. Talarovicova: None. **J.W. Irvin:** None. **H.E. Schrader:** None. **E.F. Rissman:** None.

Poster

284. Neurodevelopmental Disorders: Environmental Exposures

Location: Halls A-C

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Program#/Poster#: 284.15/B57

Topic: A.07. Developmental Disorders

Title: Nature versus nurture: Small additions to one carbon metabolism in pregnant mice alter behavior in offspring as adults

Authors: *R. F. YOSHIMURA, A. ALACHKAR, A. LO, E. MURADYAN, H. SHAHARUDDIN, Y. TU, Y. YI, O. CIVELLI Pharmacol., Univ. of California, Irvine, Irvine, CA

Abstract: One-carbon metabolism regulates the availability of methyl groups for the methylation of DNA and histone proteins. Methionine can be taken exogenously from the diet and is converted in the one-carbon metabolism cycle to the universal methyl donor responsible for biological methylation. We have previously demonstrated that subchronic treatment with a low dose of L-methionine (L-MET) in adult mice caused a number of behavioral changes (Wang et al., 2015). We subsequently saw similar effects in the offspring of females given the same L-MET treatment during pregnancy (manuscript in preparation). We wanted to determine the contribution from the maternal environment to these behaviors, so we cross-fostered the treatment groups (saline pups with L-MET mothers and L-MET pups with saline mothers) and tested them alongside of control groups in adulthood. We examined locomotor activity, T-maze performance, social interaction, prepulse inhibition and fear conditioning. The results suggest a stronger correlation between direct or epigenetic factors and aberrant behavior, in comparison to the maternal environment. This suggests that changes in dietary levels of methionine during pregnancy may have profound behavioral results that are independent of the maternal environment.

Wang L, Alachkar A, Sanathara N, Belluzzi JD, Wang Z, Civelli O. A Methionine-Induced Animal Model of Schizophrenia: Face and Predictive Validity. Int J Neuropsychopharmacol. 2015. 18(12).

Disclosures: R.F. Yoshimura: None. A. Alachkar: None. A. Lo: None. E. Muradyan: None. H. Shaharuddin: None. Y. Tu: None. Y. Yi: None. O. Civelli: None.

284. Neurodevelopmental Disorders: Environmental Exposures

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 284.16/B58

Topic: A.07. Developmental Disorders

Support: IIE-SRF

Title: Prenatal one-carbon dysregulation impairs memory

Authors: *A. ALACHKAR, R. YOSHIMURA, L. WANG, G. ABBOTT, X. XU, O. CIVELLI Pharmacol., Univ. of California Irvine, Irvine, CA

Abstract: The one-carbon metabolism, which relies on the methionine-folate cycles, has been implicated in the pathophysiology of a number of psychiatric disorders such as autism, Alzheimer's, and schizophrenia. Here we examined the effects on mouse development and behavior of perturbation of one-carbon metabolism evoked by daily methionine administration to dams in the last week of gestation. The dose of methionine is equivalent to double their normal daily dietary intake. The resulting pups (MET mice) exhibited deficits in cognitive functions and memory as shown in T-maze, social recognition, novel object recognition, novel location recognition, and fear conditioning. MET mouse brains also exhibited decreased neurogenesis and synaptic plasticity, increased gliogenesis, and abnormally reduced local excitatory synaptic connections in CA1 neurons. Strikingly, neural transcript expression of only one gene, encoding the Npas4 transcription factor, was >2-fold altered (downregulated) by prenatal methionine administration. Our data support a role for prenatal one-carbon pathway in memory, and suggest methionine metabolism as a potential therapeutic target for psychiatric disorders associated with cognitive deficits

Disclosures: A. Alachkar: None. R. Yoshimura: None. L. Wang: None. G. Abbott: None. X. Xu: None. O. Civelli: None.

Poster

284. Neurodevelopmental Disorders: Environmental Exposures

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Topic: A.07. Developmental Disorders

Support: Grant from the SLE lupus foundation

Title: In utero exposure to anti-aquaporin-4 antibodies alters brain vasculature

Authors: ***S. MADER**¹, L. BRIMBERG¹, J. CRAWFORD², A. BONNIN³, A. VO¹, J. CARRION¹, A. LA BELLA¹, S. DEWEY¹, D. EIDELBERG¹, J. BENNETT⁴, B. VOLPE¹, P. HUERTA¹, B. DIAMOND¹

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Abstract: The formation of the blood brain barrier (BBB) is key to brain development. Recently it has been demonstrated that radial glial (RG) neural progenitor cells have a strong impact on brain vessel development and BBB formation in the embryonic cortex through modulating endothelial wnt signaling. Maternal brain reactive antibodies (Abs) can penetrate the fetal brain before the embryonic BBB is established. Abs to the brain water channel protein Aquaporin-4 (AQP4) have been associated with fetal loss and may contribute to neurodevelopmental impairment in the offspring of patients with Neuromyelitis optica (NMO), a neurological autoimmune disease characterized by AQP4-IgG in the vast majority of patients. In NMO patients, AQP4 Abs bind to AQP4 on astrocytic endfeet surrounding blood vessels. AQP4 is expressed at the BBB interface and has probably an important role in BBB formation. In order to study the effect of maternal AQP4 Abs we injected intravenously human monoclonal AQP4-IgG or an isotype matched control antibody to pregnant mice at embryonic day E14.5. Western blot and qPCR results show that AQP4 is expressed at a significantly higher level in the fetal brain compared to the placenta throughout all gestational stages. Immunohistochemistry staining shows that a single exposure to AQP4-IgG during embryogenesis alters the vasculature and the number of GFAP positive astrocytes in the hippocampus and cortex of AQP4-IgG in utero exposed male mice throughout adulthood, but did not affect the vasculature of the kidney, where AQP4 is also expressed. We performed a PET study in these mice, which revealed BBB impairment in the entorhinal cortex, which was accompanied by an increased blood flow. QPCR analysis demonstrated differential expression of wnt signaling molecules and LEF1, a transcription factor regulated by wnt signaling pathway, in the cortex of AQP4-IgG exposed mice at postnatal day P0.

Currently we are studying if the altered vasculature and BBB impairment are caused by binding of AQP4-IgG to RG cells. Our findings add to the understanding of BBB formation during embryogenesis and include NMO to the list of potential conditions in which maternal Abs lead to neurodevelopmental problems in the offspring.

Disclosures: S. Mader: None. L. Brimberg: None. J. Crawford: None. A. Bonnin: None. A. Vo: None. J. Carrion: None. A. La Bella: None. S. Dewey: None. D. Eidelberg: None. J. Bennett: None. B. Volpe: None. P. Huerta: None. B. Diamond: None.

284. Neurodevelopmental Disorders: Environmental Exposures

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Topic: A.07. Developmental Disorders

Support: NIH Grant DA013137

NIH Grant HD043680

NIH Grant MH106392

Title: Evolution of the HIV-1 transgenic rat: Utility in assessing the progression of HIV-1 associated neurocognitive disorders

Authors: *K. A. MCLAURIN, R. M. BOOZE, C. F. MACTUTUS Dept. of Psychology, Univ. of South Carolina, Columbia, SC

Abstract: Due to the marked increase in life expectancy following the advent of combination antiretroviral therapy (cART), there is a critical need to examine the progression of HIV-1 associated neurocognitive disorders (HAND). Longitudinal experimental designs, in comparison to cross-sectional studies, provide an opportunity to establish age-related disease progression in HAND. The HIV-1 transgenic (Tg) rat, which has been promoted for investigating the effect of long-term HIV-1 viral protein exposure in neurocognitive deficits, was used to examine two interrelated goals. First, to establish the integrity of sensory and motor systems through the majority of the animal's functional lifespan. Strong evidence for intact sensory and motor system function through advancing age in HIV-1 Tg and control animals, was observed in cross-modal prepulse inhibition (PPI) and locomotor activity. The integrity of sensory and motor system function suggests the utility of the HIV-1 Tg rat in investigating the progression of HAND. Second, to assess the progression of neurocognitive impairment, including temporal processing and long-term episodic memory, in the HIV-1 Tg rat; the factor of biological sex was integral to the experimental design. Cross-modal PPI revealed significant alterations in the development of temporal processing in HIV-1 Tg animals relative to controls. Female HIV-1 Tg rats exhibited more pronounced alterations in the progression of temporal processing relative to male HIV-1 Tg animals. Locomotor activity revealed deficits in intrasession habituation, suggestive of a disruption in long-term episodic memory, in HIV-1 Tg animals. Understanding the progression of HAND heralds an opportunity for the development of an advantageous model of progressive neurocognitive deficits in HIV-1 and establishes fundamental groundwork for the development of neurorestorative treatments. Funded by NIH grants DA013137, HD043680, and MH106392

Disclosures: K.A. McLaurin: None. R.M. Booze: None. C.F. Mactutus: None.

284. Neurodevelopmental Disorders: Environmental Exposures

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 284.19/B61

Topic: A.07. Developmental Disorders

Title: Neurodevelopmental and behavioural consequences of maternal immune activation in male and female Wistar rats

Authors: *J. OLADIPO¹, V. FASOLINO¹, M. E. EDYE³, N. DOOSTDAR¹, G. PODDA¹, M. MANCA¹, J. MIYAN², J. C. NEILL¹

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Abstract: Background: Viral infection in pregnancy is suggested a risk for the development of autistic spectrum disorder (ASD). Maternal immune activation (mIA), using the viral mimetic poly (I:C), produces phenotypes relevant to ASD in mice when administered at gestational day (GD)12.5. However, no studies have explored mIA at GD12.5 in rats. Our aim is to characterise effects of mIA at GD12.5 on offspring neurobiology and behaviour in Wistar rats. Methods: Pregnant female Wistar rats were injected (i.p.) with poly (I:C) (10mg/kg, n=15 dams) or saline (n=18 dams) at GD12.5. All offspring were monitored for changes in morphometric parameters at GD21 (n=36-51 pups/treatment), (body weight (BW), brain weight (BrW) and placental weight (PW). BW was measured regularly until postnatal day (PD)21 alongside BrW (n=22-30 pups/treatment). The open field test (OFT) was used to measure anxiety-like and repetitive behaviour in adolescence (n=12-16 pups/treatment). Gene expression in frontal cortex (FC) of GD and PD21 offspring was measured using qPCR for genes related to synaptic development and stability alongside blood brain barrier (BBB) integrity. For data analysis between treatment groups, a nested-ANOVA was used with litter as a random variable. Results: At GD21, no effect of mIA was observed on BW or BrW. In male offspring a significant down regulation of Snap25 expression was found (p < 0.05). A significant reduction was found in female PW (p < 0.01) and at PD1 in BW for both sexes from poly (I:C) dams vs. saline (p < 0.001). Reduced BW was maintained at PD12-21 (p<0.001). BrW from offspring at PD21 was decreased in male offspring from poly (I:C) dams vs. saline (p < 0.01). A significant increase in gene expression of structural protein Shank3 was found in FC of male offspring only (p < 0.05). In females, a significant reduction in expression of BBB marker Mfsd2a was shown in the FC (p < 0.05). Similarly, only male offspring showed a significant increase in anxiety-like behaviour in the OFT (p < 0.05). Both male and female offspring from poly (I:C) dams showed increased grooming in the OFT that failed to reach statistical significance. Conclusion: To our knowledge this is the first mIA study investigating the effects of 10mg/kg poly (I:C) in Wistar rats at GD12.5. We provide an in depth developmental analysis of both male and female offspring in this model. mIA resulted in sex

specific alterations in morphometric parameters and gene expression in the FC at GD and PD21. A subtle behavioural phenotype relevant to ASD was shown in male offspring. We suggest that this one-hit exposure to poly (I:C) provides a priming model, relevant for investigating neurodevelopmental disorders at the preclinical level.

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Poster

284. Neurodevelopmental Disorders: Environmental Exposures

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 284.20/B62

Topic: A.07. Developmental Disorders

Support: Fonds de Recherche du Québec - Santé

Foundation of Stars

The Montreal Children's Hospital Foundation

Heart & Stroke Foundation

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Title: End-gestational group B Streptococcus-induced inflammation and attention deficit/hyperactivity disorder in the female rat offspring

Authors: *M.-J. ALLARD¹, M.-E. BROCHU², J. BERGERON², G. SEBIRE^{1,2} ¹McGill Univ., Montreal, QC, Canada; ²Univ. de Sherbrooke, Sherbrooke, QC, Canada

Abstract: <u>Introduction</u>: We previously reported autistic-like behaviors in male - but not female - offspring exposed to group B *Streptococcus* (GBS) placental infection and/or inflammation (live and formaldehyde-killed GBS rat models). Female offspring exposed *in utero* to killed GBS displayed increased social interaction time and forebrain injuries in the periventricular external capsule were detected at postnatal day (P) 40, corresponding to young adulthood. Sex differences have been reported in neurodevelopmental disorders including autism spectrum disorder and attention deficit hyperactivity disorder (ADHD), which might affect differently male and female subjects. We hypothesized that female rats exposed *in utero* to GBS may present neurobehavioral impairments detectable only after the juvenile period, *i.e.* during adulthood. <u>Methods</u>: Lewis dams were injected intraperitoneally on gestational day (G) 19 with either saline (controls) or formaldehyde-killed serotype Ia GBS (10⁹ CFU). Maternal weight gain from G19 to G22, litter size and mean weight of pups following natural birth were measured. Spontaneous

motor activity, anxiety, grooming and rearing behaviors were assessed at P15, P20 and P25 using the open field apparatus. Motor learning was investigated using the Rotarod test performed at P30, P35 and P40. Anxiety and anxiety-related impulsivity behaviors were assessed using the Elevated-plus-maze test on adult rats (P104-P110). Results: GBS had a negative impact on maternal weight gain, but did not affect litter size. GBS-exposed males - but not females weighted less than male controls at P1, but no difference was observed from P2. Reduced time and number of rearing episodes were detected at P20 and P25 in GBS-exposed vs control rats. Increased average time of grooming episodes was observed in GBS-exposed vs control males but not females - at P15. At P40, the latency to fall in the Rotarod test was reduced in GBSexposed females - but not males - as compared to controls. Anxiety-related impulsive behavior associated with open-arm exploration - was observed in GBS-exposed versus control females but not males - during adulthood. Discussion: End-gestational GBS-induced inflammation resulted in ADHD-like behaviors in female - but not male - offspring, which were noticed only from the late juvenile/young adulthood period. Neurobehavioral impairments might be subtler in female subjects before adulthood. Therefore, further neurobehavioral assessments during adulthood would be of interest to better understand these sex differences.

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Poster

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Topic: A.07. Developmental Disorders

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Title: Behavioural & molecular consequences of perinatal infection throughout development

Authors: *R. V. WHEELER, S. ALAM, I. C. G. WEAVER, T. B. FRANKLIN Psychology and Neurosci., Dalhousie Univ., Halifax, NS, Canada

Abstract: Human studies have indicated a link between perinatal infection (PI) and the development of autism-spectrum disorder (ASD) suggesting an epigenetic etiology. Previous research has shown that mice exposed to PI have an ASD-like phenotype, behaviourally and molecularly. However, to date, the literature has primarily focused on the effects of PI on adult male mice; there are few reports describing the behavioural and molecular effects of PI on females, or mice in early development. In the current study, we injected pregnant C57BL/6 mice with lipopolysaccharide (LPS) or polyinosinic:polycytidylic (PolyIC) acid to induce a bacterial or viral response, respectively. Male and female offspring were tested during development and at adulthood for the three core symptoms of ASD: social communication, social interaction, and

repetitive behaviours. Ultrasonic vocalizations to maternal separation, repetitive grooming, and juvenile play between unfamiliar mice were measured during early development. Olfactory habituation/dishabituation to social stimuli, marble burying, 3-chamber social preference, and free-social interaction was measured during adulthood. Additionally, the expression of several synaptic (*Nlgn2/3, Shank3, Mdga2, Mtor*) and regulatory epigenetic (*Hdac2*) genes was analyzed in the hippocampus, cerebellum and frontal cortex of offspring at postnatal day zero (P0) and at 3 months of age to discriminate prenatal from postnatal effects. Changes in gene expression were related to DNA methylation in the promotor regions of these genes. Ultimately, the PI mice displayed abnormal social behaviours, repetitive behaviours and gene expression compared to control sham-injected mice. Moreover, exposure to bacterial infection had differing effects than exposure to viral infection. Distinct sex effects which have not been previously reported were also observed. Overall, this study identified novel mechanistic insights on PI-induced ASD.

Disclosures: R.V. Wheeler: None. S. Alam: None. I.C.G. Weaver: None. T.B. Franklin: None.

Poster

284. Neurodevelopmental Disorders: Environmental Exposures

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Topic: A.07. Developmental Disorders

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Title: Porcine fetal microglial cells are transiently activated by maternal viral infection

Authors: *A. M. ANTONSON, R. W. JOHNSON

Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract: Epidemiological studies have revealed that prenatal exposure to maternal infection increases the risk of psychiatric disorders like autism and schizophrenia, though the mechanisms remain to be elucidated. We have previously established a maternal immune activation (MIA) swine model, which results in altered piglet social behaviors postnatally, in the absence of microglia activation. Thus, we sought to identify microglia activity prenatally, immediately following maternal infection. We hypothesized that MIA would elicit transient fetal microglia activation concomitant to maternal symptoms of infection. Pregnant gilts were inoculated with porcine reproductive and respiratory syndrome virus (PRRSV; 5 x 10⁵ TCID₅₀ of live virus) on gestational day (GD) 76 and cesarean sections were performed 7 and 21 days post-inoculation (dpi). Primary microglia were isolated from fetal brains and assessed for activation status through flow cytometry, phagocytosis and chemotaxis assays, and LPS and Poly I:C stimulation in culture. Maternal PRRSV treatment did not affect fetal body weights at 7 or 21 dpi; however,

fetal brain weights were reduced (p < 0.0001) at 21 dpi, but not 7 dpi, due to maternal infection. At 7 dpi, primary fetal microglia from PRRSV-infected litters expressed more MHCII (p < 0.0001), and tended to express more CD68 (p = 0.0534), compared to control litters. Additionally, these fetal microglia displayed reduced phagocytic (p < 0.0001) and chemotactic (p < 0.0001) activity compared to controls at 7 dpi. At 21 dpi, expression of MHCII was still elevated (p < 0.05) in microglia from PRRSV-infected litters, though to a lesser extent, and CD68 expression no longer differed. Interestingly, though chemotaxis remained reduced (p < 0.05), phagocytic activity returned to control levels. Intriguingly, TNF α production by primary microglia stimulated with LPS and Poly I:C was not impacted by maternal infection, though production of this pro-inflammatory cytokine was greatly reduced at GD 97 compared to GD 83 (p < 0.0001). Overall, these data suggest that the activity of fetal microglia are transiently altered by maternal viral infection, indicating a potential mechanism through which MIA could negatively impact prenatal neurodevelopment and cause altered behaviors postnatally.

Disclosures: A.M. Antonson: None. R.W. Johnson: None.

Poster

284. Neurodevelopmental Disorders: Environmental Exposures

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Title: Autism-relevant behavioral outcomes in an antigen-driven rat model of maternal autoantibody related autism

Authors: *M. D. BAUMAN¹, A. MELTZER³, K. L. JONES⁴, R. F. BERMAN², J. VAN DE WATER³

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Abstract: Maternal autoantibodies reactive to fetal brain proteins have been described by numerous researchers in a subset of mothers of children with autism spectrum disorder (ASD), but not in mothers of typically developing children. While previous passive transfer animal models in mice and nonhuman primates have yielded promising results, they did not reflect a constant exposure to the salient autoantibodies throughout gestation, as would be the case in the

clinical setting. Our research group has recently developed a novel non-passive transfer mouse model of maternal autoantibody related (MAR) ASD to directly assess the pathologic significance of prenatal exposure to epitope-specific autoantibodies in generating ASD-relevant behaviors in offspring. Here we expand this program of research from the mouse and into the laboratory rat in order to evaluate the impact of maternal autoantibody exposure in a species that exhibits complex, reciprocal play behavior. In order to generate epitope-specific autoantibodies that mimic those found in the mothers of children with ASD, female Sprague Dawley rats randomly assigned to MAR-ASD treatment received a series of immunizations containing the immunodominant peptide epitope sequences of the four primary target proteins of MAR ASD (lactate dehydrogenase A and B, collapsin response mediator protein 1, and stress-induced phosphoprotein 1). Control females were injected with saline only. Following confirmation of autoantibody production, females were paired with male breeders to produce the experimental offspring of interest. Subsequent male and female offspring were tested in a sequence of autismrelevant behaviors and developmental milestones from an early postnatal period through adulthood. Our results indicate offspring prenatally exposed to ASD-specific maternal antibodies emit fewer ultrasonic vocalizations as pups, display robust deficits in social interactions at juvenile and young adult time points, and exhibit increased self-grooming behaviors as adults. The developmental trajectory of social impairments and repetitive behaviors observed parallels features of human autism. These findings contribute to the ongoing efforts towards identification of biomarkers specific to subphenotypes of ASD and the establishment of a highly translatable rat model of ASD.

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Poster

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Title: Biodistribution of autism-specific maternal autoantibodies in both maternal and fetal tissues throughout gestation and early postnatal life

Authors: *K. L. JONES^{1,2}, J. FUNG³, D. ROWLAND³, C. SMITH³, D. KUKIS³, J. L. SUTCLIFFE^{3,4}, J. VAN DE WATER^{1,2}

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Abstract: While the etiology of autism spectrum disorders (ASD) is currently unknown, immune dysregulation has been noted consistently in individuals with ASD and their families, including the presence of immunoglobulin G (IgG) autoantibodies reactive to fetal brain proteins in 23% of mothers of children with ASD versus less than 1% in mothers of typically developing (TD) children. However, the mechanisms through which these maternal autoantibodies act to influence neurodevelopment and subsequent behaviors in the offspring have yet to be elucidated. Further, although maternal IgG is known to cross the placenta during gestation, it is important to determine the extent to which the autoantibodies access the fetal brain. Therefore the objective of this study was to establish the biodistribution of the circulating maternal autoantibodies during gestation and early postnatal life, as well as the extent to which they enter the fetal compartment. To accomplish this, maternal IgG from either autoantibody positive mothers of children with ASD or autoantibody negative TD mothers were conjugated with desferrioxamine for radiolabeling with 89Zr (half-life 78.4 hours), using established methods. The radiolabeled maternal IgG (approximately 200 uCi) in isotonic solution was then injected intravenously of timed-pregnant C57BL/6J female mice on gestational day 15. Pregnant dams were then imaged under anesthesia 2, 24, and 96 hours after injection using microPET emission scans to capture biodistribution during gestation. Additionally, resulting male and female offspring were scanned on postnatal days 0.5, 3.5, and 4.5. Each emission scan was immediately followed by a cobalt-57 transmission scan (400 s) for attenuation correction, and a magnetic resonance (MR) scan for anatomical reference. Zirconium-89 filled capillaries attached to the animal bed were used for alignment of the PET and MR scans during image co-registration. For image analysis, 3D-binned listmode data from the emission scans was reconstructed using a MAP3D algorithm, attenuation corrected and co-registered with the MR data. Regions of interest were drawn for selected organs, as well as the fetal compartment and standardized uptake values calculated. Preliminary results suggest that radiolabeled IgG from mothers of children with ASD and from TD mothers readily enters the fetal compartment beginning 24 hours post-injection. Further, radioactivity was readily detected in the brains of offspring during gestation and persisting into the first week of postnatal life.

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Poster

284. Neurodevelopmental Disorders: Environmental Exposures

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Topic: A.07. Developmental Disorders

Title: Host cell binding mechanism of the zika virus envelope protein

Authors: M. MAY¹, C. A. PEARSON¹, D. GOODE¹, R. GEGUCHADZE¹, D. C. MOLLIVER¹, R. F. RELICH², J. M. VAUGHN¹, *T. E. KING¹ ¹Biomed. Sci., Univ. of New England, Biddeford, ME; ²Indiana Univ., Indianapolis, IN

Abstract: Zika virus (ZIKV) is responsible for an ongoing and intensifying epidemic in the Western Hemisphere. We previously reported a systems biology analysis of ZIKV utilizing diverse strains (representing temporally diverse members of the African lineage, the Asian lineage, and the current outbreak in the Americas) to explore the natural history of this previously little-known virus. Our analysis highlighted a persistent change in the N-linked glycome of all Asian and American strains examined: glycosylation of the envelope (E) protein residue ASN₁₅₄, a modification proposed to mediate neurotropism in related flaviviruses. This modification was absent from examined African strains. Structural analysis indicates that this residue is modified by addition of N-acetylglucosamine (NAG) and falls within a disordered region of the E protein, suggesting that it is part of a linear epitope in vivo. We synthesized short (20-mer) peptides representing this region from strain HPF2013, which is conserved in all sequenced Western Hemisphere isolates, the Nigerian strain IbH30656, and the type strain MR 766(Uganda). A NAG-linked version of the HPF2013 peptide and an unglycosylated version were generated to explore the contribution of this modification to interaction with neuronal cells and fibroblasts, expecting interactions to be representative of ZIKV E protein/cell interactions. Non-glycosylated peptides from HPF2013, IbH30656, and MR 766 bound MDCK cells and primary dorsal root ganglia neurons significantly above a scrambled HPF2013 control peptide at equivalent levels, implicating this motif in ZIKV-cell interactions. These peptides significantly inhibited the ability of ZIKV strain MR 766 to adsorb to host cells, as measured by TCID[50], indicating that we have positively identified the binding mechanism of ancestral African ZIKV strains. Surprisingly, binding of the NAG-glycosylated (i.e., native) HPF2013 peptide to MDCK cells and DRG neurons was abolished, potentially indicating that HPF2013 and related strains target host cells with an alternative binding mechanism. Studies exploring a.) alternative HPF2013 binding motifs and b.) trafficking of NAG-glycosylated and unglycosylated peptides to the central nervous system and the placenta in vivo are ongoing.

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Poster

284. Neurodevelopmental Disorders: Environmental Exposures

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Topic: A.07. Developmental Disorders

Title: Rat model of prenatal Zika virus infection

Authors: *M. L. SHERER¹, P. KHANAL², M. PARCELLS², J. M. SCHWARZ³ ¹Psychology, ³Psychological and Brain Sci., ²Univ. of Delaware, Newark, DE

Abstract: Zika virus (ZIKV), a mosquito-borne flavivirus, has been associated with microcephaly and other neurological disorders in infants born to infected mothers. Despite being declared an international emergency by the World Health Organization, comparatively very little is known about the pathogenesis, mechanisms, or behavioral consequences of maternal ZIKV infection in the offspring. Our lab is interested in developing a working animal model to answer some of these questions. Here, we use a rat model of prenatal ZIKV infection to measure the level of infectivity, as well as the rate of viral clearance in both the mother and her pups. We use quantitative PCR to measure the effect of ZIKV on inflammatory gene expression, and examine various aspects of brain development in pups, including cortical thickness, microglia morphology, and apoptosis. Given that pregnancy is also associated with significant immunomodulation, we are also interested in the role that pregnancy has on the impact of ZIKV infection, therefore we compare viral infectivity between both pregnant and non-pregnant female rats. This model will allow us to 1) better understand the mechanisms underlying ZIKV infection and transmission to the fetus, 2) determine the impact of ZIKV infection on the developing fetal brain, and 3) in the future, measure potential behavioral deficits associated with fetal ZIKV infection later in life.

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Poster

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Topic: A.07. Developmental Disorders

Title: An investigation into the microglial response to neonatal ZIKV infection

Authors: *J. H. LAWRENCE¹, A. TURANO¹, M. S. PARCELLS², J. M. SCHWARZ¹ ¹Psychological and Brain Sci., ²Animal and Food Sci., Univ. of Delaware, Newark, DE

Abstract: Zika Virus (ZIKV), unlike other *Flaviviruses*, has an apparent selective tropism for developing neural stem cells. As a result, the fetal brain is acutely more vulnerable to ZIKV infection than the adult brain. ZIKV can cross the placental barrier to directly enter the fetal brain. In the fetal brain, ZIKV appears to specifically target developing radial glia. In embryonic mice, ZIKV infection of neural progenitor cells (NPCs) arrests the cell cycle and hinders

apoptotic cell death by 24 hours post-infection. However, by 3 days post-infection, ZIKV induces apoptosis in NPCs and stimulates pro-inflammatory cytokine expression from microglial cells - the resident immune cells of the brain. It is unclear whether neural progenitor cells are the only cells targeted by ZIKV. It is also unclear what role microglia have in the mitigation or exacerbation of cell death associated with ZIKV infection. We hypothesize that ZIKV infection of NPCs will induce microglia to express high levels of pro-inflammatory cytokines (IL-1ß and IL-6), either mitigating or contributing to the resultant cell death of developing neural cells. To test this hypothesis using a rat model, we collected hippocampal and cortical tissue from male and female Sprague Dawley rat pups and generated three distinct cell populations for culture: isolated neonatal microglia, all other developing neural cells, and a population of unsorted cells containing both microglia and all other neural cells. The cells were infected with ZIKV at 0, 0.1, 1, or 10 MOI and collected for qPCR analysis of pro-inflammatory cytokine expression, markers of microglial activation, and apoptotic cell death. A separate set of similarly-treated cell cultures were fixed for analysis of cell type-specific ZIKV infection and apoptosis. This experimental design is unique in its use of a rat animal model as well as its use of a primary cell culture model over a complex in-vivo model. These infection studies will allow us to better characterize Zika virus-host interactions in the developing brain, including how the developing neural immune system responds to this emergent virus. Our preliminary data indicate that ZIKV replication can be detected in rat neural cells following a 16-hour incubation at 1 MOI, an unprecedented result in a rat animal model. Still, the infected cell type remains to be determined. We are currently examining these samples for pro-inflammatory cytokine (IL-1 β , IL-6, and TNF- α) and type I interferon (IFN- α) expression. Ultimately, we predict that the microglial response to infection will be exacerbated by the presence of other developing neural cells, infected and dying from the infection.

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Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

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Title: Late stage spontaneous waves and their role in downstream visual areas In vivo

Authors: *A. GRIBIZIS^{1,1}, X. GE¹, J. B. ACKMAN², D. LEE¹, M. C. CRAIR¹ ¹Dept. of Neurosci., Yale Univ., New Haven, CT; ²Molecular, Cell & Developmental Biol., Univ. of California, Santa Cruz, Santa Cruz, CA

Abstract: In the developing mammalian visual system, neighboring retinal ganglion cells (RGCs) fire in correlated bursts of action potentials that are observed as propagating waves across the retina. These spontaneous waves of activity, which in mice begin around the time of birth and last until eye opening, are thought to be crucial for normal formation of visual system circuitry. The features of retinal waves have been described in detail in a number of species in vitro, but little is known about their properties in the epoch just prior to eye opening ('Stage III' or P9-P13 in mice) in vivo. Using optical imaging techniques with genetically encoded Ca2+ indicators, we report here on experiments examining the spatiotemporal and pharmacological properties of Stage III retinal waves in RGCs, the superior colliculus, lateral geniculate nucleus and visual cortex in mice in vivo. In comparison to Stage II waves, Stage III waves are smaller, faster, and of shorter duration, which is simultaneously observed in their downstream targets. We also describe the results of novel dual wavelength and fluorophore (RCaMP, GCaMP) experiments to simultaneously image presynaptic axons and postsynaptic neuronal activity. Both Stage II and Stage III waves show faithful transfer to postsynaptic targets in the SC, however Stage III wave transfer is less predictable, likely due to increased post-synaptic activity at older ages. Our data also suggest that Stage III retinal waves propagate in a wave like fashion in both the colliculus and thalamic afferents, but cortical activity is less tightly correlated to retinal activity in Stage III waves than during Stage II. We also examined the effects of pharmacological manipulations in the eye on the propagation of retinal waves to the superior colliculus and higher order visual circuits. These results demonstrate that Stage III waves have unique properties and trans-synaptic propagation compared to earlier (Stage II) spontaneous activity, which may be essential in the patterning of circuits throughout the visual system in vivo.

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Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

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Title: Origin and function of directionality in spontaneous retinal waves

Authors: *X. GE, A. GRIBIZIS, M. C. CRAIR Neurosci., Yale Univ., New Haven, CT

Abstract: Before the onset of visual experience, spontaneous retinal waves are the main source of activity in the developing superior colliculus (SC), thalamus and primary visual cortex in the mammalian visual system. Genetic and pharmacological manipulations of this spontaneous retinal activity suggest a causal link between retinal waves and circuit refinement. Previous studies found that Stage II (around the first week after birth in mice) spontaneous waves exhibit a strong directional bias. However, it remains unclear how this bias emerges, whether it changes over development and whether this spatiotemporal feature of spontaneous waves is critical for circuit refinement and the development of functional circuit properties. Here, we describe experiments that investigate the role and origin of spontaneous wave directionality during visual system development. We used wide-field Ca²⁺ imaging of retinal ganglion cell (RGC) projections in the SC in vivo and optogenetic or light stimulation of the retina to examine spontaneous and stimulus induced wave direction bias. We observe that the directional bias emerges at the end of Stage II (~P8) waves and vanishes around the time of eye opening (~P13). The biased direction (temporal to nasal) is consistent between P8 and P13. The averaged wave propagation direction distributes inhomogenously across the retina, suggesting a difference in wave initiation and propagation in different parts of the retina. To understand the origin of wave directional bias in vivo, we used selective optogenetic stimulation of starburst amacrine cells (SACs), or light stimulation after the onset of light response (~P10), to initiate waves at various locations in the retina. Our preliminary data suggests that starting from around P10, stimulated retinal waves propagate in a direction consistent with intrinsic waves, regardless of their nucleating sites. This suggests a biased retinal circuit that favors the propagation of waves in a specific direction on the retina. These results suggest a specific developmental stage in which spontaneous retinal waves exhibit a strong bias, which could potentially affect visual circuit refinement in the retina and other parts of the developing visual system.

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Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

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Title: Ontogenetic course of the establishment of the thalamo-palio-intra pallial connectivity in birds. The chick visual DVR as a study case

Authors: R. REYES-PINTO, G. MARIN, *J. MPODOZIS

Univ. of Chile, Santiago, Chile

Abstract: In spite its evident cytoarchitectonic differences, avian and mammalian pallial territories are comparable in terms of the complexity of its neural processing capabilities. This operational similarity has been attributed to a similar organization of the thalamic inputs to the pallium, and also to a seemingly common pattern of organization of the intrapallial circuits. The avian pallium contains a dorsal intraventricular protrusion known as dorsal ventricular ridge (DVR), which is formed by a dorsal neuronal stratum, known as the mesopallium and a more ventral stratum, the nidopallium. Of particular concern to us is the visual DVR, which can be regarded as a complex composed of three main layers: an internal nidopallial one, the entopallium (E), in receipt of afferents from the dorso-thalamic stage of the tecto-fugal visual system, the nucleus rotundus (Rt); an overlaying nidopallial region, the intermediate nidopallium (NI), lacking sensory inputs; and a more external mesopallial one, the ventral mesopallium (MV). Interconnections between these layers follow a "columnar/ recurrent" arrangement that features a striking resemblance with that of the interlaminar circuitry of the mammalian sensory cortex. We prompted to ask whether the structural similarities between avian and mammalian pallia arise from comparable ontogenetic trajectories. To that end, we study the ontogenetic development of the Rt-E projection, along with that of the E-M reciprocal connectivity in chicks. Minute deposits of biocytin crystals were placed in selected locations of vital slices of the brain of chick embryos. These slices were then reacted with DAB/Ni and counterstained with giemsa. Neurotracing experiments revealed the existence of a prospective Rt as early as E8. At this stage, the visual DVR appear cytoarchitectonically composed by the three main layers described in adults; axons from the prospective Rt were found reaching the nidopallium and delineating in it a prospective entopallial region. Furthermore, columnarly organized axonal processes running reciprocally and homotopically between the E and the MV were found from, at least, E12 onwards. These results suggest that the "cortical like" arrangement proper of the adult visual DVR is established in early embryonic stages, in conjunction with the arrival of the rotundal axons to E, and prior to the (previously documented) date of arrival of the tecto-visual afferents to the dorsal thalamus. This situation may be comparable to that of mammals, where the early ingrowth of thalamic fibres and the maturation of the cortex take place in conjunction, and before the arrival of sensory afferents to the dorsal thalamus.

Disclosures: R. Reyes-Pinto: None. G. Marin: None. J. Mpodozis: None.

285. Development of Visual, Auditory, and Somatosensory Circuits

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 285.04/C8

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

Support: Ey025627

Title: Hedgehog interacting protein is a candidate regulator of visual circuit formation in the superior colliculus

Authors: *U. JAVED¹, A. MONAVARFESHANI³, M. A. FOX³, J. TRIPLETT² ²Ctr. for Neurosci. Res., ¹Children's Natl. Med. Ctr., Washington, DC; ³Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: We are able experience and navigate through the world through the use of our highly elaborate visual system. In this system, parallel circuits process information about various aspects of the visual scene. For instance, there are multiple subtypes of retinal ganglion cells (RGCs) that respond to specific stimuli, such as contrast or motion. The molecular components that are essential in the formation of these sub-circuits have not been clearly elucidated. In this study, we focus specifically on the sub-circuits of the retina's projection to the superior colliculus (SC). In Isl2-EphA3 knock-in mice (EA3ki/ki), the projections of different RGCs are segregated into the rostral and caudal regions of the SC. Interestingly, the sublaminar targeting of class-specific projections was unaffected, suggesting that the molecules that regulate this process may also be differential expressed in the SC of EphA3^{ki/ki} mice. We performed RNAseq analysis of microdissected regions of the developing SC and identified the hedgehog-interacting protein (*hhip*) was as a potential candidate regulator of class-specific circuit formation. Using a combination of RNA in situ hybridization and immunohistochemistry, we found that *hhip* is expressed specifically in neurons located in the retino-recipient layer of the SC. Additionally, we found that expression of *hhip* was temporally regulated during development, peaking at postnatal day 4 (P4), a time point that overlaps with the period of laminar targeting by RGCs in the SC. Interestingly, we found that *hhip* was expressed in retinal neurons; however, the specific subset of RGCs has not been clearly identified. Taken together these data suggest that *hhip* is ideally positioned to mediate class-specific targeting of RGCs in the SC and raise the possibility that *hhip* may be involved in trans-synaptic signaling between both RGCs and neurons in the SC.

Disclosures: U. Javed: None. A. Monavarfeshani: None. M.A. Fox: None. J. Triplett: None.

285. Development of Visual, Auditory, and Somatosensory Circuits

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

Support: NIH Grant EY025627

Title: Investigating the mechanisms of parallel circuit formation by converging visual inputs in the superior colliculus

Authors: *N. GABRESKI, J. TRIPLETT

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Abstract: Integrating sensory information is a central task of the nervous system, which is necessary to create a unified percept of the outside world. In the mammalian visual system, distinct neuronal subtypes monitor different aspects of the visual scene, such as contrast or motion, and this information needs to correctly converge in integrative centers to successfully relay both qualitative and spatial information about stimuli. One such integrative center, the superior colliculus (SC), receives converging input from retinal ganglion cells (RGCs) of the eye through the retinocollicular pathway, and also from Layer 5 (L5) of the primary visual cortex (V1) through the corticocollicular pathway. We previously demonstrated that retinal input instructs the spatial alignment of V1 neurons in a process requiring the normal pattern of spontaneous waves of activity. Based on this, we hypothesized that subtypes of RGCs instruct both the spatial and functional alignment of matched L5-V1 inputs in the SC. Utilizing the Islet2^{EphA3/EphA3} knock-in mouse model in which distinct morphological and functional types of RGC inputs are segregated into two independent subdomains of the SC, we are able to separate neuronal projections from L5-V1 that align with these distinct retinal projections. To retrogradely label corticocollicular neurons making monosynaptic connections in the SC of adult mice, a Cre-based pseudotyped rabies viral tracing strategy is being used. Consistent with previous data, we have found that corticocollicular L5-V1 neurons are pyramidal cells that have complex apical and basal dendritic tufts. Current and future experiments using a combination of anatomical tracing, in vivo electrophysiology, and optogenetics will determine the morphological and functional differences of L5-V1 corticocollicular neurons aligning with distinct retinal inputs in the SC.

Disclosures: N. Gabreski: None. J. Triplett: None.

285. Development of Visual, Auditory, and Somatosensory Circuits

Location: Halls A-C

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

Support: Career Development Grant, NHRI-EX103-10316NC

General Research Project, MOST 105-2628-B-002-033-MY3

Title: Experience-regulated transcriptomic and imprintomic profilings during critical periods of mouse visual system development

Authors: *C.-L. HSU¹, C.-H. CHOU², S.-C. HUANG², C.-Y. LIN², M.-Y. LIN², C.-C. TUNG², C.-Y. LIN², P.-C. LAI², Y.-F. ZHOU³, N. YOUNGSON⁴, N. TAKAHASHI⁵, S.-P. LIN⁶, A. FERGUSON-SMITH⁵, C.-H. YANG⁷, S.-K. CHEN³, S.-F. GAU², H.-S. HUANG² ¹Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; ²Grad. Inst. of Brain and Mind Sciences, Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; ³Dept. of Life Science, Col. of Life Science, Natl. Taiwan Univ., Taipei, Taiwan; ⁴Dept. of Pharmacology, Sch. of Med. Sciences, The Univ. of New South Wales, Sydney, Australia; ⁵Dept. of Genetics, Univ. of Cambridge, Cambridge, United Kingdom; ⁶Dept. of Biotechnology, Col. of Bio-Resources and Agriculture, Natl. Taiwan Univ., Taipei, Taiwan; ⁷Dept. of Ophthalmology, Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; ⁷Dept. of Ophthalmology, Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; ⁷Dept. of Ophthalmology, Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; ⁷Dept. of Ophthalmology, Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; ⁷Dept. of Ophthalmology, Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; ⁷Dept. of Ophthalmology, Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; ⁷Dept. Of Ophthalmology, Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; ⁷Dept. Of Ophthalmology, Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; ⁷Dept. Of Ophthalmology, Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; ⁷Dept. Of Ophthalmology, Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; ⁷Dept. Of Ophthalmology, Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Visual system, part of the central nervous system, receives external visual cues and transforms it into visual and non-visual signals, which enables organisms to react to environment. However, the development of the visual system is influenced by light experience, which implicates the involvement of epigenetic regulations. We focused on the role of one of the epigenomic phenomenon, genomic imprinting, which is expressed in parent-of-origin-specific manner, during critical periods of visual system development. By manipulating light experience during critical periods of visual system development in the mice, we identified experience-regulated, isoform-specific, and brain region-specific imprinted genes. We also found imprinted microRNAs occurring in the clusters. Our results provide the first comprehensive profiling of light-experience regulation of the transcriptome and imprintome during critical periods of visual system development and potential therapeutic strategy for visual impairments and circadian rhythm disorders.

Disclosures: C. Hsu: None. C. Chou: None. S. Huang: None. C. Lin: None. M. Lin: None. C. Tung: None. C. Lin: None. P. Lai: None. Y. Zhou: None. N. Youngson: None. N. Takahashi:

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Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 285.07/C11

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

Support: National Research Foundation of Korea (NRF-2016R1C1B2016039)

KAIST (Future Systems Healthcare Project)

Title: Retinal origin of various functional maps in visual cortex

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Abstract: In higher mammals, the primary visual cortex (V1) is organized into various functional maps such as an orientation map. The topographies of these maps in the same cortical area are geometrically correlated with each other, but it remains unclear as to how such systematic organization may have developed. Recent studies have reported that the structure of each functional map is strongly correlated with the local organization of ON and OFF thalamic inputs (Kremkow et al., 2016; Lee et al., 2016). It was also proposed that a quasi-periodic orientation map can be seeded by the moiré interference of hexagonal lattices of ON and OFF retinal ganglion cells (RGC) (Paik and Ringach, 2011). Here, expanding upon the previous model, we propose that such a regular distribution of RGCs can also seed the spatial frequency, direction and ocular dominance maps in a way that their topographies are systematically correlated, as observed in experiments. First, we simulated multiple functional maps on a moiré interference of RGC by estimating the individual selectivities of model V1 neurons. As the preferred orientation is determined by the alignment of neighboring ON and OFF cells, the preferred frequency depends on the spatial distribution of neighboring ON and OFF cells in the moiré interference. Because the alignment and the local distribution changes in relation to the other property in the orthogonal direction, we successfully reproduced the orthogonal organization of the orientation map and the spatial frequency map (Nauhaus et al., 2012). Given that this periodic change in the spatial distribution causes the preferred direction to flip at the center of an iso-orientation domain, the iso-orientation domain was divided into two isodirection domains with opposite directions (Weliky et al., 1996). Next, we simulated the ocular dominance while assuming that a V1 neuron receives input from two sets of moiré interference, mimicking the contra- and ipsilateral retina. Because the early orientation map is dominated by

the contralateral eye (Crair et al., 1998), the contralateral input was assumed to be projected from the retina to V1 by local wiring that can seed a clear orientation map (Paik and Ringach, 2011), whereas more extensive wiring was assumed for the ipsilateral projection. In our simulation, the ocular dominance peaks were located where the local density of the contra-RGC is relatively high or low. This reproduced the previous observation of singularities in the orientation map which are located at the center of an ocular dominance domain (Crair et al., 1997). Our results suggest the retinal origin of multiple functional maps and their structural correlation.

Disclosures: M. Song: None. J. Jang: None. S. Paik: None.

Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 285.08/C12

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

Support: CAPES

CNPQ FAPERJ PRONEX NIH

Title: Effects of congenital hypothyroidism on development and plasticity of the visual system

Authors: *W. S. RODRIGUES JUNIOR¹, N. S. PULIMOOD², P. O. SILVA³, P. CAMPELLO-COSTA³, P. PANDOLFO⁴, A. E. MEDINA², C. A. SERFATY⁴ ¹Neurophysiol., Rio de Janeiro State Univ. (UERJ), Niteroi, Brazil; ²Pediatrics, Univ. of Maryland, Sch. of Med., Baltimore, MD; ³Neurobio., Federal Fluminense Univ., Niterói, Brazil; ⁴Neurobio., Federal Fluminense Univ., NIterói, Brazil

Abstract: Thyroid hormones are crucial for the development and proper maturation of the CNS. Severe changes in thyroid hormones levels during perinatal period can result in impaired brain development since they regulate neurogenesis, neuronal growth and synaptogenesis (Zoeller, Dowling et al. 2002; Plateroti Bernal et al. 2011). During the post natal development, the sensory experience plays a key role in the refinament and maturation of of neural circuits, since it regulates the development of intrinsic programs to modulate gene expression as both the anatomical and functional properties of neuronal circuits through patterns of neuronal activity. The objective of this study is to evaluate the role of thyroid hormones in the development of neural circuits modeled the visual system that has topographical organization characteristics and formation of eye-specific layers, properties that point to the formation of organized neuronal circuits.

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Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

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

Support: NSERC CGS-M to VJL

CIHR Grant FDN-143238 to ESR

FRSQ Grant 31036 to ESR

Title: Targeted expression of GCaMP6 to map functional retinotopy in the optic tectum of Xenopus tadpoles

Authors: *V. J. LI, A. SCHOHL, E. S. RUTHAZER Montreal Neurolog. Inst., McGill Univ., Montreal, QC, Canada

Abstract: The wiring of the developing *Xenopus laevis* retinotectal system has been well described anatomically, but the functional organization of the retinotopic map has not been thoroughly characterized. Here we report on the development of an optical approach to visualize retinotopy in the tectal neuropil of albino Xenopus larvae, based on in vivo two-photon imaging of calcium fluorescence changes in response to visual stimulation. By microinjecting messenger RNA for the genetically-encoded calcium indicator GCaMP6 into one blastomer of two-cell stage Xenopus embryos, expression of calcium indicator can be restricted to half of the developing tadpole. Because the projection from the eye to the brain crosses the midline, this approach permits the presynaptic terminals of retinal ganglion cell inputs and the postsynaptic dendritic fields of tectal neurons to be observed independently. Drifting bar stimuli were presented on a LED monitor placed adjacent to the animal under the microscope. Threedimensional stacks of calcium fluorescence images of the neuropil and tectal cell somata were collected using resonance-scanning 2-photon excitation with piezo focus control, at acquisition rates of up to 30 Hz. Correlation of fluorescence intensity changes to visual stimulus location revealed smooth maps for both azimuth and elevation. This method can uncover continuous retinotopic maps in most of the volume of the tectal neuropil, and can serve as a tool to examine the influences of various developmental factors on map organization.

Disclosures: V.J. Li: None. A. Schohl: None. E.S. Ruthazer: None.

Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 285.10/DP02/C14 (Dynamic Poster)

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

Support: NEI IRP funds

Title: Characterization of Brn3c RGCs

Authors: *T. C. BADEA¹, N. PARMHANS², E. NGUYEN², K. CHUANG², S. SAJGO², W. LUO³, J. NIU³

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Abstract: We have previously described a transcriptional combinatorial code of Pou domain transcription factors regulating Retinal Ganglion Cell (RGC) diversity in the mouse. RGCs expressing the Brn3c transcription factor constitute a small fraction of all mouse RGCs, and, depending on the particular subtype, they can also express the transcription factors Brn3a and/or Brn3b, as well as neurotrophin receptors such as cRet. We have recently described a sequential Dre to Cre recombination strategy involving a Dre-dependent conditional Cre knock-in allele at the Brn3c locus. In addition, we have described a unique cell type identified by the intersection of the cRet and Brn3c expression domains. We are taking advantage of these unique tools to characterize the dendritic arbor lamination, molecular profile and brain projections for RGCs expressing Brn3c, hopefully leading to the characterization of the physiological and circuit function of individual RGCs expressing Brn3c.

Disclosures: T.C. Badea: None. N. Parmhans: None. E. Nguyen: None. K. Chuang: None. S. Sajgo: None. W. Luo: None. J. Niu: None.

Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

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

Support: NIH Grant EY022730

Title: Thalamic contributions to the developmental acquisition of state-dependent cortical activity

Authors: *Y. MURATA¹, M. T. COLONNESE²

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Abstract: Two major checkpoints of cortical development are the acquisition of continuous background (spontaneous) activity and the state-dependent modulation of this activity. In both humans and rodents, cortical activity during early development is discontinuous, meaning it contains long (>1 second) silent periods, and oscillatory activities are poorly modulated by arousal state. Continuous background activity that is reliably modulated by state develops around the term age in humans and at the end of second postnatal week in rodents. Abnormal development of continuity is often a result of brain damage due to epileptic seizures or hypoxic-ischemic encephalopathy. However, the underlying circuit changes that instruct continuity are unknown. Thalamic lesions in adults can result in discontinuous activity and disrupt state-dependent modulation in cortex, but the changing behavior and function of thalamus around the time of continuous activity development has not been examined.

Here we use the rodent visual system as a model to examine the role of thalamus in cortical activity development. We conducted simultaneous recordings in the lateral geniculate nucleus (LGN) and visual cortex (VC) of awake head-fixed rats using linear multi-electrode arrays throughout development. We find that between P5 and P11 spontaneous LGN activity is discontinuous and not modulated by movement. By P13 activity in both VC and LGN becomes continuous and movement causes increased multi-unit firing in both structures. To determine the role of thalamus in this change, we pharmacologically silenced LGN. This treatment reduces firing and continuity in VC at all ages. Furthermore silencing LGN causes movement to suppress, rather than increase, VC activity. Thalamic single-units isolated with spike sorting showed that the developmental acquisition of continuity and movement dependence occur at the neuron level. Thalamic bursting only emerged on P16, a few days after thalamic acquisition of movement modulation, suggesting that the mechanisms initiating the development of movement modulation and bursting are different.

Together our results demonstrate that maturation of LGN circuitry, not intra-cortical or thalamocortical connections, plays a determinative role in the developmental acquisition of continuity and state-dependence observed in cortex. Our study has implications for understanding the circuit basis of human EEG development, which could improve diagnosis and treatment of preterm and neonatal infants.

Disclosures: Y. Murata: None. M.T. Colonnese: None.

285. Development of Visual, Auditory, and Somatosensory Circuits

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

Support: NSERC Discovery Grants to DEM, KRD & NAC

Canadian Institutes of Health Grant MOP102653

Title: The profile of the critical period for darkness-induced recovery of vision in monocularly deprived kittens

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Abstract: In Long-Evans rats (He et al., *Nat Neurosci* 10:1134, 2007) and 3 month-old kittens (Duffy & Mitchell *Curr Biol* 23:382, 2013), a 10 day period of total darkness has been shown to precipitate fast improvement of the visual acuity of the deprived eye induced by a short early period of monocular deprivation (MD). For rats, the period of darkness was imposed when the animals were considered adult (at P70 – 100 days) and beyond the upper limit of the age (~7weeks) of vulnerability of neural connections with the deprived eye in the rat visual cortex to the effects of MD. On the other hand, 10 days of darkness when imposed on adult cats at 1 yr of age promoted no recovery of vision in the deprived (amblyopic) eye (Holman et al., *Soc Neurosci* 780.02, 2014), a result that implied that the benefits of darkness in felines were confined to an early critical period.

To document the profile of the critical period for the benefits of darkness, the same 10 day period of darkness was imposed on 15 kittens of progressively older ages each having received an identical early one week period of MD from P30-37 days. Longitudinal measurements were made by use of a jumping stand to assess the recovery of grating acuity of the two eyes at regular intervals, as well as the extent and time course of any recovery that occurred in the period following the period of darkness. Total darkness over 10 days was ensured by use of a custom-built darkroom facility (Mitchell *Clin Exp Optom* 96: 363, 2013).

In terms of the grating acuity of the derived eye, the effects of 10 days of darkness appeared quite uniform with respect to both the speed and the extent of the recovery of acuity when darkness was applied at any age prior to P180. However, thereafter there was a precipitous decline in the benefits of darkness such that no darkness-induced improvement in the acuity of the deprived eye was evident at all after P195 days. It is known that darkness can influence the levels of many putative molecules that serve to either facilitate or inhibit neural plasticity in the visual system. The protracted period during which darkness is efficacious may represent a period

during which at least a subset of such molecules retain sufficient susceptibility to environmental manipulation to promote significant recovery. The abrupt termination of the critical period for darkness-induced recovery may reflect the age at which alteration of the slowest developing plasticity mechanisms is no longer possible with darkness. From a clinical perspective, the results imply that for darkness to be effective for treatment of human amblyopia it should be applied only in childhood.

Disclosures: D.E. Mitchell: None. N.A. Crowder: None. K.R. Duffy: None.

Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

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

Support: CNPq

FAPERJ

Title: Cell architecture alterations and loss of retinal ganglion cells followed by pupillary light reflex alteration in prenatal hypoxia ischemia rats

Authors: *G. D. TAVEIRA¹, L. S. FONSECA², M. N. OLIVEIRA³, F. TENORIO², P. C. BARRADAS², T. KRAHE³

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Abstract: Prenatal hypoxia-ischemia (HI) is one of the main causes of neurodevelopmental impairment in the newborn and is associated with cerebral palsy, attention problems, hyperactivity, epilepsy, and sensory alterations, including visual processing problems. The retina is widely recognized as a neural circuit model used in the study of the development and plasticity of neuronal circuits. Thus, the investigation of the effects of prenatal HI on retinal development offers great potential to elucidate mechanisms related to the effects of HI during pregnancy. Here we studied the effects of prenatal HI on retinal morphology and function. Specifically, we evaluated the number of retinal ganglion cells (RGCs) and the pupillary light reflex (PLR) after prenatal HI of Wistar rats. Prenatal HI was induced by occlusion of uterine arteries for 45 minutes on the eighteenth gestational day (HI group, n = 23 rats from 6 litters). Control animals were obtained from pregnant females submitted to the same surgical procedures except for the occlusion of the uterine arteries (SH group, n = 24 rats from 6 litters). Histological procedures were made at postnatal (P) day 2, 9, 23 and 30. Hematoxylin and eosin staining revealed a

significant reduction in retinal layer thickness of HI animals compared to controls for all ages except P30. Immunohistochemical labeling of RGCs with Brn3- α showed a significant reduction in the number of RGCs in the HI group compared to control. PLR was evaluated in a separate group of animals at P30 (HI, n = 7; SH, n = 8) after two hours of dark adaptation. Both groups showed equal pupil constriction times and sizes, but HI animals were not able to sustain pupillary constriction under continuous illumination. Sustainability of the PLR is attributed to the activation of intrinsically photosensitive RGCs (ipRGCs). Therefore, our results suggest that prenatal HI could be preferentially eliminating ipRGCs.

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Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

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

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Title: Developmental refinement of interhemispheric connections in ferret visual cortex

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Abstract: Visual corticocortical connections in adult animals link cortical areas in the same hemisphere (intrahemispheric) as well as in the opposite hemisphere (Interhemispheric or callosal). The adult pattern of callosal connections between visual areas in both hemispheres in a number of species is thought to emerge from an immature state during development through refinement of some aspects of this circuit. We studied the postnatal refinement of callosal connections projecting from multiple cortical areas to ferret (*Mustela putorius furo*) primary visual cortex (area 17) during the period from just before eye opening (4 weeks) to 10 weeks of age. Our goal was to determine (a) whether callosal projections from multiple visual cortical

areas to area 17 refine with a similar rate and (b) whether the developmental refinement of callosal projections parallels that of intrahemispheric cortical circuits. To follow the development of callosal connections we injected the bidirectional tracer CTb into area 17 of juvenile ferrets, and visualized the distribution and pattern of callosal projecting cells in area 17, 18, 19, 21, and Ssy of the contralateral hemisphere. We analyzed the refinement of retrogradely labeled callosal cells in each source area by quantifying their areal and laminar distribution. Throughout development, the greatest proportion of callosal projections arises from the infragranular layers in most visual areas. At 4 weeks of age we find the greatest proportion of callosal inputs to area 17 arising from Ssy, with a lesser and comparable proportion arising from areas 17, 18, 19, and 21. From 5 to 6 weeks of age the proportion of callosal projections from Ssy to contralateral area 17 declines significantly, while the proportion from contralateral area 18 increases. From 6 weeks to 10 weeks of age there is minimal change in the proportion of callosal input to area 17 arising from areas 18 and Ssy. In contrast, we find no significant change in the proportion of total callosal inputs to area 17 arising from contralateral areas 17, 19, and 21 during this postnatal period. We observe the same trends in the postnatal refinement of intrahemispheric feedback to area17; around the time of eye opening the major ipsi- and contralateral corticocortical input is provided by area Ssy, but within a week or two thereafter area 18 supplants Ssy as the major corticocortical input. This suggests that the refinement of both interand intrahemispheric connections follows a broadly similar developmental trajectory during the period just after eye opening.

Disclosures: R. Khalil: None. C. Gonzalez: None. J.B. Levitt: None.

Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 285.15/C19

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

Title: Binocular integration of orientation selectivity in the developing ferret visual cortex

Authors: *D. E. WHITNEY¹, G. B. SMITH³, B. HEIN⁴, M. KASCHUBE⁵, D. FITZPATRICK²

¹Neurosci., ²Max Planck Florida Inst., Jupiter, FL; ³Max Planck Florida Inst. For Neurosci., Jupiter, FL; ⁴Frankfurt Inst. For Advanced Studies, Frankfurt, Germany; ⁵Frankfurt 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 exquisitely organized into a smoothly varying columnar map that emerges in an activity-dependent manner during early postnatal life. Recently, we reported that the initial absence of orientation selectivity can be accounted by high trial-to-trial

variability at a columnar scale. Thus, response variability appears to be a limiting feature of the developing cortex, and its reduction crucial for the emergence of feature-specific representations. However, variability may also beneficially provide a flexible and dynamic mechanism for the developing cortex to explore and select relevant feature-specific input.

To test whether response variability might be associated with a constructive role in forming coherent cortical representations, we chose to investigate how binocular inputs for orientation tuning become matched in ferret visual cortex. In rodents, this is an activity-dependent process that begins around eye-opening as orientation selectivity emerges (Wang et al., Neuron, 2010), but it is unclear if a similar process occurs in a species with cortical columns. Using functional imaging of GCAMP6s at both the columnar and cellular level, we show that the monocular presentation of drifting gratings to either eye evokes robust and modular responses. Furthermore, as in rodents, orientation-tuning is initially mismatched through the eyes, resulting in dissimilar orientation maps. The initial mismatch in orientation preference is gradually eroded, and within 5-7 days of visual experience monocular orientation maps match at near adult-levels. We next tested the idea whether a gradual reduction in trial-to-trial variability to responses evoked by monocular stimuli precisely coincides with a developmental matching of orientation tuning through the two eyes. Indeed, we find both processes occur simultaneously. Moreover, because of the higher trial-to-trial variability, we observe with dimension reduction techniques that stimuli presented to each eye evoke activity in overlapping neural populations. In principle, Hebbian-based learning rules could then associate orientation-selective responses through the two eyes to a common neural population. In summary, while early trial-to-trial variability is a limiting factor for the emergence of orientation-selectivity in visual cortex, variability might also serve as an important framework to align binocular inputs for forming a coherent percept of the visual scene through both eyes.

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Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

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

Support: NIH R01 DC005798

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Gordon and Betty Moore Foundation

Title: Rapid tissue dynamics during the formation of a mammalian central synapse

Authors: *D. R. JACKSON¹, J. HEDDLESTON², S. RAY¹, M. MOREHEAD¹, S. PIDHORSKYI¹, P. S. HOLCOMB³, S. SIVARAMAKRISHNAN¹, S. M. YOUNG, JR⁴, T.-L. CHEW², T. DEERINCK⁵, M. H. ELLISMAN⁵, G. A. SPIROU⁶ ¹Sensory Neurosci., West Virginia Univ., Morgantown, WV; ²Advanced Imaging Ctr., HHMI

Janelia Res. Campus, Ashburn, VA; ³Sensory Neurosci. Res. Ctr., West Virginia Univ. Sch. of Med., Morgantown, WV; ⁴Res. Group Mol. Mechanisms of Synaptic Function, Max Planck Florida Inst., Jupiter, FL; ⁵Natl. Ctr. for Microscopy and Imaging Res., Univ. of California San Diego, La Jolla, CA; ⁶Sensory Neurosci., West Virginia Univ. Sch. Med., Morgantown, WV

Abstract: The calyx of Held (CH) is a giant nerve terminal that monoinnervates the medial nucleus of the trapezoid body (MNTB) within the brainstem and is critical for relaying interaural timing information for sound localization. This synapse exhibits hallmark developmental features of strengthening and pruning necessary to sculpt a precise neural circuit. Using serial block-face scanning electron microscopy (SBEM) on tissue collected from neonatal littermate mice (24-48 sampling), we previously found that around birth most principal cells host many small competing inputs (20+). Then, largely between postnatal (P) days 2 and 4, most inputs are pruned and selected terminals (2-3) grow at rates of 200 μ m² per day. Finally, a single input is strengthened into a calyx by P6. Importantly, this allows for the establishment of a large yet precise circuit ahead of the onset of hearing (P10). To date, imaging techniques have likely underestimated the actual growth dynamics of a developing neural circuit, owing to poor temporal resolution. Here, we examine the temporal dynamics of synaptic organization in the MNTB as it relates to the ultrastructure revealed with SBEM. We employed the lattice lightsheet (LLS) microscopy that offers fast acquisition with minimal bleaching. Acute coronal brainstem slices (300-600 µm thickness) were collected daily in neonates ranging from P0-14. Following 4D image acquisition, data was imported into syGlass, a custom software package designed in-house, for immersive virtual reality aided-analysis. Points were manually placed through time to track growth dynamics of calyces and their emanating processes including filopodia and growth cone tipped collateral branches extending as far as 75 µm. We found an age-dependent relationship with motility of these calyceal collaterals that form a fluid field around the host calyx. Growth cones, perhaps the most dynamic feature of this system, extend fastest during the growth of the calyx yet tend to slow as monoinnervation is established (P2: 65±11 μm/hr; P3: 288±20 μm/hr; P4: 176±33 μm/hr; P5: 90±7 μm/hr). Instantaneous velocities are unprecedented however for growth cones in any system (P2: 270±18 µm/hr; P3: 1318±101 μm/hr; P4: 1037±79 μm/hr; P5: 713±54 μm/hr). This is because movement of calyceal collaterals are stochastic where as much time is spent extending and retracting as pausing. These data are effective in monitoring the navigation patterns of assembling neural circuits in an intact system. Moreover, these observations reveal the dynamic nature of growth and retraction of developing neurites that may otherwise be missed without sufficient temporal resolution.

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Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 285.17/C21

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

Support: JSPS KAKENHI Grant Number 26430025

Title: Influence of thyroid hormone deficiency on mouse auditory cortex

Authors: *M. CHANG¹, H. D. KAWAI²

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Abstract: Auditory system requires thyroid hormone for normal development and function. Lack of thyroid hormone during development causes such symptoms of psychiatric disorders as auditory hallucination. Since auditory hallucination is thought to involve abnormal cortical connectivity, we suspected that hypothyroidism affected the distribution of neurons and laminar formation in the auditory cortex. Previous studies had suggested that the retrogradely labeled callosal projection neurons were mis-distributed within auditory cortex in the adult hypothyroid rats. Whether hypothyroidism affects the distribution of corticothalamic and subcortical projection neurons in primary auditory cortex (A1) is a remaining question. In this study, we investigated this using mice treated with methimazole, an antithyroid drug, starting at embryonic day 10 until the day of experiments up to postnatal day 60.

To identify corticothalamic and subcortical projection neurons using the immunofluorescence technique, antibodies against transcription factors Forkhead-box protein 2 (Foxp2) and COUP-TF interacting protein 2 (Ctip2) were used, respectively. Dendrites and dendritic spines of layer 5 neurons were also analyzed by the Golgi staining. We found that Foxp2-immunopositive cell density and distribution were not affected, whereas Ctip2-immunopositive cells were misdistributed without changing their cell density in layer 5 of hypothyroid mice. Further, we also found abnormal spine morphology in layer 5 pyramidal neurons in hypothyroid mice. In summary, hypothyroidism in mice induces misdistribution of subcortical projection neurons and abnormal spine morphology of pyramidal neurons in layer 5 of A1 during development and in the adult. These changes may underlie the symptoms of psychiatric disorders such as auditory hallucination.

Disclosures: M. Chang: None. H.D. Kawai: None.

285. Development of Visual, Auditory, and Somatosensory Circuits

Location: Halls A-C

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

Support: NIMH 1P50MH094271

NINDS 1R01NS080565

Title: Transcriptional profiling of the tonotopic critical period in mouse primary auditory cortex

Authors: B. KALISH¹, E. E. DIEL², M. E. GREENBERG¹, *T. K. HENSCH² ¹Dept. of Neurobio., Harvard Med. Sch., Boston, MA; ²Harvard Univ., Cambridge, MA

Abstract: Sensory experience drives neural circuit refinement during critical periods of heightened plasticity, but little is known about the genetic regulation of these developmental windows. The primary auditory cortex (A1) exhibits a critical period for thalamocortical connectivity between postnatal days P12-P15, during which continuous tone exposure alters the tonotopic topography of A1. We hypothesized that a coordinated, multicellular transcriptional program governs this window for patterning of the auditory cortex. To generate a robust multicellular map of gene expression, we performed droplet-based, nuclear single cell RNAsequencing (scRNA-seq) of A1 homogenates across three developmental time points spanning the tonotopic critical period (P10, P15, P20). We also tone-reared mice (7kHz pips) during the 3day critical period and collected A1 at P15 and P20. Using semi-supervised clustering and marker genes, we identified and profiled neuronal (glutamatergic and GABAergic) and nonneuronal (oligodendrocytes, microglia, astrocytes, and endothelial) cell types. This yielded cell type-specific and shared developmental gene programs. Next, we clustered genes based on their temporal expression kinetics and described ontological categories for these clusters. We were able to subgroup glutamatergic neurons into cortical layer-specific populations and GABAergic interneurons into known subtypes. This data is a rich resource for the identification of potential new tools for cell type-specific studies of A1. Furthermore, we sought to identify novel candidate genes that may execute auditory critical period plasticity. By comparing normally-reared and tone-reared mice, we found hundreds of genes in both glutamatergic and GABAergic cells with altered expression as a result of sensory manipulation in the critical period. Based on expression patterns, we identified genes as potentially positive or negative regulators of plasticity, and further validated cell-type specific expression of select genes using *in situ* hybridization. This paves the way for assessing therapeutic reopening of critical periods in A1 following removal of molecular 'brakes' on plasticity. To our knowledge, our study represents the first single cell transcriptomic analysis of the developing auditory cortex and is a powerful discovery platform with which to identify mediators of tonotopic plasticity.

Disclosures: B. Kalish: None. E.E. Diel: None. M.E. Greenberg: None. T.K. Hensch: None. Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

Location: Halls A-C

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

Support: NIH Grant DC008860

NIH Grant DC000023

Otonomy

Title: Cochlear purinergic receptors contribute to *In vivo* spontaneous activity in the developing auditory system

Authors: *T. A. BABOLA^{1,3}, A. GRIBIZIS⁴, A. P. LOMBROSO⁵, J. ISSA², S. LI³, B. LEE³, M. C. CRAIR⁴, D. E. BERGLES³

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Abstract: Spontaneous electrical activity is a prevalent feature of the developing nervous system and has been shown to influence the maturation and survival of neurons, as well as refinement of circuits in the brain. In the developing auditory system, bursts of activity originate in the cochlea and propagate through the brainstem and midbrain to the auditory cortex before hearing onset. Although spontaneous activity is initiated in the cochlea, the sequence of events that lead to burst firing of spiral ganglion neurons remains uncertain, in part, because there have been few mechanistic studies performed in vivo. To define the patterns of activity that occur in auditory circuits in the brain and determine the mechanisms responsible, we developed an in vivo method for imaging synchronized neuronal activity in unanesthetized mice before hearing onset. In neonatal prehearing mice, spontaneous activity in the inferior colliculus (IC) was imaged using the genetically encoded calcium indicator, GCaMP6s, expressed in neurons under the SNAP25 promoter. Widefield epifluorescence imaging revealed that groups of neurons located along the tonotopic axis exhibit periodic spontaneous increases in calcium at this age (P7-9). These events were bilateral, spatially restricted and oriented along the tonotopic axis, consistent with the excitation of adjacent hair cells in the cochlea by supporting cells. Bilateral cochlear ablation eliminated these bursts of activity, demonstrating that that they originate within the developing cochlea. Unilateral cochlear ablation revealed that although activity from one cochlea is bilaterally represented, there is a strong contralateral bias, consistent with previous in vivo

electrophysiological studies of sound evoked activity. When NBQX, an AMPA receptor antagonist, was applied to the round window membrane, activity in the contralateral IC was suppressed, mimicking unilateral cochlear ablation, indicating that permeation through the round window membrane is sufficient to enable pharmacological manipulation of the cochlea. To test the involvement of the metabotropic ATP receptor, P2ry1, which is highly expressed by inner supporting cells in the cochlea, in generating spontaneous activity, a selective P2ry1 antagonist (MRS2500) was applied to the round window membrane. Activity in the IC was dramatically reduced following acute P2ry1 antagonism, an effect that was mimicked in high frequency zones in P2ry1 knockout mice. These studies provide the first in vivo evidence that ATP release within the cochlea promotes burst firing in central auditory circuits.

Disclosures: T.A. Babola: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Otonomy. **A. Gribizis:** None. **A.P. Lombroso:** None. **J. Issa:** None. **S. Li:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Otonomy. **B. Lee:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Otonomy. **M.C. Crair:** None. **D.E. Bergles:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Otonomy. **M.C. Crair:** None. **D.E. Bergles:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Otonomy.

Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 285.20/C24

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

Support: NIH Intramural

Title: Investigating the perinatal expression of tyrosine hydroxylase in the mouse spiral ganglion

Authors: *T. SANDERS, M. W. KELLEY NIDCD, NIH, Bethesda, MD

Abstract: The afferent innervation to the cochlea is composed of the spiral ganglion neurons (SGNs), which transmit mechanosensory input from the hair cells centrally to the cochlear nucleus as an electrochemical signal. There are two populations of SGNs in the mammalian cochlea: Type 1 SGNs which constitute 90-95% of the total population, and form contacts with inner hair cells (IHCs); and Type 2 SGNS which constitute the remaining 5-10% of the total population and form contacts with outer hair cells (OHCs). Recently, Tyrosine Hydroxylase (TH), an enzyme involved in the synthesis of catecholamines, has been shown to be expressed exclusively by Type 2 SGNs predominantly in the apical region of the adult mouse cochlea. To further understand the development of this TH expressing population in the mouse we undertook an immunohistochemical study analyzing the proportion of TH+ SGNs in the basal,

mid and apical turns of the cochlea across a range of prenatal and early postnatal time points. TH protein expression was first detected in the spiral ganglion at embryonic day 16 (E16). At post natal day 0 (P0) an average of 3.9% of SGNs were TH+ in the basal turn, 8.9% in the mid turn and 7.2% in the apical turn (n=5). Interestingly, while the proportion of TH+ SGNs remained relatively stable until P5 in both the basal and apical turn, in the mid turn the proportion significantly decreased to 2% at P5 (P<0.05, one way ANOVA with posthoc Tukey's test, n=5). In all three turns the proportion of TH+ SGNs appears to decrease further at P7 (n=5). Consistent with this data we observed strong TH staining in the terminals surrounding the OHCs in the apical and mid turns of the cochlea beginning at P0. The amount of staining below hair cells in the mid turn appears to decrease at P5, whereas the apical staining remains consistent. However, at least some of this staining may be due to dopaminergic efferent terminals in the same region which also express TH. No staining was observed in the terminals surrounding the IHCs at any age.

Overall this work shows that TH, a newly described Type II SGN marker, is expressed more widely in the early postnatal cochlea than in the mature cochlea. Future studies will focus on determining the phenotype of the population which turn off TH expression, and the role TH may be playing in the development of these neurons.

Disclosures: T. Sanders: None. M.W. Kelley: None.

Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 285.21/C25

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

Title: Pharmacological reprogramming of lateral line progenitors to a multipotent state

Authors: M. KHALIL¹, *J. R. MEYERS²

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Abstract: The zebrafish lateral line is a series of mechanosensory organs termed neuromasts that detect the flow of water along the body; the homology between the cells and genes in the lateral line and those in the inner ear have made this an attractive system for studying development and regeneration of mechanosensory cells and organs. The neuromasts are deposited along the head and trunk by a migratory clusters of cells termed primordia comprised of mesenchymal, multipotent progenitors that organize into epithelial rosettes which are deposited from the back end of the primordium where they differentiate to form the sensory hair cells, non-sensory supporting cells, and the surrounding mantle cells. The migration of the primordium and deposition of neuromasts have been well studied and is known to be coordinated by Wnt at the leading, migratory edge, and FGF and Notch signaling in the trailing region where the
neuromasts organize and are deposited. We have utilized pharmacological alterations in these signaling cascades to alter the fates of the progenitor cells during migration and deposition and to attempt to revert the differentiating cells back to a progenitor-like state. Mimicking the signaling environment of the leading edge of the primordium by simultaneously activating Wnt signaling with the GSK3 β inhibitor 1-azakenpaullone and inhibiting FGF signaling with the FGFRTK inhibitor PD166866 after all of the neuromasts have been deposited leads to the supporting cells reverting to a migratory mesenchymal-like state. These cells retain progenitor-like properties as following washout of the drug cocktail, they re-epithelialize into neuromasts, though with improper positioning and numbers, and produce physiologically active and innervated hair cells within each neuromast. We also show that inhibition of MEK with PD0325901 is sufficient to induce a similar reprogramming of the supporting cells to a migratory mesenchymal state that can reform new neuromasts upon washout of the drug. Together these data demonstrate that lateral line supporting cells can be reverted to a migratory progenitor-like state that are capable of producing new sensory organs including new mechanosensory hair cells.

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Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

Location: Halls A-C

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

Support: G. Harold and Leila Y. Mathers Foundation

Title: Developmental features of primary sensory cortex and subcortical areas in the prosimian galago (Otolemur garnettii)

Authors: *E. C. TURNER, J. H. KAAS

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Abstract: The primate cerebral cortex undergoes striking changes during its development, but much is still unknown regarding how the primary sensory systems develop to reach their mature organization. We used a combination of immunohistochemical markers in the prosimian galago (Otolemur garnettii, n=8), aged P0 to P72, to follow the cortical and subcortical development of areas connected to primary sensory cortex (visual, somatosensory, and auditory). In addition to standard Nissl and cytochrome oxidase staining, we used vesicular glutamate transporter 2 (VGluT2) protein, which is primarily expressed in glutamatergic feedforward thalamocortical connections, and neuronal nuclei (NeuN) protein, which identifies all neurons, to characterize architectonic features. We also used calcium-binding proteins (CBPs) parvalbumin (PV) and calbindin (CB), as while the function of CBPs remains debated, they are known to be observed in

well-defined subpopulations of neurons. We find that all sensory thalamic nuclei, including the lateral geniculate nucleus (LGN), medial geniculate nucleus (MGN), and ventroposterior nucleus (VPN), show distinct subdivisions, related to the representation of their respective sensory inputs, that differ from expressions in the adult galago. For example, there is evidence for developmental changes in the connections of the magnocellular (M), parvocellular (P), and koniocellular (K) pathways to visual cortex; the P layers of the LGN are the only layers to express PV at P0, in contrast to full expression throughout the LGN layers in the adult. Similarly, VGluT2 expression at P0 in the LGN is evenly distributed across the M, P, and K layers, in contrast to the adult galago which has the strongest reactivity in the M layers, followed by the P layers, and most weakly in the K layers. In all primary sensory areas, layer IV is easily identifiable with PV reactivity, in contrast to the surrounding non-primary areas which show minimal reactivity across cortex. One exception to this is middle temporal visual area (MT), which contains strong neuropil and cell immunoreactivity for PV in layer IV at P0. These results, similar to those reported for MT in marmosets (Warner et al., 2012), suggest that area MT may serve as a primary-like area early during development. These architectonic maps of galago cortex can reveal more about the hierarchical developmental of cortical areas and the functional roles of these areas in early postnatal development.

Disclosures: E.C. Turner: None. J.H. Kaas: None.

Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

Location: Halls A-C

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

Support: State of Baden-Württemberg

DFG SFB 1134/A03

Title: Rapid and slow axon initial segment plasticity in rodent somatosensory cortex

Authors: N. JAMANN¹, M. KAISER², R. J. WAGENER³, J. MAURER², C. CORCELLI⁴, J. F. STAIGER⁵, C. SCHULTZ⁴, *M. ENGELHARDT⁶

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Abstract: The axon initial segment (AIS) is an electrogenic microdomain with potential impact on neuronal excitability in the context of activity-dependent cortical plasticity in adult networks. Its length and location (25-40µm in most cortical pyramidal neurons, usually at the proximal axon) and molecular infrastructure (clustering of voltage-gated ion channels) as well as its unique innervation pattern by axo-axonic GABAergic synapses make it a prime candidate for modulating neuronal input-output properties. Recent studies have highlighted its role in cellular mechanisms reminiscent of homeostatic plasticity, both under physiological and pathophysiological conditions. Structural AIS plasticity (changes in length or position) has been observed both *in vitro* and *in vivo* and is thought to fine-tune neuronal activity when networks are either activity-deprived or hyperexcited.

In a previous study we showed that in the rodent somatosensory cortex (barrel field), the AIS undergoes partially activity-dependent structural plasticity during development and also retains a level of plasticity in adult circuits after bilateral whisker-trimming in adult mice (postnatal day (P) 180). AIS length changes were observed within time frames of days to weeks. However, the question whether AIS plasticity also occurs rapidly after hours, e.g. as a response to sudden changes in network state, has yet to be addressed in vivo. Therefore, we set out to investigate whether rapid AIS plasticity, so far only described in vitro, also occurs in the mouse barrel cortex (S1BF). We employed confocal analysis of immunofluorescent AIS stainings as well as electrophysiological recordings in acute slices prepared from mice that were subjected to either sensory deprivation or stimulation (exposure to enriched environment, EE). Our data show that sensory deprivation via whisker trimming from birth until P15 results in slow, significant AIS lengthening in layer II/III of S1BF. Additionally the previously described shortening of AIS length after the onset of active whisking (P12) is delayed, but not completely abolished, by deprivation. Accordingly, our preliminary electrophysiological data indicate that the shift in firing patterns during normal S1BF development correlates with AIS shortening after P15 and that sensory deprivation causes increased excitability. Strikingly, stimulation of the barrel network via EE results in significant rapid shortening of the AIS within 3 hours by 10%. This effect could no longer be observed 6 hours after EE, suggesting that rapid AIS plasticity could serve as a fast modulator of neuronal activity in excited networks until a homeostatic balance is achieved.

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Poster

285. Development of Visual, Auditory, and Somatosensory Circuits

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

Support: MINECO Grant BFU2015-64432-R

ERC Grant CoG-647012

Title: Embryonic thalamic calcium waves predate cortical map formation

Authors: *N. ANTÓN-BOLAÑOS¹, H. GEZELIUS², L. PÉREZ-SAIZ¹, F. J. MARTINI¹, A. FILIPCHUK¹, A. ESPINOSA¹, A. SEMPERE-FERRÀNDEZ¹, J. P. LÓPEZ-ATALAYA¹, M. VALDEOLMILLOS¹, G. LÓPEZ-BENDITO¹

¹Inst. Neurociencias de Alicante UMH-CSIC, San Juan de Alicante, Spain; ²Sci. for Life Lab., Tomtebodavägen, Solna, Sweden

Abstract: Sensory systems are represented in the primary sensory areas of the brain organized in anatomical and functional maps. For instance, the whisker pad representation in the barrel field of S1 in rodents. Many intrinsic and extrinsic factors have been proposed to shape sensory maps during early development. Thalamic spontaneous calcium waves propagate from the thalamus to the cortex perinatally, before sensory maps are formed. Recently, we have shown their role in controlling sensory area size (Moreno-Juan et al., 2017), being a good candidate to influence cortical map formation as an extrinsic factor. Despite these recent evidences, the role of prenatal spontaneous subcortical activity in cortical map formation remains to be elucidated. To this end, we developed a mouse model in which thalamic neurons are hyperpolarized due to the overexpression of the inward rectifier potassium channel Kir2.1 (Th^{kir}). Th^{kir} mice show a disruption of prenatal thalamic spontaneous calcium waves and lack the somatosensory map representation in the cortex. Remarkably, although thalamocortical axons (TCAs) reach their cortical target in S1, they do not segregate into cortical barrels, thus losing their characteristic point-to-point refined organization. To unfold whether these topographical defects are controlled by gene expression changes, we performed genome-wide transcriptomic analysis in the somatosensory thalamus at different embryonic stages. We also tested to what extent the lack of a somatotopic map alter the function of the somatosensory system. In vivo extracellular recordings upon whisker stimulation in Th^{kir} mice show a clear reduction of the cortical responses in S1, which is in agreement with a decrease of whisker-specific cFos induction after novelty exposure in these mice. Hence, by generating a mouse model in which prenatal spontaneous calcium waves are suppressed in the somatosensory thalamus, we disrupt the formation and function of the barrel-field map in the somatosensory cortex. Our results suggest that cortical somatosensory map formation relies on the spontaneous calcium waves of the thalamus, giving a main role to the prenatal thalamic activity in cortical acquisition of sensory map representations.

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285. Development of Visual, Auditory, and Somatosensory Circuits

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

Support: NIH Grant HD081168

Title: Differences in state-dependent responses to sensory feedback between somatosensory and motor cortex in developing rats

Authors: *J. C. DOOLEY, M. S. BLUMBERG

Dept. of Psychological and Brain Sci., Univ. of Iowa, Iowa City, IA

Abstract: In the developing rat, myoclonic twitches occur in skeletal muscles across the entire body during active sleep (AS, or REM sleep). The sensory feedback from these twitches strongly drive neural activity throughout the sensorimotor system. Further, early in development, the motor function of primary motor cortex (M1) has not yet developed, and thus neural activity in M1 is primarily related to sensory input. We have recently shown that in the region within M1 that represents the forelimb, reafference from forelimb twitches results in substantially more neural activity than reafference from wake movements. This raised the following question: Is this state-dependent difference in the magnitude of reafferent responses unique to M1, or is it a more general feature across sensorimotor cortex. To answer this question, using 9- to 11-day-old rats, we recorded from neurons in the regions of M1 and primary somatosensory cortex (S1) that are responsive to forelimb movements. We then examined the neural activity in the period immediately after forelimb twitches and wake movements. Consistent with previous findings, we found that both S1 and M1 show strong responses to forelimb twitches. However, unlike M1, S1 shows equally strong responses to wake movements. This finding indicates that M1 and S1 process wake-related movements differently and highlights the unique ability of AS-related twitches to activate the entire somatomotor neuraxis. Combined with our previous work showing state-dependent gating of reafference in the medullary external cuneate nucleus, this result suggests a segregation of the sensory pathways providing reafference to S1 and M1 in the developing rat. Determining the mechanisms and pathways involved in this segregation is one focus of current work.

Disclosures: J.C. Dooley: None. M.S. Blumberg: None.

285. Development of Visual, Auditory, and Somatosensory Circuits

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 285.26/C30

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

Support: NSERC Grant RGPIN-2016-06518

Title: Distribution of P2X2 and P2X3 purinergic receptors in newborn and developing opossums, Monodelphis domestica

Authors: *A. BEAUVAIS, E. CORRIVEAU-PARENTEAU, J.-F. PFLIEGER Sci. biologiques, Univ. De Montréal, Montreal, QC, Canada

Abstract: We use the opossum Monodelphis domestica as a model of sensorimotor development given its immaturity at birth, comparable to a rat embryo of 13-14 days, allowing easier access to early developmental stages. Previous experiments using in vitro preparations of newborn opossums have shown that motor responses to mechanical stimulation of the face are affected by application of the non-specific purinergic receptor antagonists PPADS. Purinergic receptors are involved in a large number of functions in adult mammals and are expressed early in the central nervous system of rodents. Firstly, to identify if P2X2R and P2X3R purinergic receptors may be involved in sensorimotor responses in neonatal opossums, we have used immunofluorescence on fixed tissues to examine their distribution in the neuraxis and spinal ganglia from the day of birth (P0) to P13. P2X2R labeling was observed from P1 onwards and solely in some fibers at the periphery of the spinal cord, in the presumptive white matter under the pial surface and mostly in the dorsomedial part of the cord. Some of these fibers seemed to end by a growth cone. At older ages, labeled fibers increased in number and were observed more laterally and ventrally in the cord, still predominantly located at the periphery of the cord but an increasing proportion of them adopt a radiating orientation. In the head tissues, P2X2R labeling was not observed at P0 but was present at P5, P9 and P13 in the trigeminal ganglion and the brainstem. In the latter, labeled fibers were seen mostly at the periphery, but also centrally in the presumptive pons. No neuronal cell bodies appeared to be labeled anywhere in the central nervous system. P2X3R immunolabeling was noticed only from P5 to P13, being restricted to the skin covering the back of the lids and the cornea. These results suggest that both P2XR subtypes are expressed in neonatal opossums, particularly P2X2R. Pharmacological experiments should allow us to determine if they are involved in the control of the precocious sensorimotor behaviors in opossums.

Disclosures: A. Beauvais: None. E. Corriveau-Parenteau: None. J. Pflieger: None.

286. Monamines

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 286.01/C31

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NSERC grant

Title: Use of optogenetics to trigger and characterize somatodendritic dopamine release in the mouse mesencephalon

Authors: *B. DELIGNAT-LAVAUD, L.-E. TRUDEAU

Pharmacol. and Physiol. and Neurosciences GRSNC, Univ. De Montreal, Montréal, QC, Canada

Abstract: Most dopamine (DA) release in the brain occurs from axon terminals through a classical exocytotic mechanism. However, DA can also be released from the somatodendritic (STD) compartment of DA neurons. The molecular mechanisms of STD DA release are unclear. STD DA release has been previously studied in mouse brain sections by combining extracellular field stimulation with cyclic voltammetry or with patch-clamp recordings of D2-mediated currents. An important limitation of these approaches is that extracellular stimulation nonselectively depolarizes DA neurons as well as local terminals and cell bodies of GABA and glutamate neurons, in addition to axon terminals containing 5-HT, which can complicate analysis of cyclic voltammetry recordings. Here we further explored this unconventional form of transmitter release in mouse brain slices by combining optogenetics to selectively activate DA neuron cell bodies and cyclic voltammetry. For this purpose, a mouse line expressing the lightactivated channelrhodopsin (ChR2) was crossed with a DA-specific Cre driver mouse line (Ires-Dat-Cre) to selectively express ChR2 in DA neurons. We find that train pulses of blue light can effectively trigger an electrochemical response of small amplitude, which can be increased in magnitude by blocking the membrane DA transporter (DAT) and the D2 autoreceptor. The capacity of this releasable DA pool appears to be small, as repeated train pulses with an interval of 5-10 min lead to a large decrement of the response. We are presently exploring strategies to increase the size of the releasable STD DA pools.

Disclosures: B. Delignat-Lavaud: None. L. Trudeau: None.

286. Monamines

Location: Halls A-C

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Program#/Poster#: 286.02/C32

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH R01 DA035913

Title: Input-specific, dopaminergic modulation of synaptic gain in medial prefrontal cortex

Authors: *K. BURKE, K. J. BENDER UCSF, San Francisco, CA

Abstract: The prefrontal cortex (PFC) supports working memory and decision-making tasks by integrating excitatory and neuromodulatory inputs from across the brain. Performance on these tasks is dependent on the neuromodulator dopamine, as well as long-range excitatory input from several limbic, thalamic, and cortical regions. The dopamine D1 receptor family, including D1 and D5 receptors, has been previously shown to suppress glutamatergic transmission in PFC, but whether D1/D5 receptors differentially regulate these PFC inputs remains unclear. Here, we show that D1/D5 receptors preferentially suppress action potential-evoked calcium influx in a subset of excitatory long-range inputs to PFC. By analyzing trial-to-trial fluctuations in presynaptic calcium influx in axonal boutons, we show that dopamine likely alters voltagedependent recruitment of calcium channels, in turn lowering vesicular release probability. Interestingly, dopamine alters release probability at these synapses without altering short-term facilitation or depression. This allows dopamine to modulate synaptic gain, a process more commonly associated with changes in postsynaptic efficacy. Moreover, because dopamine acts presynaptically, this mechanism can alter the relative input strength of modulated and unmodulated inputs. Taken together, these findings suggest that D1/D5 receptors can bias information transfer in PFC through previously unidentified cellular mechanisms.

Disclosures: K. Burke: None. K.J. Bender: None.

Poster

286. Monamines

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 286.03/C33

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant DA038456

NIH Grant DA04523

Title: Localization and trafficking of dopamine D2 autoreceptors

Authors: *B. G. ROBINSON, J. R. BUNZOW, J. T. WILLIAMS Vollum Inst., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Dopamine D2 autoreceptors are G protein-coupled receptors (GPCRs) that regulate the excitability of dopamine neurons. In the midbrain, D2 receptors are activated by the somatodendritic release of dopamine from neighboring neurons. D2 receptor signaling and distribution was investigated in this study by combining electrophysiology with the imaging of tagged receptors. Surprisingly, upon agonist exposure, GFP-tagged D2 receptors on dopamine neurons in the substantia nigra pars compacta (SNc) were not internalized. D2 receptors were seen to internalize in other neuron types indicating a specific interaction within dopamine neurons. Additionally, tagged D2 receptors were found to be clustered on the somata and dendrites of dopamine neurons. Furthermore, specific dopamine release areas corresponding to individual D2 receptor clusters were identified using localized theta stimulation. These results are at odds with structural data that show the majority of D2 receptors reside at extrasynaptic locations. In this study, the distribution, lack of trafficking, and localized activation of receptor clusters indicate a purposeful placement within dopamine synapses of a subset of midbrain D2 autoreceptors.

Disclosures: B.G. Robinson: None. J.R. Bunzow: None. J.T. Williams: None.

Poster

286. Monamines

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 286.04/C34

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant DA004523

Title: Subcellular D2-receptor localization in the substantia nigra pars compacta of an EGFP transgenic mouse line

Authors: *S. R. SESACK¹, S. HETELEKIDES¹, M. TRINKLE¹, J. BALCITA-PEDICINO¹, B. G. ROBINSON², J. R. BUNZOW³, S. A. AICHER³, J. T. WILLIAMS² ¹Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; ²Vollum Inst., ³Physiol. & Pharmacol., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Dopamine (DA) neurons in the substantia nigra pars compacta (SNc) project to forebrain areas involved in goal-directed behavior and are subject to plasticity induced by psychostimulant addiction. SNc DA cells are extensively autoregulated by somatodendritic DA release acting on D2-receptors (D2Rs), although neither the exact mechanism for DA release nor the precise subcellular distribution of D2Rs have been fully characterized. Our electrophysiological evidence suggests that a vesicular-type DA release mechanism produces spontaneous inhibitory postsynaptic currents in SNc DA neurons in a manner that resembles classical synaptic transmission. We therefore hypothesized that D2Rs are distributed in clusters along restricted regions of the somatodendritic membrane. To investigate this, we utilized transgenic mice with an EGFP tag incorporated into the N-terminus of the D2R and light microscopic immunofluorescence labeling for EGFP, which provided further evidence for clustered D2Rs. We next sought to verify our observations by electron microscopic examination of silver-enhanced immunogold labeling for EGFP. In the SNc of the transgenic mice, EGFP-D2R labeling was found predominately in dendrites and less frequently in axon varicosities and glia. While most immunolabeling was observed in the cytoplasm, roughly 29% of EGFP-D2R gold particles contacted the plasma membrane. Gold particles were relatively uniformly distributed along mainly extrasynaptic regions, although the proportion of total labeling found on the membrane increased as dendritic diameter decreased (i.e. in a distal direction). Enrichment of EGFP-D2R gold to dendrodendritic synapses and dendritic spines was found only rarely. Axon varicosities expressing EGFP-D2R typically formed symmetric synapses likely to mediate inhibition. Surprisingly, a few putative axon initial segments with marked EGFP-D2R along the plasma membrane were also found. As most SNc neurons are dopaminergic, these axon initial segments likely derive from DA cells, marking the first observation of autoreceptors at this compartment in a monoamine cell population. The relatively uniform ultrastructural distribution of D2Rs along dendritic membranes is inconsistent with observations from light microscopy and electrophysiology, indicating that further experiments are needed to determine the precise distribution of functional D2Rs in the SNc. These findings have important implications for understanding the modulation of DA cell activity in health and addiction.

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Poster

286. Monamines

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 286.05/C35

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Brain Canada / Krembil Foundation

Title: Understanding the selective vulnerability of neurons in Parkinson's disease: An *In vitro* study of the relationship between axonal growth, bioenergetics, and survival of noradrenergic neurons of the locus coeruleus

Authors: *S. BURKE NANNI, M.-J. BOURQUE, L.-E. TRUDEAU Departments of Pharmacol. and Physiology, and Neuroscience, GRSNC, Univ. de Montréal, Montreal, QC, Canada

Abstract: In Parkinson's disease (PD) several restricted neuronal populations, including those of the substantia nigra pars compacta (SNc) and locus ceruleus (LC), gradually degenerate. It has emerged that mitochondrial dysfunction is likely central to the disease mechanism, and this has led to a hypothesis that one important common characteristic of vulnerable neurons is that they are particularly active from a bioenergetic point of view and thus more susceptible to any mitochondrial impairment. This high basal activity of mitochondria would in turn be associated with elevated production of reactive oxygen species and chronic oxidative stress. We have recently examined different dopaminergic neuronal sub-groups including those of the SNc, ventral tegmental area and olfactory bulb and provided evidence for a tight link between neuronal vulnerability, basal bioenergetics and the size of the axonal arbor of neurons, raising the hypothesis that elevated basal mitochondrial function in vulnerable neurons is in part secondary to the development and maintenance of a particularly large axonal arbor. In the objective of further testing the generality of this hypothesis, we have begun to examine noradrenergic neurons of the LC. We first isolated primary mouse LC neurons from neonatal TH-GFP transgenic mice. After 7 days in vitro and immunostaining for TH and MAP2, confocal image capture coupled with semi-automated tracing confirmed that LC noradrenergic neurons also develop a very large axonal arbor in vitro, with a size even larger than that of SNc dopamine neurons. Measurements of basal and maximal oxygen consumption rate performed with a Seahorse bioanalyzer revealed that the rate of oxidative phosphorylation in LC neurons is comparable to that of SNc neurons. We are now extending these experiments to examine the relationship between basal bioenergetics and firing. These data contribute to validating the hypothesis that the bioenergetic phenotype of neurons plays an important role in determining their vulnerability in PD.

Disclosures: S. Burke Nanni: None. M. Bourque: None. L. Trudeau: None.

Poster

286. Monamines

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 286.06/C36

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH grant AG043817

Title: Comparison of transitional vs surgical menopause on monoamine and amino acid levels in the brain

Authors: *T. LONG^{1,2}, J. K. YAO^{1,2}, J. LI¹, Z. KIRSHNER¹, D. NELSON¹, G. G. DOUGHERTY², R. B. GIBBS¹

¹Pharmaceut. Sci., Univ. of Pittsburgh, Pittsburgh, PA; ²Med. Res. Service, Veterans Affairs Pittsburgh Healthcare Syst., Pittsburgh, PA

Abstract: Loss of ovarian function has important effects on neurotransmitter production and release. To date, there has been little direct comparison of the effects between surgical and transitional menopause on neurotransmitter pathways in the brain. In this study, effects on monoamine and amino acid levels were evaluated in adult ovariectomized (OVX) rats and in rats that underwent selective and gradual ovarian follicle depletion by daily injection of 4vinylcyclohexene-diepoxide (VCD). Tissues from the hippocampus (HPC), frontal cortex (FCX), and striatum (STR) were dissected and analyzed at 1- and 6-week (w) following OVX or VCD treatments (n=6-8). Tissues from gonadally intact rats were collected at proestrus (n=8) and diestrus (n=8) periods to represent neurochemical levels during natural states of high and low estrogens. Seven different monoamines and their metabolites were quantified along with eight different amino acids. In the FCX of gonadally intact rats, higher levels of serotonin (5-HT) were detected at proestrus than at diestrus, and the ratio of 5-hydroxyindoleacetic acid (5-HIAA)/5HT in the FCX and HPC was lower at proestrus than at diestrus, suggesting an effect on 5-HT turnover in these regions. No other significant differences between proestrus and diestrus were observed. In OVX- and VCD-treated rats, substantial changes were observed in the HPC at 1-w following treatment. Specifically, levels of norepinephrine (NE), 5-HIAA and eight tested amino acids were significantly reduced in both menopausal models compared to proestrus and diestrus. The largest effects were the decreases (up to 80%) in the levels of the amino acids. By six weeks the levels of NE, 5-HIAA and all tested amino acids had returned to normal. In the FCX, dopamine (DA) levels were elevated at 6-w after OVX relative to diestrus rats. A similar trend was observed at 1-w (but not 6-w) following VCD treatment. In the STR, NE levels were elevated at 1-w following OVX, and HVA levels were elevated at 1-w following VCD treatment, relative to proestrus and diestrus. These effects were not observed at 6 weeks. Collectively, our results demonstrate that surgical and transitional loss of ovarian function have significant neurochemical effects on the brain, which are region-specific and time-dependent. In many instances OVX and VCD treatments produced similar effects. Most notable changes are the substantial decreases in amino acid levels in the HPC with corresponding effects on NE and 5-HT pathways. The effects likely contribute to the surgical and transitional menopause on hippocampal function and cognitive performance.

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286. Monamines

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Boettcher Young Investigator Award

NARSAD Young Investigator Award

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Title: Benzodiazepines and their dual administration with ethanol increase accumbal transient dopamine release events

Authors: *G. KRZYSTYNIAK¹, K. J. PULTORAK², S. SCHELP⁴, D. R. RAKOWSKI², E. B. OLESON³, Z. BRODNIK⁵, R. A. ESPAÑA⁶

¹Univ. of Colorado At Denver, Denver, CO; ³Neurobio. and Anat., ²Univ. of Colorado Denver, Denver, CO; ⁴Univ. of Colorado, Denver, Denver, CO; ⁵Univ. of Colorado at Denver, Denver, CO; ⁶Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: All drugs of abuse increase the concentration of dopamine in the nucleus accumbens. Benzodiazepines are anxyolitic drugs that are also susceptible to abuse. Benzodiazepines are allosteric modulators of the GABA_AR, and are known to alter dopamine transmission. Previous studies have demonstrated that benzodiazepines disinhibit dopamine neurons but also decrease the concentration of dopamine released in the nucleus accumbens. Here we sought to characterize how benzodiazepines alter dopamine transient release events in the awake and behaving rat. Using fast scan cyclic voltammetry we demonstrated that the benzodiazepines diazepam (trade name valium) and zolpidem (trade name ambien) dose-dependently increase the frequency and decrease the amplitude of dopamine transient release events in the nucleus accumbens core and shell in a dose dependent manner. These effects were occluded by the administration of flumazenil, a benzodiazepine antagonist. This study implies that an increase in frequency, indifferent of amplitude, may be adequate to provide abuse liability to a drug. Further research shows that ethanol administration interrupted by a benzodiazepine augments the frequency of dopamine transient release events yet drastically decreases the amplitude.

Disclosures: G. Krzystyniak: None. K.J. Pultorak: None. S. Schelp: None. D.R. Rakowski: None. E.B. Oleson: None. Z. Brodnik: None. R.A. España: None.

286. Monamines

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Topic: B.01. Neurotransmitters and Signaling Molecules

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Title: Noradrenaline in the monkey thalamus: Axonal innervation and adrenoceptors

Authors: I. PÉREZ-SANTOS^{1,2}, N. PALOMERO-GALLAGHER^{2,3}, *C. CAVADA¹, K. ZILLES^{2,3}

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Abstract: The role of noradrenaline (NA) in the thalamus is relevant because of its key position in cortico-thalamo-cortical pathways and in relaying information from subcortical structures to the cortex. We have mapped the thalamic NA innervation in adult macaque monkey brains using immunohistochemistry against the NA synthesizing enzyme, and the NA transporter. We also analyzed the distributions of Alpha1 and Alpha2 adrenoceptors using quantitative *in vitro* receptor autoradiography.

The maps of the two different NA markers are highly coincident. NA axons are present in all thalamic nuclei, albeit highly heterogeneously distributed. The most innervated ones are the midline nuclei. High NA axon densities are also present in some intralaminar nuclei (paracentral -Pcn- and parafascicular), and in the medial sector of the mediodorsal nucleus (MDm). The ventral motor nuclei receive a moderate NA innervation, with the ventromedial subdivision of the ventral anterior nucleus (VAvm) and area X holding the highest innervation among these nuclei. The lateral geniculate nucleus (GL) holds the lowest NA innervation. Densities and distribution of Alpha1 and Alpha2 receptors are also considerably heterogeneous throughout the thalamic nuclei, with Alpha1 densities being higher than Alpha2 densities. The nuclei with the highest Alpha1 densities are the MDm, the parvocellular part of the ventral posterior medial nucleus, medial pulvinal (PulM), and midline nuclei. The nucleus with the lowest Alpha1 consultion of alpha2 receptor densities are highest in the lateral dorsal, centromedian, PulM, inferior pulvinar, and midline nuclei.

Thus, location and densities of NA axons and Alpha receptors match only partially. Matching

distributions of NA axons and Alpha1 receptors are present in MDm, VAvm, area X and midline nuclei. However, PulM exhibits moderate NA innervation but comparatively high Alpha receptor densities. A reverse relationship is present in other nuclei; for instance, in Pcn there is dense NA innervation while Alpha receptors are scarce. These differences between NA axonal distribution and Alpha receptor densities suggest interesting local modulations of NA-based signal transmission caused by the receptors. The high NA axon innervation in nuclei with low Alpha receptor densities could point out to a more prominent role of Beta family receptors on noradrenergic signalling within these nuclei. And the presence of conspicuously high receptor densities in poorly/moderately innervated regions may reveal nuclei in which Alpha receptors considerably modulate NA-driven signal transmission.

Disclosures: I. Pérez-Santos: None. N. Palomero-gallagher: None. C. Cavada: None. K. Zilles: None.

Poster

286. Monamines

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 286.09/C39

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: A role for norepinephrine in modulating glutamatergic synaptic transmission during and following intermittent hypoxia

Authors: *M. A. KHUU, J. E. BARNARD, A. J. GARCIA, III Univ. of Chicago, Chicago, IL

Abstract: Several recent studies have proposed that the efficacy for neuromodulation may be state dependent. This ongoing study seeks to examine the interaction between adrenergic neuromodulation and acute intermittent hypoxia (AIH) on glutamatergic synaptic transmission and synaptic plasticity in area CA1 of the hippocampus. Electrophysiological recordings of the evoked field excitatory postsynaptic potential (fEPSP) were made in the CA1 neuronal population of adult mouse hippocampal brain slices. AIH was achieved by exposing preparations to three hypoxic intervals (95% N2, 5% CO2 duration=180 sec) separated by periods of recovery (95% O2, 5% CO2 duration=180 sec). Enhanced adrenergic tone (10 microM norepinephrine, NE) augmented the suppressive action of AIH on the evoked fEPSP. Interestingly, NE also suppressed paired pulse facilitation only after AIH. Yohimbine, an α 2 adrenergic receptor antagonist, mimicked the effects of NE following AIH, which implicates α 2 adrenergic receptor involvement. Our findings suggest that that increased adrenergic tone may play a protective role by reducing excessive glutamatergic synaptic transmission during and following intermittent hypoxia. These findings may ultimately be important to understanding the neurological aspects

associated in conditions, like sleep apnea, where oscillations in blood gases are accompanied with enhanced adrenergic tone.

Disclosures: M.A. Khuu: None. J.E. Barnard: None. A.J. Garcia: None.

Poster

286. Monamines

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant DA032895

Title: Role of nuclear membrane adrenergic receptors and organic cation transporter 3 in norepinephrine-induced stimulation of astrocyte BDNF expression

Authors: D. S. WHEELER, K. BENTON, D. CIBICH, E. DASSOW, P. FLETCHER, M. HERBST, B. KURTOGLU, A. TAYLOR, D. C. LOBNER, *P. J. GASSER Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: Deficits in neurotrophic and neuroprotective processes are believed to contribute to the detrimental effects of chronic stress and chronic elevations of glucocorticoids, and to the pathophysiology underlying major depressive disorder. Recent studies demonstrated that astrocytes respond to norepinephrine with increases in the synthesis and release of brain-derived neurotrophic factor (BDNF). Treatment of cultured astrocytes with the glucocorticoid corticosterone has the opposite effect on BDNF expression. We are examining potential cellular mechanisms by which glucocorticoids and norepinephrine may interact to regulate astrocyte BDNF expression. We have recently demonstrated that organic cation transporter 3 (OCT3), a corticosterone-sensitive monoamine transporter, is densely expressed in the outer nuclear membranes of astrocytes, and that beta1-adrenergic receptors are also localized to the nuclear envelope of these cells, suggesting that NE may activate G-protein-mediated signaling cascades in the nucleus, and that OCT3 gates the access of norepinephrine to nuclear membrane adrenergic receptors. We hypothesized that nuclear beta-1ARs are functionally coupled to stimulatory G-proteins in the nuclear compartment, and that norepinephrine-induced increases in BDNF expression are mediated by activation of nuclear cAMP signaling. To test this hypothesis, we examined A) the localization of Gs-related signal transduction machinery to the nuclear compartment, using immunofluorescence; and B) the effects of norepinephrine treatment on BDNF protein expression in astrocytes cultured from wild-type or OCT3-deficient mice. Gsalpha-like immunostaining was observed in both cytosolic and nuclear compartments in triton X-100-permeabilized cells, but only in the cytosolic compartment in cells permeabilized with digitonin. Phosphodiesterase 4B-like immunoreactivity was observed primarily in the nuclear

compartment in triton-100-permeabilized astrocytes, and nuclear PDE4B-like immunoreactivity was not observed in digitonin-permeabilized cells. Treatment of wild-type mouse astrocytes with norepinephrine resulted in dose-dependent increases in BDNF as measured by western blot. The same treatment had no effect on BDNF expression in OCT3-deficient astrocytes. Together, these data indicate that nuclear membrane beta-1 ARs couple to intranuclear signal transduction machinery; that access of norepinephrine to nuclear membrane receptors is mediated by OCT3, and that nuclear membrane beta-1 ARs mediate norepinephrine effects on BDNF expression.

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Poster

286. Monamines

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Program#/Poster#: 286.11/C41

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Z01MH002768

Title: Disruption of the axonal trafficking of tyrosine hydroxylase mRNA impairs catecholamine biosynthesis in the axons of sympathetic neurons

Authors: A. ASCHRAFI¹, *A. E. GIOIO², L. DONG³, B. B. KAPLAN⁴ ¹NIH/NIMH, Bethesda, MD; ²Lab. of Mol. Biol., NIH, NIMH-, Bethesda, MD; ³Genet. Engin. Core, Natl. Inst. of Health, Natl. Eye Inst., Bethesda, MD; ⁴NIMH, Bethesda, MD

Abstract: Tyrosine hydroxylase (TH) is the enzyme that catalyzes the rate-limiting step in the biosynthesis of the catecholamine neurotransmitters. In a previous communication, we provided evidence that TH mRNA is trafficked to the axon and that the mRNA is locally translated. In addition, we identified a 50bp sequence element in the 3'untranslated region (3'UTR) of TH mRNA that directs TH mRNA localization to distal axons. In the present study, the hypothesis was tested that local translation of TH plays a key role in the biosynthesis of the catecholamine neurotransmitters in the axon and/or presynaptic nerve terminal. Toward this end, a targeted deletion of the axonal transport sequence element was developed, using the lentiviral delivery of the CRISPR/Cas9 system, and two guide RNA sequences flanking the 50bp *cis-* regulatory element in rat superior cervical ganglion (SCG) sympathetic neurons. Deletion of the axonal transport element present in the 3'UTR of TH mRNA reduced TH mRNA levels in the distal axons and reduced the axonal proteins levels of TH and TH phosphorylation at SER40 in SCG neurons, as well as diminished the axonal levels and release of dopamine and norepinephrine. Conversely, the local translation of exogenous TH mRNA in the distal axon enhanced TH levels

and activity, as measured by Ser40 phosphorylation, and enhanced axonal norepinephrine levels. Taken together, our results provide evidence to support the hypothesis that TH mRNA trafficking and local synthesis of TH plays a key role in the synthesis of catecholamines, allowing for a rapid response to alterations in the need for neurotransmitter synthesis and release.

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Poster

286. Monamines

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: DA-000266

NIDA/IRP

Title: Absence of translin/trax, a microRNA degrading RNase complex, blocks cocaine's ability to increase dopamine tone

Authors: X. FU¹, A. SHAH¹, M. NIWA¹, D. FUKUDOME¹, A. SAWA¹, J.-L. CADET², J. KEIGHRON², G. TANDA², *J. M. BARABAN¹ ¹Johns Hopkins Univ., Baltimore, MD; ²NIDA, Baltimore, MD

Abstract: Recent studies indicate that the microRNA system plays a key role in regulating behavioral responses to drugs of abuse. However, its role in reward pathways is poorly understood. Accordingly, we have initiated studies aimed at understanding the role of the microRNA system in regulating dopamine (DA) signaling. While synthesis of nearly all microRNAs is mediated by a single enzyme, Dicer, multiple enzymes are involved in degrading microRNAs. Hence, selective deletion of individual microRNA degrading enzymes, such as the translin/trax RNase complex, leads to restricted increases in a subset of microRNAs and can be used to define the function of this subpopulation.

As the translin/trax (TN/TX) RNase complex is expressed by midbrain DA neurons, as well as D1R+ and D2R+ striatal neurons, we tested whether translin KO mice, display abnormal dopamine signaling. We found that these KO mice exhibit marked impairment in the ability of cocaine to enhance locomotor activity in an open field arena. This deficit does not reflect degeneration of DA neurons as TH immunostaining and striatal DA levels are normal in these mice. In addition, we confirmed that cocaine levels in forebrain and serum samples from WT and translin KO mice are not significantly different. Microdialysis studies demonstrated that the ability of cocaine to increase extracellular DA in the nucleus accumbens is blunted in translin KO mice in parallel with their reduced locomotor response to cocaine. In contrast, the ability of

amphetamine to elevate DA levels in the accumbens is unaffected. Preliminary fast scan voltammetry studies to monitor evoked DA release in the nucleus accumbens have not revealed any difference in DA max or clearance rates between translin KO mice and age-matched WT mice. However, the ability of cocaine to increase evoked release of DA in the nucleus accumbens is impaired in translin KO mice. Although this effect of cocaine is thought to underlie its rewarding properties and is mediated by its interaction with the dopamine transporter, how it enhances evoked DA release is poorly understood. Accordingly, further studies aimed at defining how translin deletion blocks this response may provide valuable clues to understanding the biological basis of cocaine's reinforcing properties.

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Poster

286. Monamines

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Leon Black Family Foundation

Title: Glutamate counteracts dopamine/PKA signaling via dephosphorylation of DARPP-32 Ser97 and alteration of its cytonuclear distribution

Authors: *A. NISHI¹, M. MATAMALES², V. MUSANTE³, E. VALJENT⁴, M. KUROIWA¹, Y. KITAHARA¹, H. REBHOLZ⁵, P. GREENGARD⁵, J.-A. GIRAULT², A. C. NAIRN³ ¹Dept. of Pharmacol., Kurume Univ. Sch. of Med., Kurume-Shi, Japan; ²Inserm UPMC Inst. du Fer A Moulin, Paris, France; ³Psychiatry, Yale Univ., New Haven, CT; ⁴Inserm U1191, CNRS UMR5302, IGF, UM, Montpellier, France; ⁵Lab. of Mol. and Cell. Neurosci., The Rockefeller Univ., New York, NY

Abstract: The interaction of glutamate and dopamine in the striatum is heavily dependent on signaling pathways that converge on the regulatory protein DARPP-32. The efficacy of dopamine/D1 receptor/PKA signaling is regulated by DARPP-32 phosphorylated at Thr34 (the PKA-site), a process that inhibits protein phosphatase 1 (PP1), and potentiates PKA action. Activation of dopamine/D1 receptor/PKA signaling also leads to dephosphorylation of DARPP-32 at Ser97 (the CK2-site), leading to localization of phospho-Thr34 DARPP-32 in the nucleus where it also inhibits PP1. In this study, the role of glutamate in the regulation of DARPP-32 phosphorylation at 4 major sites was further investigated. Experiments using striatal slices revealed that glutamate decreased the phosphorylation states of DARPP-32 at Ser97 as well as Thr34, Thr75 and Ser130 by activating NMDA or AMPA receptors in both direct and indirect pathway striatal neurons. The effect of glutamate to decrease Ser97 phosphorylation was mediated by activation of PP2A. In vitro phosphatase assays indicated that the PP2A/PR72 heterotrimer complex was likely responsible for glutamate/Ca²⁺-regulated dephosphorylation of DARPP-32 at Ser97. As a consequence of Ser97 dephosphorylation, glutamate induced the nuclear localization in cultured striatal neurons of dephospho-Thr34/dephospho-Ser97 DARPP-32. It also reduced PKA-dependent DARPP-32 signaling in slices and in vivo. Taken together, the results suggest that by inducing dephosphorylation of DARPP-32 at Ser97 and altering its cytonuclear distribution, glutamate may counteract dopamine/D1 receptor/PKA signaling at multiple cellular levels.

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Poster

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G12MD007601

Title: Role of Selenoprotein P in dopaminergic transmission and modulation by methamphetamine

Authors: *D. TORRES¹, C. CHAO², J. T. YORGASON⁴, S. KORUKAWA⁵, M. ANDRES³, S. C. STEFFENSEN⁴, F. P. BELLINGER²

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Abstract: Methamphetamine (METH) is a powerful psychostimulant drug with neurotoxic properties. METH increases dopamine (DA) signaling by inhibiting DA reuptake, resulting in increased oxidative stress and eventual degeneration of DAergic terminals. Selenium (Se) is an antioxidant trace element that is incorporated into selenoproteins, a family of proteins with multiple functions that include protection from oxidative stress. Se protects against METH toxicity and may also play a role in the DA system. Selenoprotein P (Sepp1) is vital for proper brain function as it is responsible for supplying neurons with Se, and may also play a role in DAergic transmission. Fast-scan cyclic voltammetry (FSCV) is an electrophysiological technique that can be used to measure DA release and reuptake kinetics. We used FSCV to measure DA release in nucleus accumbens (NAc) slices in order to investigate the role of Sepp1 in DAergic transmission and modulation by METH. Sepp1-KO mice had reduced basal DA reuptake rates compared to wild-type controls, suggesting reduced dopamine transporter (DAT) activity. Sepp1-KOs also had decreased levels of basal stimulated DA release. However, KOs displayed a dramatic increase in stimulated DA release in response to METH compared to wildtype mice. The DA receptor 2 (D2R) agonist quinpirole prevented the large METH-induced increase in stimulated DA release in Sepp1-KOs, while the D2R antagonist sulpiride dramatically increased METH response in wild-type slices but not in Sepp1-KO slices. These results suggest that D2R dysfunction in Sepp1-KO mice potentiates METH-induced DA release.

Disclosures: D. Torres: None. **C. Chao:** None. **J.T. Yorgason:** None. **S. Korukawa:** None. **M. Andres:** None. **S.C. Steffensen:** None. **F.P. Bellinger:** None.

Poster

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NSF grant EF1451125

Title: Activity of local inhibitory neurons gates locus coeruleus noradrenergic activity

Authors: *V. BRETON-PROVENCHER, M. SUR Picower Inst. for Learning and Memory, MIT, Cambridge, MA

Abstract: Locus coeruleus noradrenergic (LC-NE) neurons send broad neuromodulatory projections to the central nervous system. While these neurons are thought to be involved in a variety of functions, including arousal, attention, and cognitive shifts, the behavioral contexts and the underlying mechanisms that drive LC-NE neurons remain largely unknown. Here, we examined the microcircuit mechanism of LC-NE activity by investigating a population of local GABAergic neurons located in the ventromedial region of the LC (LC-GABA). We monitored pupil size as a behavioral marker of LC-NE activity, and performed cell type-specific optogenetic manipulations and electrophysiological recordings within the LC of awake headfixed mice. We found that activating LC-GABA neurons resulted in constriction of the pupil, similar to direct inactivation of LC-NE activity, suggesting a functional coupling between these cell types. In support of this hypothesis, simultaneous recordings using multi-channel extracellular probes revealed an ongoing interaction between putative LC-GABA and LC-NE activity, in which GABAergic activity followed the increase in NE associated with pupil dilation. Importantly, LC-GABA neurons may critically gate LC-NE function, as increasing their activity impaired responses of LC-NE neurons to novel sensory stimuli. We are currently investigating brain-wide inputs to LC-NE and LC-GABA neurons using monosynaptic rabies virus tracing to identify upstream brain regions that regulate this gating mechanism. Overall, these results demonstrate the existence of a local population of interneurons in the LC capable of rapidly controlling overall NE activity by modulating LC responses to unexpected sensory stimuli.

Disclosures: V. Breton-Provencher: None. M. Sur: None.

Poster

286. Monamines

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NINDS Grant 5R01NS062019

Title: PEDOT nanocomposites based electrodes for high sensitivity detection of tonic and phasic dopamine *In vivo*

Authors: *I. M. TAYLOR¹, X. CUI² ¹Bioengineering, ²Dept. of Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Dopamine (DA) signaling over multiple time courses is responsible for the regulation of a variety of vital life functions. Furthermore, dysfunction in dopaminergic signaling pathways has been implicated in the onset of various neurological disorders. When studying DA signaling pathways, it is absolutely necessary to monitor extracellular DA, as changes in the concentration and kinetics of DA release and uptake are highly correlated to physiological functionality. Fast

scan cyclic voltammetry (FSCV) has been performed at carbon fiber microelectrodes (CFEs) for in vivo DA detection for decades, but the technique is limited by issues resulting from moderate sensitivity, poor sensor longevity and the inability to measure resting DA concentrations. We have developed poly(3,4-ethylene dioxythiophene) (PEDOT) nanocomposites based electrode coatings capable of electrochemically measuring both sub second DA fluctuations as well as resting DA concentrations with high sensitivity and selectivity. PEDOT/graphene oxide coated CFEs exhibit an 880% increase in sub second, transient DA detection sensitivity using FSCV, decreased the lower limit of detection for DA by 50% and minimally altered electron transfer kinetics compared to bare CFEs. PEDOT/carbon nanotube coated CFEs exhibit a 4700% increase in sensitivity for the detection of resting DA levels using pulse voltammetric methods compared to bare CFEs, exhibited a sub-50 nM lower limit of detection for DA and are capable of stable continuous recording over multiple hours. Both PEDOT coated CFEs are capable of in vivo DA recording in the rat dorsal striatum. PEDOT/graphene oxide coated CFEs electrodes exhibit a 5.5X increase in measured current resulting from electrically stimulated in vivo DA release. PEDOT/carbon nanotube coated CFEs are capable of measuring the resting DA concentration in the rat dorsal striatum as well as pharmacologically induced tonic DA changes. Overall, PEDOT-based coatings significantly improve DA detection capabilities both in vitro and in vivo and is expected to considerably improve our understanding of the DA function signaling over multiple time courses.

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Poster

286. Monamines

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NSF EPSCoR RII-2 FEC OIA1632891

Title: High sensitivity electrochemical probes for detection of glutamate and dopamine in brain tissue

Authors: *T. A. MURRAY¹, C. TAN², M. HOSSAIN², P. DOUGHTY¹, C. PERNICI¹, J. SCOGGIN¹, S. SIDDIQUI¹, P. ARUMUGAM¹ ¹Biomed. Engin., ²IFM, Louisiana Tech. Univ., Ruston, LA

Abstract: Enhanced neurochemical microsensors were developed for brain slice recordings where higher sensitivity is required to detect small, dynamic changes in glutamate and dopamine levels. Dopamine sensors were created by electrophoretically depositing <100-nm thick multiwall carbon nanotubes (MWCNT) onto platinum microelectrode arrays and then drop

casting 0.2 µL of 5% wt. nafion solution. Glutamate sensors were created by drop casting a mixture of 0.1 U/µL glutamate oxidase, 1% bovine serum albumin and 0.125% glutaraldehyde followed by curing at room temperature for 72 h. A size-exclusion polymer, mphenylenediamine, was electrochemically deposited to the enzyme coated MEA to prevent ascorbic acid, an interferent, from reaching the electrode surface. Coronal slices from adult murine brain were maintained in artificial cerebral spinal fluid. Caudate putamen was used to test dopamine sensors and parietal cortex was used to test glutamate sensors. Current responses to biphasic stimulation, were recorded on a F.A.S.T. 16mkIII system (Quanteon, Kentucky). Responses (Fig. 1) were compared to previously acquired standard curves from 1-40 µM of glutamate and dopamine concentrations. The high randomness and open pores present in the three dimensional MWCNT film contributed to a significant increase in the electroactive area and adsorption sites for dopamine. In vitro calibration studies showed that with nafion coating, the MWCNT modified microelectrode had a 100-fold increase in DA sensitivity (20 nA/ μ M). The glutamate microsensor showed a sensitivity of 25 pA/ μ M, which is much higher than similar commercial probes (< 15 pA/ μ M) reported in the literature. Future work includes combining these microsensors into a single probe and further refinement of the coatings for chronic, in vivo recordings.



Figure 1. Responses to biphasic stimulation (platinum wires, 300 mA, 2Hz) of dopamine (left) and glutamate (right). Stimulation artifact is denoted by blue arrows. Black and blue traces are recorded from electrode 1 and 8, respectively, on modified CenMeT 8-TRK microelectrode arrays.

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286. Monamines

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Presidential Grant

Title: Enhancing fast-scan cyclic voltammetry detection of dopamine with tryptophan derivatives

Authors: *S. E. THOMPSON¹, E. RAMSSON²

¹Cell and Mol. Biol., ²Biomed. Sci., Grand Valley State Univ., Allendale, MI

Abstract: Fast-Scan Cyclic Voltammetry (FSCV) is an analytical tool which is used to detect sub-second changes in dopamine (DA) concentration in the striatum. In FSCV, repeated adsorption and oxidation of DA occurs on the carbon fiber microelectrode surface. Using background subtraction, the current produced can be used to create a calibration curve for [DA] that allows correlation with in vivo measures. We are exploring the electrodeposition of various coatings as a method for improving the detection limit for this technique. The goal of these coatings is to increase the amount of DA adsorbed onto the electrode surface, thus increasing the oxidation current, which is proportional to the concentration of DA. The goal of this project is to investigate different tryptophan derivatives plated onto the surface of the electrodes for their ability to improve FSCV sensitivity for DA. The coated electrodes must not only be investigated for their ability to improve the detection limit of DA, but their selectivity and durability must be investigated. Coated electrodes will be tested in vitro in the presence of other neurotransmitters which are present in the striatum. The durability of the coatings on the electrodes will be tested through extended FSCV cycling and collection periods. Improving the limit of detection of FSCV for DA will help with monitoring low levels of DA which are present in patients with Parkinson's and Alzheimer's. We hope with this project, to provide a simpler, a cost-effective and time-efficient means of increasing the FSCV sensitivity for DA to aid in the research of these diseases.

Disclosures: S.E. Thompson: None. E. Ramsson: None.

286. Monamines

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Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Investigation of the basal dopamine levels in the zebrafish telencephalon using fast-scan controlled adsorptive voltammetry

Authors: K. BAUSTERT¹, *E. RAMSSON²

¹Dept. of Chem., ²Biomed. Sci., Grand Valley State Univ., Allendale, MI

Abstract: Zebrafish are widely used organisms for a variety of different studies in neuroscience. The neurotransmitter dopamine (DA) is a molecule important in learning and memory, motivation, and movement in higher vertebrates. Quantification of DA in the zebrafish brain has recently taken place via amperommetry and fast scan cyclic voltammetry (FSCV). FSCV is a technique that uses a triangle waveform with background subtraction to measure DA oxidation current as a proportional measure to DA concentration. This can be used to quantify small changes in neurotransmitter concentration but is limited to acquiring only concentration changes, and not basal DA levels, due to this subtraction. Fast scan controlled-absorptive voltammetry (FSCAV) allows for inquiries into basal DA levels and changes to those levels. Since little is known about the basal DA concentrations present within the zebrafish telencephalon, we used FSCAV to investigate these levels.

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Poster

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Tubitak project # 113S083

Ataturk University BAP # 2008/126

Ataturk University BAP # 2015/038

Title: Escitalopram and serotonin reuptake parameters in animal model of chronic pain: an *In vivo* voltammetric study

Authors: *H. AHMET¹, U. OKKAY, 25240², K. NALCI², B. TOGAR³, N. TASPINAR², I. AYDIN²

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Abstract: We investigated the character of chemotherapy-evoked neuropathic pain using rats treated with paclitaxel. The novel method of in vivo voltammetry was utilized to determine if changes in extracellular seratonin levels and release in rats occur after chronic paclitaxel therapy. Peripheral neuropathy is the dose-limiting side effect of paclitaxel. The cause of the pain is not known. This study investigated whether changes in seratonin homeostasis occur after paclitaxel treatment in specific brain areas.

Materials:

Analgesiometer (Randal- Selitto) was used to measure the mechanical allodynia. 2 milimolar seratonin was given to the areas and uptake activities were recorded with in vivo voltammetry. 270-310 g rats are important for in vivo voltammetry studies for true brain stereotaxic coordinates.

Coordinates	X	Y	Z
CA3 (Cornus Ammonis)	3,4	-4	-3,8

Results:

Paclitaxel-evoked pain was detected by analgesiometer test on 7th day and it continued to 50th day. Pain tests were done on 0, 8, 15, 21, 30, 50 th days.

n Control Mean±SD Paclitaxel Mean±SD Paclitaxel+Escitalopram Mean±SD

0.day	5 125,00±0,00	124,00±2,24	116,00±12,45*
8. day	5 114,00±11,40	63,00±40,40*	59,00±12,94*
15. day	5 109,00±5,48	70,00±33,35	41,00±11,94*
21.day	5 108,00±9,75	65,00±55,11	43,00±9,083*
30.day	5 115,00±9,35	26,00±52,72*	27,00±20,80*
50.day	5 115,00±10,61	14,00±19,49*	15,00±18,71*

* p<0,05 according to the control group, # p<0,05 according to paclitaxel group Serotonin reuptake time(t_{80}) from synaptic cleft were decreased in paclitaxel group when compared to control group. And escitalopram turned this decrease back and even longer than control group.

Conclusion:

This study showed that serotonin uptake rate was increased in CA3 region in the brain in paclitaxel -treated animals. And we can use escitalopram to ameliorate of increased serotonin reuptake times. Also we observed clinical improvement in escitalopram group. Support:

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Chinese Scholarship Council (XZ)

Title: Isoflurane anesthesia alters monoamine levels in prefrontal cortex (PFC) of C57BL/6J (B6) mouse

Authors: *X. ZHANG¹, A. BAER¹, N. R. HARP¹, H. A. BAGHDOYAN², R. LYDIC² ¹Anesthesiol., Univ. of Tennessee Med. Ctr., Knoxville, TN; ²Anesthesiol. & Psychology, Univ. of Tennessee, Knoxville, TN

Abstract: Previous studies quantifying isoflurane-induced changes in brain chemistry typically have been limited to measures of a single neurotransmitter (*J Neurochem* 119: 419, 2011).

During anesthesia, however, levels of multiple, interacting neurotransmitters change simultaneously (Sleep 35: 1325, 2012). This ongoing study is quantifying several neurotransmitters in brain samples obtained by in vivo microdialysis. Dialysis probes were aimed for the PFC (histology pending) and samples (25 microliters) were collected during wakefulness (n=9 mice) and isoflurane (1.3%) anesthesia (n=12 mice). Dialysis samples were derivatized, separated with LC/MS and quantified using the AnalystTM data system (Applied Biosystem, version 1.4.2). A between-groups design enabled evaluation of state-dependent increases (+) and decreases (-) in levels of histamine (HA), dopamine (DA), serotonin (5-HT), and norepinephrine (NE). Compared to levels measured during wakefulness, percent change values during anesthesia were: HA = -30.8%, DA = +39.6%, 5-HT = +7.4%, and NE = +41.6%. Independent t-test comparisons revealed that these isoflurane-induced changes were statistically significant for HA (t=2.2, df=16, p=0.03), DA (t=1.91, df=12, p=0.04), and NE (t=1.95, df=17; p=0.03). We are unaware of previously reported measures of monoamines from PFC of B6 mice during isoflurane anesthesia. Many studies have shown that monoamines promote cortical activation and behavioral arousal. Microdialysis data indicate that stimulants increase levels of HA (Eur J Pharm 558:96, 2007), and 5-HT, NE, and DA (Neuropsychopharmacology 27:699, 2002) in PFC of rat. The present results encourage efforts to determine the extent to which increased levels of monoamines resulted from isoflurane-induced changes in monoamine synthesis, degradation, and/or reuptake. Combining metabolomic data (http://www.abstractsonline.com/pp8/index.html#!/4071/presentation/6411) with measures of neurotransmitters offers unique opportunities to increase understanding of the mechanisms through which volatile anesthetics alter brain chemistry. Together, these analytic approaches may provide new insights into mechanisms of anesthetic action.

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Poster

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Title: Structure-activity relationships of N,N-diallyltryptamine hallucinogens

Authors: *L. M. KLEIN¹, M. A. GEYER², S. D. BRANDT³, A. L. HALBERSTADT² ¹Neurosciences, UCSD, San Diego, CA; ²Psychiatry, UCSD, La Jolla, CA; ³Pharm. and Biomolecular Sci., Liverpool John Moores Univ., Liverpool, United Kingdom

Abstract: N,N-Dialkyltryptamines such as psilocybin and N,N-dimethyltryptamine (DMT) are a potent class of serotonergic hallucinogens. Recently, designer hallucinogens derived from N,Ndiallyltryptamine (DALT) have been marketed online as New Psychoactive Substances, resulting in numerous cases of acute toxicity. Tryptamine hallucinogens act primarily as 5-HT_{1A} and 5-HT_{2A} receptor agonists, but it is not clear whether DALT derivatives have a similar mechanismof-action. To investigate the pharmacology and structure-activity relationships of these compounds, we assessed the binding affinities of DALT and 11 ring-substituted derivatives at 44 receptor and transporter sites. A subset of the compounds were also assessed for induction of the head twitch response, a 5-HT_{2A} receptor-mediated behavioral response in rodents that is often used as a proxy for human hallucinogen effects. Binding assays confirmed that DALT and its ring-substituted derivatives bound non-selectively to most 5-HT receptors, including 5-HT_{1A} and 5-HT_{2A}. DALT significantly increased HTR counts in male C57BL/6J mice relative to vehicle $(ED_{50} = 3.42 \text{ mg/kg IP})$. The 4-hydroxy and 5-methoxy derivatives induced the HTR with almost twice the potency of DALT; 4-acetoxy- or 5-fluoro-substitution produced even greater increases in potency. By contrast, 5-bromo substitution did not significantly alter HTR potency relative to DALT. 2-Methyl or 2-phenyl substitution abolished HTR activity, as did 7-ethyl substitution. An ordinary least-squares (OLS) regression revealed that 5-HT_{1A} and 5-HT_{2A} binding affinities significantly predicted HTR potency (R^2 =0.904, $R^2_{adjusted}$ =0.840). These results confirmed that 5-HT_{2A} and 5-HT_{1A} receptors made positive and negative contributions, respectively, to HTR potency. To our knowledge, this is the first quantification of the relative contributions of 5-HT_{2A} and 5-HT_{1A} receptors to the behavioral effects of tryptamine hallucinogens. These findings improve our understanding of how tryptamine hallucinogens produce their effects, and allow for better prediction of the abuse potential of novel derivatives of DALT.

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Poster

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HKU Strategic Research Theme

Title: Serotonin restricts synaptic plasticity and decreases excitability of vestibular circuits via distinct receptors

Authors: *Y.-S. CHAN¹, L. HAN¹, Y. H. LI², K. L. K. WU¹, D. K.-Y. SHUM¹ ¹Sch. of Biomedic. Sci., Fac. Med., Univ. Hong Kong, Hong Kong, China; ²Sch. of Life Sci. and Technology, Xi'an Jiaotong Univ., Xi'an, China

Abstract: The medial vestibular nucleus (MVN) is a major relay station for postural coordination, eye movement control and spatial cognition. Microinjection of 5-HT to the MVN impaired performance of vestibular-mediated motor balance and negative geotaxis in both adult and juvenile (P13-16) rats. We hypothesize that serotonergic neurotransmission modulates the activity of neurons in the MVN circuitry for vestibular-related behaviors. Specific activation of 5-HT₇ receptors in the MVN restricted circuit plasticity by abolishing NMDAR-mediated LTD via a protein kinase A (PKA)-dependent postsynaptic mechanism. Activation of 5-HT_{1A} receptor attenuated the excitability of vestibular circuits, as evidenced by reduction of both evoked excitatory and inhibitory transmission (eEPSC and eIPSC), through a presynaptic mechanism. These results reveal that 5-HT_{1A} and 5-HT₇ receptors within the MVN exhibit differential modulatory mechanisms but these in concert accomplish synergistic influence on motor coordination.

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Title: Electrophysiological properties of target-specific populations of rat dorsal raphe projection neurons

Authors: *E. W. PROUTY¹, W.-J. GAO¹, B. D. WATERHOUSE²

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Abstract: Serotonin (5-HT)-containing neurons in the dorsal raphe (DR) nucleus project throughout the mammalian forebrain and are implicated in a host of physiological processes and neuropsychiatric disorders. These neurons have been characterized in terms of their electrophysiology and show differences in presynaptic drive, passive membrane properties, and excitability, but studies to determine if these attributes align with specific brain functions or specific forebrain terminal fields have only recently begun. The goal of this study was to link DR neurons to a specific functional role in sensory versus cognitive circuit operations by characterizing cells according to both their efferent connectivity and electrophysiological properties; specifically, comparing cells projecting to the medial prefrontal cortex (mPFC), a region implicated in decision-making and emotion with cells projecting to the lateral geniculate nucleus (LGN) of the thalamus, a subcortical relay for visual information. We have previously shown that over 90% of the DR neurons projecting to these regions are serotonergic. After injecting the retrograde tracer FluoroGold (FG) into the mPFC or LGN of Sprague-Dawley rats, brainstem sections from these animals were collected and whole-cell patch clamp recordings were made from FG-labeled neurons within the DR. Initial results show that these two populations of projection neurons have similar passive membrane properties; however, cells targeting the mPFC respond to depolarizing current steps with a higher firing frequency than LGN-projecting cells. This greater excitability of mPFC vs LGN-projecting cells may suggest that greater stimulus-driven 5-HT release is required to optimize the function of cognitive and limbic circuits within the mPFC than for early stage visual processing in the LGN. Interestingly, LGN-projecting neurons display a greater frequency of putative AMPA-mediated spontaneous postsynaptic currents (t = 3.05, p = 0.0081), an indication that these cells receive greater presynaptic excitatory drive than those projecting to mPFC. Such target-specific differences in the synaptic regulation and excitability of DR projection neurons provide insight into the functional organization of the DR 5-HT system and indicate that this type of characterization may be a valuable strategy for untangling the diverse psychological and behavioral roles of 5-HT in the forebrain. Ongoing studies will determine if the electrophysiological profile of these DR projection neurons changes from late adolescence to adulthood. (NIMH MH101178 to BDW)

Disclosures: E.W. Prouty: None. W. Gao: None. B.D. Waterhouse: None.

Poster

286. Monamines

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 286.25/C55

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIDA R01-DA16736 (MPG)

Department of Veteran Affairs grant RX000458

Fund for Anesthesiology Research (MPG, FG)

Title: Neurochemical profile in mice genetically depleted of brain serotonin: A proton magnetic resonance spectroscopy study

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Abstract: Introduction: Serotonin (5HT) neurons originating in the dorsal raphe innervate nearly all areas of the brain. 5HT is involved in mediating neuronal development as well as behavioral and physiological processes including, sleep, aggression, aging, control of food intake and body weight. In addition, the dysfunction in the 5HT neuronal system has been linked to neuropsychiatric conditions such as depression, anxiety, obsessive compulsive disorder and suicide. Serotonin selective reuptake inhibitors (SSRIs) are the most common treatment for depression and their therapeutic effect is generally attributed to their ability to increase the synaptic levels of 5HT. Tryptophan hydroxylase-2 (TPH2), expressed selectively in 5HT neurons, is the initial and rate limiting enzyme in the biosynthesis of 5HT. Genetic ablation of the TPH2 gene provides a hypo-serotonergic animal model to examine the influence of 5HT innervation on development and behavior. TPH2^{-/-} mice show intense compulsivity and impulsivity, social communication deficits, exaggerated aggression and decreased levels of anxiety like behavior. 5HT neurons in these mice, although lacking 5HT, retain their characteristic electrophysiological properties as well as relatively normal brain development and elaboration of the 5HT neuronal system. This suggests that behavioral phenotypes of the TPH2^{-/-} may be mediated via the absence of a modulatory effect of 5HT on other neurotransmitter systems such as Glutamate (GLU) and GABA. Methods: TPH2^{-/-} mice were generated by deleting exon1 of the Tph2 gene and were on a mixed C57BL/6-Sv129 background. High resolution magic angle spinning (HR-MAS) proton magnetic resonance spectroscopy (¹H-MRS) ex vivo (translation potential to clinical MRS) was used to assess unbiased neurochemical profiles in 12 different brain regions of 8 week-old WT (n=8) and KO (n=12) mice. Results: MRS analysis showed significant decrease in GLU and GABA levels in Hippocampus (dentate gyrus, HP), significant increase in Glu and GABA levels in Cerebellum (CB), no change in GLU and GABA levels in Anterior Striatum (AST). Discussion: Animal and clinical studies suggested, although with conflicting findings, that a deficit in GABAergic activity may be crucial in the pathophysiology of mood disorders. The 5HT innervation in ACC, CB and HC is substantial and 5HT release has modulatory effects on the release of the GLU and GABA neurotransmitters. Changed concentrations of GLU and GABA may reflect a developmental

insult related to 5HT deficits in the critical period or a compensatory effect both associated with the unique behavioral phenotypes of the TPH^{-/-}mouse.

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Poster

286. Monamines

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 286.26/C56

Topic: B.07. Synaptic Transmission

Support: NIH2RO1-AG-12418

Title: Serotonin-7 receptors in the hippocampus vary with time of day but not aging

Authors: *M. J. DUNCAN, K. M. FRANKLIN, J. T. SMITH Dept Anat/Neurobiol, Univ. of Kentucky Dept. Neurosci., Lexington, KY

Abstract: Activation of serotonin-7 (5-HT₇) receptors modulates circadian rhythms, memory, REM sleep, and depression, processes which are deleteriously affected by aging. Endogenous regulation of these receptors is not well understood, although pharmacological regulation has been reported. Chronic treatment with selective serotonin re-uptake inhibitors known to increase extracellular serotonin levels leads to down-regulation of 5-HT7 receptors. Because endogenous serotonin release exhibits a daily rhythm with higher levels at night, we hypothesized that 5-HT₇ receptors exhibit 24-h variations characterized by lower nighttime expression. Our previous studies of Syrian hamsters showed that aging decreases 5-HT₇ receptors in the dorsal raphe nucleus, a brain region in which these receptors affect circadian rhythms and REM sleep, but not in the several other circadian substrates, such as the suprachiasmatic nucleus. Here we tested whether aging reduces 5-HT7 receptors in the hippocampus, a likely substrate for the effects of 5-HT₇ receptor drugs on memory and depression. Male Syrian hamsters (young, 3-5 months; old, 17-21 months) exposed to a daily alternating cycle of 14 h light:10 h dark were euthanized at 4 times of day (zeitgeber times [ZT]1, 6, 13, & 19; ZT12 = time of lights:off; N=8-13/tim/age). Coronal sections through the hippocampus were processed for 5-HT₇ receptor autoradiography using [³H]8-OH-DPAT [2 nM] as the radioligand and SB-269970 [1 µM] to define nonspecific binding. Tissue sections and radioactive standards were apposed to X-ray films to generate autoradiograms that were assessed by computer-assisted microdensitometry. Robust specific 5-HT7 receptor binding was observed in the hippocampal dentate gyrus (DG), CA1, and CA2 but not in CA3. In the DG and CA1, specific 5-HT7 receptor binding sites exhibited 24-h rhythms with troughs at night (P<0.005; P<0.05, respectively), in support of the hypothesis. Specific 5-HT₇ receptor binding in the CA1 and DG were not significantly affected by age or by

interactions between time and age. In conclusion, these data indicate that 5-HT7 receptors in the hippocampus are influenced by time of day but not by aging. Furthermore, these findings suggest that the therapeutic effectiveness of 5-HT₇ drugs may persist in old age but will depend on the daily time of administration.

Disclosures: M.J. Duncan: None. K.M. Franklin: None. J.T. Smith: None.

Poster

286. Monamines

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 286.27/C57

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Towards a zebrafish serotonin receptor pharmacology

Authors: *H. SCHNEIDER¹, P. SURESH², S. GERONGAY², D. HUYNH² ¹Dept. of Biol., Depauw Univ., Greencastle, IN; ²DePauw Univ., Greencastle, IN

Abstract: Serotonin (5-hydroxytryptamine; 5-HT) is a neurotransmitter in the brain of vertebrate animals and humans. 5-HT is involved in the regulation neurological states such as mood, pain, and anxiety and has been linked to drug dependency. Zebrafish (*Danio rerio*) could represent a tool for the pre-clinical development of pharmacotherapies of human disorders with a link to the serotonin system. We identified 23 serotonin receptor (htr) genes in the zebrafish genome. Every human htr gene appears to have an ortholog in zebrafish, except for three htr3 receptor types. Because of a teleost genome duplication event several but not all human serotonin receptor genes have duplicates in the zebrafish genome. For a pharmacological characterization of zebrafish serotonin receptors, we have cloned and sequenced htr2cl1, htr2aa, htr2ab and htr7c entire coding regions. At the protein level, zebrafish amino acid sequences are about 70% identical to human serotonin receptors. Expression of serotonin receptors in HEK293 cells enables the isolation of htr proteins for binding assays which are being carried out to determine the degree of similarity between zebrafish and human serotonin receptor pharmacology.

Disclosures: H. Schneider: None. P. Suresh: None. S. Gerongay: None. D. Huynh: None.

Poster

286. Monamines

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 286.28/C58

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Functional interplay between early growth response protein (EGR) and specificity protein (Sp) transcription factors is critical for activation of the human tryptophan hydroxylase 2 (TPH2) promoter in RN46A cells

Authors: H. KANEKO¹, Y. NAWA¹, M. TSUBONOYA¹, T. HIROI¹, R. TAKAHASHI², *H. MATSUI³

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Abstract: Tryptophan hydroxylase 2 (TPH2) is the key enzyme in the synthesis of neuronal serotonin. Although previous studies suggest the NRSF-mediated negative regulation of the human TPH2 (hTPH2) gene, the mechanisms by which the hTPH2 gene expression is activated remains unresolved. An in silico analysis, 5'-flanking region revealed the presence of two consecutive GC-rich motifs (GCB1, -207/-199 and GCB2, -182/-174), within which the putative transcription factor binding sites, including early growth response proteins (EGRs) and specificity proteins (Sps), were found. A 2-kb region of the hTPH2 gene (-1850/+141) was cloned into pGL4-Basic and its 5'-untranslated region (+10/+121; a region containing repression elements including NRSE) was deleted to yield TPH2-100. Furthermore, the 48-bp segment (-124/-77) adjacent to GCBs was deleted from TPH2-100 to prepare TPH2-131. Quantitative realtime RT-PCR analysis revealed the expression of Egr-1, Egr-2, Egr-3, Egr-4, Sp1, Sp3, Sp4, Nab1 and Nab2 genes in rat raphe RN46A cells. Reporter assays showed that the promoter activity of hTPH2-131 was about 2-times that of hTPH2-100, suggesting that the 48-bp downstream segment contains a not-yet-identified inhibitory element. Interestingly, this 48-bp segment is well conserved between humans and rodents. Overexpression of EGRs (EGR1, 2, 3 and 4) and/or Sps (Sp1, Sp3 and Sp4), either alone or in combination, only caused marginal effects on the hTPH2-100 promoter activity. Whereas overexpression of EGRs or Sps alone exhibited only a marginal effect on the hTPH2-131 promoter activity, when both were coexpressed, the joint effect was additive. These results indicate that EGRs and Sps apparently bind to the GCBs in a noncompetitive manner and act together to increase the hTPH2-131 promoter activity. Moreover, these results suggest that the 48-bp segment has an inhibitory effect on the joint function of EGRs and Sps via the GCBs. To analyze these GCBs further, mutant reporters were prepared and co-expression studies were performed using EGR4 and Sp3 expression vectors. Although mutation of either the GCB1 or the GCB2 reduced the hTPH2-131 promoter activation by EGR4 and Sp3, the GCB2 mutation produced a greater reduction compared to that of the GCB1 mutation. Double mutations of the GCB1 and GCB2 brought a further reduction. These results indicate that the GCB2 is critical for the hTPH2-131 promoter activation by EGR4 and Sp3, and the GCB1 might play an accessary role. Collectively, these results imply that the hTPH2 promoter activation by EGRs and Sps is dependent on the GCBs and is subjected to the inhibitory modulation mediated by the unique downstream 48-bp segment.

Disclosures: H. Kaneko: None. Y. Nawa: None. M. Tsubonoya: None. T. Hiroi: None. R. Takahashi: None. H. Matsui: None.
286. Monamines

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 286.29/C59

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Fletcher Jones Endowment

Title: Regulation of tetrahydrobiopterin (BH4), a required cofactor for serotonin & dopamine synthesis, in the nematode *C. elegans*

Authors: *C. M. LOER

Univ. of San Diego, San Diego, CA

Abstract: The rate-limiting synthetic enzymes for serotonin and dopamine synthesis, for phenylalanine catabolism, for ether lipid metabolism, and the nitric oxide synthases (NOSs) all require the cofactor 5,6,7,8-tetrahydrobiopterin (BH4) for catalytic function. We have recently described the function and expression of genes required for BH4 synthesis and regeneration in the nematode C. elegans (Loer et al., 2015, Genetics 200: 237). Worms that can't synthesize BH4 are serotonin- and dopamine-deficient, and have leaky, fragile cuticles caused by aberrant lipid metabolism in the epidermis; BH4-deficiency also alters susceptibility to bacterial pathogens. In humans, regulation of BH4 levels is important for many physiological functions. BH4 is synthesized in four steps starting with GTP; the first step is performed by GTP Cyclohydrolase I (GTPCH1, C. elegans gene cat-4). Like in many biochemical synthesis pathways, the first enzyme in the pathway is regulated by 'end product feedback inhibition.' This typically occurs by the end product itself binding to another site on the enzyme – allosteric regulation. For BH4, it is more complicated - in mammals, GTPCH1 inhibition by BH4 requires an additional small protein called GTPCH1 feedback regulatory protein (GFRP). Crystal structures show that the analog BH2 binds at the interface between pentamers of GTPCH1 & GFRP to inhibit GTPCH1 function, mainly interacting with GTPCH1 (Maita et al., 2004, J Biol Chem 279: 51534). Phe, which stimulates GTPCH1, also binds at the interface between the two proteins, mainly interacting with GFRP. C. elegans also encodes a likely ortholog of GFRP (gene *gfrp-1*); sequence analysis and structural predictions suggest the worm GFRP will function like the mammalian protein, binding both BH4 and Phe. In mammals, GTPCH1 and GFRP can also bind the selective inhibitor 2,4-Diamino-6-hydroxypyrimidine (DAHP), which is structurally similar to both GTP (substrate) and BH4 (pathway end product). At low concentrations, DAHP apparently inhibits GTPCH1 like BH4 by binding with GFRP; this inhibition is GFRP-dependent. At higher concentrations, DAHP acts as a competitive inhibitor, binding at the GTPCH1 active site like GTP; this inhibition is GFRP-independent (Xie et al., 1998, J Biol Chem 273: 21091). Although DAHP treatment has no obvious effect on wildtype C.

elegans, serotonin levels (a proxy for BH4 levels) are reduced by DAHP in worms with a reduction-of-function mutation [*cat-4(e3015)*] that already have less BH4. We plan to test whether this presumed inhibition of worm GTPCH1 is GFRP-dependent by knocking down *gfrp-1* gene function in DAHP-treated *cat-4(e3015)* worms.

Disclosures: C.M. Loer: None.

Poster

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 287.01/C60

Topic: B.03. G-Protein Coupled Receptors

Support: MH073676

NS031373

MH065215-14

Title: Therapeutic potential of mGlu1 PAMs in the treatment of schizophrenia via regulation of striatal dopamine release through an endocannabinoid-dependent mechanism

Authors: *S. E. YOHN¹, D. J. FOSTER¹, D. P. COVEY², P. M. GARCIA-BARRANTES¹, J. F. CHEER², C. W. LINDSLEY¹, P. J. CONN¹ ¹Vanderbilt Univ., Nashville, TN; ²Univ. of Maryland, Baltimore, MD

Abstract: Given the shortcomings of current antipsychotics, novel therapeutic approaches are needed that exhibit clinical efficacy with reduced adverse effect liability. Activation of the muscarinic M₄ receptor has previously been demonstrated to have antipsychotic-like efficacy in several preclinical models. Recently our laboratory reported that these antipsychotic effects are mediated through a novel mechanism wherein activation of M4 receptors reduces dopamine release through a CB₂ cannabinoid-dependent mechanism. Herein, we present new data suggesting that metabotropic glutamate 1 receptor (mGlu₁) plays a critical role in mediating these M₄-dependent dopaminergic effects. Bath application of an mGlu₁ negative allosteric modulator (NAM) VU0458650 blocks M₄-induced reductions in striatal dopamine and M₄mediated antipsychotic-like effects. A key role for mGlu₁ in regulating DA release could provide important new insights into mechanisms by which loss of mGlu1 signaling could contribute to symptoms observed in schizophrenia patients. Our laboratory has recently developed a novel highly-selective mGlu₁ positive allosteric modulator (PAM) VU6004909, which has an excellent pharmacokinetic (PK) profile for use in in vivo studies. Through use of both ex vivo and in vivo fast-scan cyclic voltammetry (FSCV), we report that application of VU6004909 reduces striatal DA release. Moreover, administration of VU6004909 possesses antipsychotic-like efficacy in

rodent models of schizophrenia, suggesting that $mGlu_1$ selective compounds may alleviate the positive symptoms of schizophrenia. Taken together, these results indicate that **i**) $mGlu_1$ activation plays a regulatory role in the antipsychotic-like effect of M₄ PAMs and **ii**) highlight the potential of a novel intervention strategy utilizing selective $mGlu_1$ compounds.

Disclosures: S.E. Yohn: None. D.J. Foster: None. D.P. Covey: None. P.M. Garcia-Barrantes: None. J.F. Cheer: Other; DA022340; DA042595. C.W. Lindsley: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent on allosteric modulators of GPCR. P.J. Conn: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent on allosteric modulators GPCR.

Poster

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 287.02/C61

Topic: B.03. G-Protein Coupled Receptors

Support: Dystonia Medical Research Foundation

NIH Grant MH073676-12

NIH Grant NS031373-22

Title: Hindbrain cholinergic projections to the substantia nigra pars reticulata regulate direct pathway dopamine signaling

Authors: *M. S. MOEHLE¹, T. PANCANI¹, S. YOHN¹, N. BYUN¹, J. DICKERSON¹, D. REMKE¹, C. JONES¹, Z. XIANG¹, C. NISWENDER¹, J. WESS², G. WILSON¹, C. LINDSLEY¹, J. ROOK¹, J. CONN¹ ¹VCNDD, Vanderbilt Univ., Nashville, TN; ²NIH, NIDDK, Bethesda, MD

Abstract: Dysregulation of dopamine (DA) release within the basal ganglia from midbrain dopaminergic neurons of the substantia nigra pars compacta (SNpc) underlies pathological states in several psychiatric and movement disorders. Within the basal ganglia, DA acts on two separate non-overlapping pathways, the direct and indirect pathway. Because of the critical modulatory role of DA, great effort has been placed on understanding both how DA modulates the basal ganglia direct and indirect pathways and how other neurotransmitter systems regulate DA signaling. One such regulator of DA is the muscarinic acetylcholine receptor subtype 4 (M4). Recently, our lab has shown that M4 activation on direct pathway spiny projection neurons (SPNs) can inhibit DA release from SNpc terminals in the striatum through an endocannabinoid dependent mechanism. However, we now report that M4 activation can block D1 DA signaling

in addition to blockading DA release. Using a wide range of behavioral, electrophysiological, optogenetic, pharmacological, and imaging techniques, we directly tested how M4 signaling opposes D1 activation. Interestingly, we have found that cholinergic projections from the hindbrain regulate D1 DA signaling at the level of the substantia nigra pars reticulata (SNr) through M4. Additionally, these data suggest that M4 activity may tonically inhibit direct pathway SPNs and D1 DA signaling. We additionally present data that suggests that elimination of cholinergic projections from the hindbrain has widespread effects on basal ganglia dependent behaviors. Taken together, our data demonstrate a new role for M4 in modulating DA signaling, extend the current model of basal ganglia processing, and suggest an important role of hindbrain cholinergic projections in regulating the basal ganglia.

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Poster

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 287.03/C62

Topic: B.03. G-Protein Coupled Receptors

Support: Grant from CHDI Foundation

Title: M₄ receptor activation normalizes dopaminergic signaling in Huntington's Disease mouse models

Authors: *M. L. LAVERY¹, D. J. FOSTER¹, S. E. YOHN¹, M. S. MOEHLE¹, J. W. DICKERSON¹, D. P. COVEY², W. PENG¹, Z. XIANG¹, J. M. ROOK¹, J. F. CHEER², P. CONN¹

¹Vanderbilt Ctr. for Neurosci. Drug Discovery, Vanderbilt Univ., Nashville, TN; ²Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Huntington's Disease (HD) is a genetic and neurodegenerative disease characterized by multiple symptom stages resulting in motor and cognitive deficits. Currently, the treatments for HD only address late-stage symptom management, indicating the need for further understanding of the disease, and development of treatments for early-stage symptoms. While the exact causes behind the neurodegeneration observed in HD are not clear, there are several circuit level changes that precede motor symptoms including hyperactive neurotransmission in

the striatum. We theorize that modulation of this imbalanced basal ganglia circuitry through activation of the M₄ subtype of the muscarinic acetylcholine receptor can provide a novel therapeutic strategy for treating HD. Through studies utilizing fast-scan cyclic voltammetry, we have confirmed that two animal models of HD, BACHD and YAC128 HD, are hyperdopaminergic compared to wild-type littermates at young ages prior to the onset of motor phenotypes. Both electrical stimulation, as well as optical stimulation in mice expressing channel rhodopsin 2 in dopamine neurons, induced larger dopamine transients in ex vivo brain slices of HD mice. In addition, in vivo voltammetry recordings made from anesthetized HD mice demonstrated larger electrically evoked dopamine transients than littermate controls. The increased striatal dopamine release in HD mice did not correlate with changes in motor function when tested in an open field paradigm. However, the BACHD model did show increased social aggression behavior compared to littermates as assessed using a tube dominance test. Through voltammetry, we have also found that the nonselective muscarinic acetylcholine receptor agonist oxotremorine-M, induced sustained reductions of dopamine release more potently in BACHD mice than in their littermates. This sustained inhibition of DA release could be potentiated by the selective M₄ positive allosteric modulator (PAM), VU0467154, demonstrating that increased M₄ signaling can normalize excessive striatal dopamine release in HD mice. Previous studies have demonstrated that chronic administration of M4 PAMs can delay the onset of motor deficits in these mouse models. Collectively, these data suggest that these mice display excessive dopamine transmission in the striatum that correlates with increased social aggression. Furthermore, we found that activation of the M4 receptor could normalize this excessive dopamine transmission suggesting that this receptor may be a novel therapeutic target with which to normalize neurotransmission in early disease stages.

Disclosures: M.L. Lavery: None. **D.J. Foster:** None. **S.E. Yohn:** None. **M.S. Moehle:** None. **J.W. Dickerson:** None. **D.P. Covey:** 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.; DA041827. **W. Peng:** None. **Z. Xiang:** None. **J.M. Rook:** None. **J.F. Cheer:** 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.; A022340. **P. Conn:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent holder.

Poster

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 287.04/C63

Topic: B.03. G-Protein Coupled Receptors

Support: NIH Grant R01 MH073676

T32 MH 64913-14

Clinical Neuroscience Scholars-Dan Marino Foundation

Title: M₁ Allosteric modulators without agonist activity in the medial prefrontal cortex may provide the optimal profile for cognition enhancement

Authors: *S. P. MORAN, C. A. DOYLE, Z. XIANG, H. C. PLUMLEY, J. W. DICKERSON, D. H. REMKE, C. M. NISWENDER, D. W. ENGERS, C. W. LINDSLEY, J. M. ROOK, P. J. CONN

Pharmacol. Dept., Vanderbilt Univ., Nashville, TN

Abstract: The M₁ subtype muscarinic acetylcholine (ACh) receptor (mAChRs) has emerged as an exciting potential target for novel therapeutic agents for the treatment of schizophrenia. Recently, highly-selective M₁ positive allosteric modulators (PAMs) have provided compelling evidence that M₁ PAMs have cognition-enhancing effects and actions that predict reduction in negative symptoms in rodent models of schizophrenia. Specifically, M₁ PAMs can enhance a novel form of mAChR-induced long-term depression, termed mLTD, at the hippocampoprefrontal cortex (PFC) synapse. mLTD at this synapse is disrupted in rodent models of schizophrenia and is thought to contribute to hippocampo-PFC communication deficits observed in schizophrenia patients. To better understand the pharmacological properties necessary for in vivo efficacy, our lab and others have developed M1 allosteric modulators with diverse pharmacological properties. One such property is intrinsic agonist activity, in addition to PAM activity, in an in vitro system used for compound characterization, such as a calcium mobilization assay. While application of M₁ PAMs with no intrinsic agonist activity, such as VU0550164 and VU0453595, do not induce mLTD by themselves at this hippocampo-PFC synapse, interestingly, M1 PAMs with agonist activity (ago-PAMs) such as MK-7622 and PF-06764427 robustly induce mLTD. Furthermore, in contrast to a VU0453595, a PAM with no observable agonist activity in vitro, these M1 ago-PAMs failed to improve novel object recognition, an assay of cognition in rodents. Thus, it is possible that structurally distinct M₁ PAMs could have fundamentally different effects on important aspects of CNS function, depending on the presence of observable agonist activity in vitro. These findings suggest that ago-PAM activity is not required for cognition enhancement and may, in fact, be detrimental.

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287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 287.05/D1

Topic: B.03. G-Protein Coupled Receptors

Support: NIH Grant R01-MH099054

Title: Mechanisms underlying persistent cholinergic excitation of corticofugal neurons

Authors: *A. L. BAKER, A. T. GULLEDGE

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Abstract: In the rodent medial prefrontal cortex (mPFC), pyramidal neurons in layer 5 broadly express G_a-coupled M1-type muscarinic ACh receptors, but the ionic mechanisms underlying cholinergic excitation of pyramidal neurons are not well understood. Recent work in our lab has characterized a long-lasting cholinergic excitation of corticofugal neurons in layer 5 of the mouse mPFC. Here we explore the mechanisms underlying cholinergic excitation in retrogradely-labeled corticopontine neurons in the mPFC of wild-type mice or mice expressing channelrhodopsin-2 in cholinergic neurons. Persistent cholinergic excitation did not depend on network activity, as it remained robust in the presence of glutamate (kynurenate, 3 mM) and GABA (gabazine, 10μ M) receptor blockers (n = 11). Cholinergic excitation was not contingent on intracellular calcium signaling, as it was not diminished in magnitude or duration following chelation of intracellular calcium with BAPTA (10 or 30 mM; n = 12 and n = 15, respectively), despite elimination of calcium-dependent cholinergic afterdepolarizations. However, blockade of the M-current with bath application of XE991 (10 μ M; n = 11) reduced the magnitude and duration of cholinergic excitation by $19 \pm 11\%$ and $29 \pm 24\%$, respectively, suggesting that Kv7 channels play a role in generating and maintaining this excitation. To test the role of calcium conductances, we combined intracellular BAPTA with removal of extracellular calcium (replaced with equimolar MgCl₂). Removal of calcium decreased both the magnitude and duration of cholinergic excitation, by $42 \pm 8\%$ and $49 \pm 20\%$, respectively, and was reversible upon reintroduction of extracellular calcium (n = 13). Moreover, combining intracellular BAPTA with the nonselective calcium conductance blocker cadmium (200 µM) similarly reduced the magnitude (by $47 \pm 8\%$) and duration (by $59 \pm 10\%$) of cholinergic excitation. The effects of blocking the M-current and eliminating calcium conductances were additive, such that removal of calcium in the presence of XE991 generated a $65 \pm 8\%$ decrease in peak cholinergic excitation, and a 76 \pm 13% reduction in response duration (n = 7). Together, these results indicate that cholinergic excitation of cortical brainstem-projecting neurons involves suppression of the M-current and activation of a calcium-permeable cation conductance.

Disclosures: A.L. Baker: None. A.T. Gulledge: None.

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 287.06/D2

Topic: B.03. G-Protein Coupled Receptors

Title: Allosteric modulators enhance responses evoked at muscarinic receptors following alkylation by acetylcholine mustard or acetylethylcholine mustard

Authors: *J. ELLIS, G. ELMSLIE

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Abstract: Mustard analogs of many receptor ligands have been used to irreversibly label or inactivate receptors. In aqueous solution, mustard groups cyclize to form aziridinium ions that closely mimic quaternary amines. Such a reactive intermediate may therefore bind in the same orientation within the same binding pocket of the receptor that accommodates the related reversible ligand. Once oriented, it is capable of alkylating any of a number of nearby amino acid side chains. In principle, a mustard analog of an agonist might yield irreversible activation of the receptor. However, acetylcholine mustard has been widely reported to possess agonist activity at muscarinic receptors only until alkylation is achieved, at which point the receptor becomes inactivated. Therefore, we were surprised to find that agonist activity persisted after irreversible binding of acetylcholine mustard and its analog, acetylethylcholine mustard, at Gq-linked muscarinic receptors (M₁, M₃, and M₅) expressed in CHO cells. The most interesting results were obtained when measuring the receptor-stimulated release of labeled arachidonic acid (AA) from intact cells. After cells were preincubated with cyclized agonist mustards and thoroughly washed, the basal AA release was found to be elevated to a small but significant degree. This elevation could be prevented if atropine was include in the preincubation phase, but was not inhibited by the presence of atropine in the assay phase. By contrast, inclusion of amiodarone in the assay phase dramatically increased the response of cells that were preincubated with the agonist mustards. We have previously shown amiodarone to be a positive allosteric modulator (PAM) at these receptors. Once again, atropine in the assay itself did not prevent the effect of amiodarone, but atropine in the preincubation phase prevented it completely. At the M₁ subtype, the well-known M₁-specific PAM BQCA enhanced the mustard-induced activity in a manner similar to that observed for amiodarone. The agonist mustards were also found to irreversibly stimulate the metabolism of inositol phosphates (IP), by a similar paradigm. The IP response to the irreversible agonists was more robust than the AA response, and the effects of the PAMs were therefore typically smaller in the IP response. These studies demonstrate that the allosteric sites for amiodarone, BQCA, and other ligands that were investigated are still available following the mustard treatment, and that receptors that have been alkylated by acetylcholine

mustard and acetylethylcholine mustard do adopt active conformations that can be further enhanced by appropriate allosteric ligands.

Disclosures: J. Ellis: None. G. Elmslie: None.

Poster

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 287.07/D3

Topic: B.03. G-Protein Coupled Receptors

Title: Expression of GABAergic interneuron-related genes in mice with genetic deletion of mGlu2 or mGlu3 metabotropic glutamate receptors

Authors: *M. CANNELLA¹, T. IMBRIGLIO², R. VERHAEGHE^{1,2,3}, D. BUCCI¹, F. SCALABRÌ¹, A. SIMEONE⁴, S. MACCARI^{5,3}, G. BATTAGLIA¹, F. NICOLETTI^{1,2} ¹IRCCS Neuromed, Pozzilli, Italy; ²Univ. Sapienza, Rome, Italy; ³Intl. Lab. Associated (LIA) Univ. of Lille 1, CNRS, Sapienza Univ. of Rome, IRCCS Neuromed Pozzili, France Italy, France; ⁴Consiglio Nazionale delle Ricerche, Inst. of Genet. and Biophysics, Naples, Italy; ⁵Univ. Lille, CNRS, UMR 8576, UGSF, Lille, France

Abstract: Group II metabotropic glutamate (mGlu) receptors (mGlu2 and mGlu3) are implicated in the pathophysiology of schizophrenia and are considered as potential targets for new antipsychotic drugs. Changes in the expression of mGlu2 and mGlu3 receptors have been found in the prefrontal cortex of mice exposed to prenatal stress. These mice show epigenetic abnormalities in biochemical markers of GABAergic interneurons and show a psychotic-like phenotype in the adult life (Matrisciano et al., Neuropharmacology, 2013). Moving from these findings, we decided to examine whether the lack of mGlu2 or mGlu3 receptors affects the expression profile of biochemical markers of GABAergic interneurons in the prefrontal cortex and hippocampus at postnatal day (PND)1, PND9, and PND30. The following markers were examined: parvalbumin, somatostatin, reelin, glutamic acid decarboxylase 1 and 2 (GAD67/65), cannabinoid receptor 1 (CB1), neuropeptide Y (NPY), GABA transporter 1 (GAT1), aristaless related homeobox (ARX), distal-less homeobox 2 (DLX2) and the α_2 and δ subunits of GABA_A receptors. The most prominent finding was a marked reduction of parvalbumin mRNA and protein levels in the prefrontal cortex of mice with genetic deletion of mGlu3 receptors. This finding suggests that mGlu3 receptors play a prominent role in the development of fast-spiking parvalbumin-positive GABAergic interneurons, which are known to be defective in schizophrenia. Of note, polymorphic variants of the gene encoding for the mGlu3 receptor (the GRM3 gene) have been repeatedly associated with schizophrenia. Perhaps, these variants influence the development of specific populations of GABAergic interneurons in the cerebral

cortex and hippocampus, causing abnormalities in cognitive functions that lie at the core of the pathophysiology of schizophrenia.

Disclosures: M. Cannella: None. T. Imbriglio: None. R. Verhaeghe: None. D. Bucci: None. F. Scalabrì: None. A. Simeone: None. S. Maccari: None. G. Battaglia: None. F. Nicoletti: None.

Poster

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 287.08/D4

Topic: B.03. G-Protein Coupled Receptors

Title: Activation of mGlu1 metabotropic glutamate receptors plays a key role in excitotoxic neurodegeneration in the mouse retina

Authors: M. ROMANO¹, F. LIBERATORE², D. BUCCI¹, G. MASCIO¹, A. PULITI^{3,4}, M. MADONNA¹, *R. GRADINI^{2,1}, G. BATTAGLIA¹, V. BRUNO^{2,1}, F. NICOLETTI^{2,1} ¹IRCCS Neuromed, Pozzilli, Italy; ²Univ. Sapienza, Roma, Italy; ³Univ. of Genoa, Genoa, Italy; ⁴Inst. Giannina Gaslini, Genoa, Italy

Abstract: Several lines of evidence demonstrate a role for excitotoxicity in retinal ganglion cell (RGC) death and most studies focus on activation of the N-methyl-d-aspartate (NMDA) receptors. To our knowledge, no studies have addressed the role of metabotropic glutamate (mGlu) receptors in mechanisms of retinal degeneration. We have recently found that only mGlu1, but not mGlu5, receptors are coupled to polyphosphoinositide hydrolysis in the mouse retina (Romano et al., 2016), even if both subtypes are expressed in the retina. Here we have studied the role of the mGlu1 receptors on retinal degeneration using the monosodium glutamate (MSG) model. A single systemic injection of MSG to C57BL/6J mouse pups at postnatal day 9 induced a severe retinal degeneration as assessed by immunohistochemistry and stereological counts of the RGCs using the specific marker, brain-specific homeobox/POU domain protein 3A (Brn3a). The selective mGlu1 receptor negative allosteric modulator, JNJ16259685 (2.5 mg/kg, s.c.), was highly protective against MSG-induced retinal degeneration, suggesting that activation of mGlu1 receptors contributes to excitotoxic neuronal death in the mouse retina. Accordingly, MSG-induced RGC death was attenuated in crv4 mice lacking mGlu1 receptors because of a spontaneous recessive mutation of the *Grm1* gene. These findings suggest that mGlu1 receptor is a key player in mechanisms of excitotoxic retinal degeneration and that it might represent a candidate drug target for the treatment of degenerative ocular diseases.

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Poster

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 287.09/D5

Topic: B.03. G-Protein Coupled Receptors

Support: NIMH Grant R01MH100064-03

Title: Comparative subcellular expression patterns of metabotropic glutamate receptor 3 in the prefrontal cortex of the adult rat and monkey

Authors: *J. L. CRIMINS, A. F. T. ARNSTEN, C. D. PASPALAS Neurosci., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Layer III pyramidal neuron networks of the dorsolateral prefrontal cortex (dlPFC) that subserve working memory are vulnerable to the effects of schizophrenia, aging, and Alzheimer's disease. These networks interconnect on dendritic spines with glutamatergic N-Methyl-Daspartate receptor synapses, which are crucial for delay-related firing during a working memory task. On the basis of data from rodents, group II metabotropic glutamate receptors are generally thought to weaken excitatory glutamatergic signaling through Gi/Go-dependent inhibition of glutamate release from presynaptic axons (mGluR2), and/or increased glutamate uptake through excitatory amino acid transporters on astroglia (mGluR3). In monkey layer III dlPFC, we discovered that mGluR3 are also expressed within spine synapses. From this postsynaptic site, mGluR3 strengthen network connectivity and enhance working memory-related neuronal firing via inhibition of cAMP-PKA opening of potassium channels. Yet, rodent literature remain at odds with these data, suggesting that mGluR3 stimulation would primarily increase astroglial glutamate removal from the synaptic cleft, ultimately leading to reduced postsynaptic excitation. To test whether this discrepancy might reflect true species differences, we used immunoelectron microscopy to map mGluR3 in the layer III neuropil of rat medial PFC, the region underlying working memory. We have found that, in addition to classical astroglial expression, mGluR3 are concentrated within preterminal axons where they are positioned to modulate extrasynaptic glutamate release (volume transmission). A postsynaptic component similar to that of nonhuman primates, albeit less extensive, was also identified within the spine synapse. Shared postsynaptic localization in PFC of both species introduces the possibility that mGluR3-mediated enhancement of working memory circuits is phylogenetically conserved.

Disclosures: J.L. Crimins: None. A.F.T. Arnsten: None. C.D. Paspalas: None.

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 287.10/D6

Topic: B.03. G-Protein Coupled Receptors

Support: NSF DMS 1121606

Title: Allosteric modulation and thermodynamic constraints in occupancy models of oligomeric G protein-coupled receptors

Authors: *G. D. SMITH¹, R. HAMMACK²

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Abstract: Terrell Leslie Hill diagrammatic method of quantifying free energy transduction in biochemical cycle kinetics (e.g., the sliding filament model of muscle contraction) is readily applied to stochastic models of ligand-receptor binding. Hill's method uses the Markov Chain Tree Theorem to express the steady-state probability distribution of a receptor occupancy model in terms of the unimolecular rate constants associated to its state-transition graph G, with each spanning tree of G rooted in a given state contributing to that state's occupancy measure. To facilitate application of Hill's diagrammatic method to G protein-coupled receptor oligomers, we have characterized the structural properties of "reduced graph powers" - denoted $G^{(N)}$ - that are the transition graphs for the master Markov chain for N identical (but not independent) M-state receptor models with transition graph G of size |G|=M. Most significantly, we provide a construction of minimum cycle bases of $G^{(N)}$ that elucidates the combinatorics of allosteric modulation and thermodynamic constraints on equilibrium association constants in occupancy models of oligomeric G protein-coupled receptors.



Disclosures: G.D. Smith: None. R. Hammack: None.

Poster

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 287.11/D7

Topic: B.03. G-Protein Coupled Receptors

Support: NRF-2016R1D1A1B03934176

Title: Role of metabotropic glutamate receptors in low Mg²⁺-induced Ca²⁺-spikes in cultured rat hippocampal neurons

Authors: S. JEON, J. YANG, *S. YOON

Dept. of Physiology, Col. of Med., Catholic Univ. of Korea, Seoul, Korea, Republic of

Abstract: Reducing $[Mg^{2+}]_0$ to 0.1 mM has elicited repetitive $[Ca^{2+}]_i$ spikes in cultured rat hippocampal neurons, driven by glutamatergic synaptic transmission. Group 1 metabotropic glutamate receptors (mGluRs), positively affect postsynaptic neuronal excitability. Present study was investigated to determine whether group 1 mGluRs are involved in the synapticallyinducced [Ca²⁺]_i spikes induced by HEPES-buffered Hank's salt solution containing 0.1 mM MgCl₂ and 10 µM glycine in cultured rat hippocampal neurons from embryonic day 17 fetal Sprague-Dawley rats using digital imaging methods for Ca². Reduction of [Mg²⁺]₀ to 0.1 mM induced synchronized and repetitive $[Ca^{2+}]_i$ spikes within 30 s at day 11.5. The group 1 mGluR agonist, DHPG (1 μ M), significantly increased the frequency and the area under the curve of the 0.1 mM $[Mg^{2+}]_{0}$ -induced $[Ca^{2+}]_{i}$ spikes. The mGluR5 antagonist MPEP (25 μ M) antagonist significantly inhibited the frequency and the area under the curve of the $[Ca^{2+}]_i$ spikes, but the mGluR1 antagonist LY367385 (100 μ M) did not affect the [Ca²⁺]_i spikes. The phospholipase C inhibitor U73122 (1 μ M) significantly inhibited the [Ca²⁺]_i spikes. The IP₃ receptor antagonist 2-APB (30 μ M) or the ryanodine receptor antagonist TMB-8 (10 μ M) also inhibited the [Ca²⁺]_i spikes. All these results suggest that mGluR 5 is involved in the 0.1 mM $[Mg^{2+}]_0$ -induced $[Ca^{2+}]_1$ spikes in cultured rat hippocampal neurons through release of Ca²⁺ from IP₃ and ryanodinesensitive stores. This work was supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (NRF-2016R1D1A1B03934176).

Keywords: $[Ca^{2+}]_i$ spike, IP₃ receptor, hippocampal neurons, low Mg²⁺, metabotropic glutamate receptor, ryanodine receptor

Disclosures: S. Jeon: None. J. Yang: None. S. Yoon: None.

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 287.12/D8

Topic: B.03. G-Protein Coupled Receptors

Support: 14182MFD977

NRF-2016R1A2B1010564

Title: Synaptic ERK2 phosphorylates and regulates metabotropic glutamate receptor 1 *In vitro* and in neurons

Authors: *J. YANG¹, L. MAO², J. Q. WANG^{2,3}, E. CHOE¹

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Abstract: A synaptic pool of extracellular signal-regulated kinases (ERK) controls synaptic transmission, although little is known about its underlying signaling mechanisms. Here, we found that synaptic ERK2 directly binds to postsynaptic metabotropic glutamate receptor 1a (mGluR1a). This binding is direct and the ERK-binding site is located in the intracellular C-terminus (CT) of mGluR1a. Parallel with this binding, ERK2 phosphorylates mGluR1a at a cluster of serine residues in the distal part of mGluR1a-CT. In rat cerebellar neurons, ERK2 interacts with mGluR1a at synaptic sites, and active ERK constitutively phosphorylates mGluR1a under normal conditions. This basal phosphorylation is critical for maintaining adequate surface expression of mGluR1a. ERK is also essential for controlling mGluR1a signaling in triggering distinct postreceptor signaling transduction pathways. In summary, we have demonstrated that mGluR1a is a sufficient substrate of ERK2. ERK that interacts with and phosphorylates mGluR1a is involved in the regulation of the trafficking and signaling of mGluR1.

Disclosures: J. Yang: None. L. Mao: None. J.Q. Wang: None. E. Choe: None.

Poster

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 287.13/D9

Topic: B.03. G-Protein Coupled Receptors

Title: Role of ubiquitination in glutamate receptors trafficking

Authors: *R. GULIA¹, R. SHARMA², S. BHATTACHARYYA¹ ¹Biol. Sci., Indian Inst. of Sci. Educ. and Res., Mohali, India; ²Biol. Sci., Indian Inst. of Sci. Educ. and Res., Mohali, SAS Nagar, Knowlwdge city, Sec-81, India

Abstract: Glutamate is a major excitatory neurotransmitter in the central nervous system (CNS). Glutamate receptors which bind to glutamate are categorized into two groups: ionotropic glutamate receptors (ion channels) and metabotropic glutamate receptors (GPCRs). Group I metabotropic glutamate receptors (mGluRs) consists of two members viz., mGluR1 and mGluR5. They are predominantly localized at the post-synaptic side are key players in the process of neuronal development as well as in synaptic plasticity in the CNS. These receptors are positively coupled to Gq pathway. Accurate temporal and spatial localization of these receptors at postsynaptic surface is crucial for normal signaling, and their spatiotemporal localization is maintained by trafficking of the receptor. Trafficking also play crucial role(s) in the regulation of these receptors. Thus, studies of the trafficking of these receptors are of utmost importance. Furthermore, these receptors upon activation result in the endocytosis of AMPA receptors, leading to mGluR-dependent Long-term depression (mGluR-LTD). We are interested in investigating the cellular and molecular mechanisms that govern the group I mGluR trafficking and it effect on mGluR-mediated AMPAR endocytosis. Our data suggest that ubiquitination plays critical role in the trafficking of these receptors. We have found that these receptors get ubiquitinated upon binding with the ligand and subsequently get endocytosed. We further demonstrated that lysine residue present at the C-terminus of mGluR1 plays critical role in the endocytosis of mGluR1. In addition, E3 ligase, Siah-1A was found to be involved the ubiquitination of mGluR1. Acute knockdown of Siah-1A also enhanced group1 mGluR mediated AMPAR endocytosis in primary neurons.

Disclosures: R. Gulia: None. R. Sharma: None. S. Bhattacharyya: None.

Poster

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 287.14/D10

Topic: B.03. G-Protein Coupled Receptors

Title: Enhanced GABA uptake underlying the anti-absence activity of mGlu5 metabotropic glutamate receptor positive allosteric modulators

Authors: R. CELLI¹, I. SANTOLINI¹, M. VERGASSOLA², V. D'AMORE¹, R. GRADINI^{1,3}, G. VAN LUIJTELAAR⁴, G. BATTAGLIA¹, *V. BRUNO^{3,1}, R. T. NGOMBA⁵, A. PITTALUGA², F. NICOLETTI^{1,3}

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Abstract: Pharmacological activation of mGlu5 metabotropic glutamate receptors reduces the frequency of spike-and-wave discharges (SWDs) in the WAG/Rij rat model of absence epilepsy. Recent evidence suggests a protective role for group I metabotropic glutamate receptors as potential candidates for the treatment of absence epilepsy. The evidence that the anti-absence activity of the mGlu5 positive allosteric modulator (PAM), VU0360172, was reversed by intrathalamic infusion of the GABA transporter inhibitor, tiagabine, raised the possibility that mGlu5 receptors regulate the expression/activity of the high affinity GABA transporter, GAT-1, in the ventrobasal thalamus. Accordingly, systemic treatments with VU0360172 was able to upregulate GAT-1 expression in the thalamus after 1 hour. The drug was also able to up-regulate GAT-1 expression in the somatosensory cortex, but this effect required three days of repeated administrations. Here, we provide new evidence in support to this hypothesis by demonstrating that systemic treatment with VU0360172 could enhance GABA uptake in the thalamus. Symptomatic WAG/Rij rats were treated with VU0360172 (3 mg/kg, s.c.) or its vehicle either acutely (single injection) or repeatedly (twice a day for three days). All animals were killed one hour after the last injection. [³H]-GABA uptake was measured in superfused isolated synaptosomes prepared from the thalamus and somatosensory cortex. Both single and repeated administrations of VU0360172 significantly enhanced GABA uptake in thalamic synaptosomal preparations, but not in cortical synaptosomes, where acute treatment with VU0360172 unexpectedly reduced GABA uptake. These findings demonstrate that mGlu5 receptors regulate GABA uptake in the thalamus and support the view that mGlu5 PAMs can be developed as antiabsence drugs. We are currently exploring the mechanisms responsible for the short-onset enhancement of GABA uptake caused by mGlu5 receptor activation focusing on changes in GAT-1 intracellular trafficking.

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Poster

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 287.15/D11

Topic: B.03. G-Protein Coupled Receptors

Support: NHMRC APP1123722

NHMRC APP1084775

Title: Coincident activation of adenosine A1 receptors and metabotropic glutamate receptor 5 modulates neuronal signalling

Authors: *K. J. GREGORY¹, S. HELLYER¹, S. ALBOLD¹, A. CHRISTOPOULOS², T. WANG¹, L. T. MAY¹

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Abstract: G protein-coupled receptors (GPCRs) are highly 'druggable' proteins and represent the largest class of targets for current therapeutics. Two distinct neuromodulatory GPCRs, the adenosine A_1 receptor (A_1AR) and the metabotropic glutamate receptor subtype 5 (mGlu₅), have been implicated in Alzheimer's disease (AD) pathology and as potential targets to treat cognitive impairments and disease progression. Both receptors are found on the same CNS-resident cell types, in particular neurons and astrocytes, and are expressed within key regions of the brain implicated in AD pathology. GPCR discovery programs generally only consider GPCR activity in isolation, without factoring in the influence of other GPCRs or stimuli present. Importantly, both glutamate and adenosine are often present within culture medium and/or released from cultured cells. GPCR cross-talk and/or heteromerisation can introduce pharmacological heterogeneity and offer new avenues for targeted drug development. We sought to test the hypothesis that coincident activation of co-located GPCRs modulates signalling in primary neurons. Primary cultures were derived from striatum and cortices of E16 mice. After 6-8 days in culture, high-throughput signalling assays were performed (iCa^{2+} mobilisation, inositol phosphate (IP1) and cAMP accumulation). We first confirmed the presence of functional mGlu₅ and A₁AR in both cultures by assessing the ability of: 1) A₁AR selective agonist (MeCCPA) to inhibit forskolin stimulation of cAMP accumulation; and 2) mGlu₅ selective allosteric agonist (VU0424465) to stimulate iCa²⁺ and IP1 accumulation. Coincident activation of A₁AR enhanced mGlu₅-mediated iCa²⁺ mobilisation in response to both orthosteric and allosteric agonists in striatal and cortical neurons. The maximal response and potency (~3 fold) of both DHPG (mGlu5 orthosteric agonist) and VU0424465 were significantly increased. In contrast, A1AR activation had no effect on mGlu5-mediated IP1 accumulation in cortical neurons. Conversely, coincident activation of mGlu₅ had little influence on A₁AR-mediated inhibition of cAMP accumulation. Collectively, our data demonstrate that coincident activation of mGlu₅ and A₁AR differentially modulates intracellular signalling pathways in primary neurons. Future work exploring the underlying mechanisms may reveal new strategies for targeting these GPCRs to treat AD and cognitive disorders.

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287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

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Title: Involvement of mGluR I in EphB/ephrinB reverse signaling induced retinal ganglion cell apoptosis in a rat chronic ocular hypertension model

Authors: *Y. ZHAO¹, Q. LI², X.-Y. LI¹, F. GAO², P. CUI¹, X.-H. SUN³, Z. WANG³ ¹Inst. of Brain Sci., Shanghai City, China; ²Dept. of Ophthalmology at Eye & ENT Hosp., Shanghai City, China; ³Inst. of Brain Science, Dept. of Ophthalmology at Eye & ENT Hospital, State Key Lab. of Med. Neurobiology, Shanghai Key Lab. of Visual Impairment and Restoration, Collaborative Innovation Ctr. for Brain Science, Fudan Univ., Shanghai City, China

Abstract: Our previous study has demonstrated that EphB/ephrinB reverse signaling was activated in a chronic ocular hypertension (COH) rat model, which contributed to retinal ganglion cell (RGC) apoptosis by modulating GluA2 subunit of AMPA receptors. Here, we explored whether and how mGluR I is involved in RGC apoptosis by activated EphB/ephrinB reverse signaling in COH rats using whole-cell patch-clamp techniques in retinal slices. We found that the frequency of spontaneous firing of RGCs was increased in both EphB2-Fc intravitreally injected retinas and COH retinas, which was blocked by pre-injection of either the tyrosine kinase inhibitor PP2 or the selective mGluR1/mGluR5 antagonists LY367385/MPEP. Co-immunoprecipitation experiments showed an interaction between ephrinB2 and mGluR1 in both normal and EphB2-Fc-injected retinas. These results suggest that activation of EphB/ephrinB reverse signaling increased RGC excitability through affecting mGluR I in COH retinas. In addition, numerous TUNEL-positive signals were observed in the COH and the EphB2-Fc-injected retinas, which were significantly reduced by intravitreally pre-injecting LY367385 or MPEP. Together, our results suggest that activation of EphB/ephrinB reverse signaling increase RGC excitability and apoptosis through interacting with mGluR I in COH retinas, in addition to the GluA2-containing AMPA receptors.

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287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

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Topic: B.03. G-Protein Coupled Receptors

Support: NIH Grant 5R01NS064025-08

Title: Tonic and phasic activation of GIRKs contribute to low dentate granule cell excitability

Authors: *J. GONZALEZ, S. A. EPPS, S. J. MARKWARDT, J. I. WADICHE, L. OVERSTREET-WADICHE

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Abstract: The dentate gyrus (DG) is a main entry point for neural activity into the hippocampal formation, integrating sensory and spatial information from the cortex in a manner that generates a neural representation ("engram") of a context. This role within the trisynaptic circuit requires that only a small fraction of the principal granule cells (GCs) are active at any given time. This sparse neural activity is enforced by powerful networks of inhibitory GABAergic interneurons in combination with low intrinsic excitability of GCs. Although the cellular and circuit properties that dictate synaptic inhibition are well studied, less is known about mechanisms that confer low GC intrinsic excitability. Using an electrophysiological approach here we demonstrate that intact G-protein mediated signaling is required to maintain the characteristic low resting membrane potential that differentiates dentate granule cells from CA1 pyramidal cells and immature adult born GCs. We show that intact G-protein signaling enables constitutive G-protein gated inwardly rectifying potassium channels (GIRK) activity, resulting in part from tonic GABA_B receptormediated stimulation of GIRKs. Perforant path electric stimulation evokes a phasic activation of GIRKs by synaptic GABA_B receptors on mature GCs, but adult born new GCs completely lack functional GIRK activity, with both tonic and phasic GABA_B-receptor mediated GIRK signaling developing only after 3-4 weeks of maturation. Using transgenic mice and optogenetic tools we show that GABA_B evoked phasic GIRK activation is interneuron specific, arising primarily from neuronal nitric oxide synthase (nNOS)-expressing interneurons rather than parvalbumin or somatostatin-expressing interneurons, and requires expression of GIRK2 subunits. Together these results demonstrate that G-protein mediated signaling robustly contributes to the low intrinsic excitability that differentiates mature and developing dentate GCs, and suggests that nNOS-expressing interneurons are principal gate-keepers of GABA_B-receptor synaptic inhibition.

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287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 287.18/D14

Topic: B.03. G-Protein Coupled Receptors

Title: The unorthodox kynurenine metabolite, cinnabarinic acid, potently inhibits MK-801induced hyperactivity by interacting with mGlu4 metabotropic glutamate receptors

Authors: F. FAZIO¹, M. ULIVIERI², L. LIONETTO³, M. CURTO², G. MASCIO¹, A. TRAFICANTE¹, G. GIANNINO², V. BRUNO^{2,1}, *G. BATTAGLIA¹, M. SIMMACO⁴, F. NICOLETTI^{2,1} ¹I.R.C.C.S. Neuromed, Pozzilli, Italy; ²Univ. Sapienza, Roma, Italy; ³Advanced Mol. Diagnostic, IDI-IRCCS, Roma, Italy; ⁴S. Andrea Hospital, Univ. Sapienza, Roma, Italy

Abstract: The kynurenine pathway is the quantitatively main branch of L-tryptophan degradation in the mammalian body and gives rise to a plethora of metabolites, collectively known as "kynurenines". Some of these metabolites, such as kynurenic acid, quinolinic acid, xanthurenic acid and cinnabarinic acid interact with glutamate receptors thus influencing excitatory transmission in the central nervous system (CNS). Kynurenic acid, an antagonist of NMDA receptors at the glycine co-activation site, has been implicated in the pathophysiology of schizophrenia, and its levels are reduced in prefrontal cortex tissues and in the cerebrospinal fluid of schizophrenic patients. The higher levels of kynurenic acid in patients affected by schizophrenia are in line with the glutamatergic hypothesis of schizophrenia, which postulates that a reduced activity of NMDA receptors causes a hypofunction of GABAergic interneurons resulting into a hyperactivity of pyramidal neurons. Recently, metabotropic glutamate (mGlu) receptors have also been included in the glutamatergic theory, because drugs that activate mGlu2 and mGlu4 receptors show efficacy in experimental animal models of psychosis. Because cinnabarinic acid acts as an orthosteric agonist of mGlu4 receptors (Fazio et al., Mol. Phamacol., 2012), we decided to examine whether systemic administration of cinnabarinic acid could affect MK-801-induced hyperactivity in mice, which models positive symptoms of schizophrenia. We were surprised to find that very low systemic doses of cinnabarinic acid (0.125 mg/kg) were able to reduced MK-80-induced hyperactivity without affecting basal motor activity. Cinnabarinic acid was less potent and efficacious in mGlu4 receptor knockout mice, suggesting that, at least, part of the antipsychotic-like activity of the compound was mediated by the activation of mGlu4 receptors. These findings suggest that the expected reduction in cinnabarinic acid synthesis in schizophrenia (which might be a downstream consequence of the reduced kynurenine monooxygenase activity) might contribute to the pathophysiology of the disease, and raise the attracting possibility that cinnabarinic acid may display therapeutic activity against positive symptoms of schizophrenia.

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Poster

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

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Program#/Poster#: 287.19/D15

Topic: B.03. G-Protein Coupled Receptors

Title: Phospholipase C as a point of convergence between D1 receptor and mGlu5 receptor stimulation in the parkinsonian striatum

Authors: I. SEBASTIANUTTO¹, N. MASLAVA¹, L. DI MENNA², *F. NICOLETTI^{3,2}, M. CENCI¹

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Abstract: In all current models of L-DOPA-induced dyskinesias (LIDs), the occurrence of abnormal involuntary movements (AIMs) is accompanied by the activation of extracellular signal-regulated kinase 1 and 2 (ERK1/2) signaling in striatal neurons. This activation is a consequence of the supersensitivity of D1 receptor (D1R) signaling caused by dopamine (DA) denervation. Antagonists of mGlu5 metabotropic glutamate receptor ameliorate L-DOPA-induced dyskinesias, and blunt D1R agonist-induced ERK1/2 posphorylation (pERK1/2). In DA-denervated striatal slices, D1R-mediated pERK1/2 can be inhibited by antagonizing mGlu5 receptor or its downstream signaling mediator, phospholipase C (PLC) (Fieblinger et al., 2014). In the present study, we set out to (i) examine the effects of PLC inhibition *in vivo*, and to (ii) investigate its role in the DA-denervated striatum.

Rats sustained unilateral 6-OHDA lesions of the nigrostriatal DA pathway, followed by chronic treatment with the D1/D5 receptor agonist SKF38393 to induce AIMs. Dyskinetic rats receiving challenge injections of the PLC inhibitor U73122 (30 mg/kg) had significantly lower AIM scores compared to vehicle-treated animals. Moreover, in vivo administration of U73122 blunted SKF38393-induced striatal activation of ERK1/2. These results prompted the question whether the activation of D1R in the DA-denervated striatum could lead to PLC activation. To answer this question, we used an assay of PLC activity in striatal slices estimating the production of [³H]-inositolmonophosphate (InsP) following SKF38393 incubation. Compared to baseline conditions, D1R stimulation induced a significant increase in [³H]-InsP in the DA-denervated striatum, but not in the intact striatum. In addition, the coapplication of SKF38393 and the mGlu5 receptor agonist DHPG induced a marked increase in inositol phospholipid hydrolysis that was (i) higher than the levels obtained when either agonist was applied alone, and (ii)

specific to the DA-denervated striatum. In conclusion, these results are the first to indicate that the stimulation of D1R and mGlu5 receptor in the DA-denervated striatum converge on PLC activation. This signalling cascade is causally linked with the occurrence of dyskinesia.

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Poster

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

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Topic: B.03. G-Protein Coupled Receptors

Support: Commission for Technology and Innovation CTI R&D grant 17485.1

Title: Metabotropic glutamate receptors 4 and 7 modulate thalamus - lateral amygdala synaptic transmission and plasticity

Authors: ***A. C. CIOBANU**¹, C. FLORES NAKANDAKARE², E. VAN DEN BURG², R. LUTJENS³, R. STOOP²

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Abstract: Thalamic nuclei project to the lateral amygdala (LA) and these connections are important for fear and anxiety. The group III metabotropic glutamate receptors 4 (mGluR4) and 7 (mGluR7) are expressed in the LA and they are involved in acquisition and extinction of fear. Here, we aimed to investigate the role of these receptors in thalamo-LA synaptic transmission and plasticity using two new specific compounds displaying anxiolytic effects in rodents: ADX88178 (positive allosteric modulator for mGluR4) and ADX71743 (negative allosteric modulator for mGluR7).

Whole-cell patch-clamp recordings were performed on pyramidal neurons in the LA to assess the roles of mGluR4 and mGluR7 on spontaneous activity, evoked thalamic synaptic transmission and long term potentiation (LTP). ADX88178 decreased and ADX71743 increased the frequency of spontaneous EPSCs. When thalamic inputs were electrically stimulated, ADX88178 decreased and ADX71743 increased the amplitude of the evoked responses. Moreover, both the positive allosteric modulator for mGluR4 and the negative allosteric modulator for mGluR7 decreased the LTP seen in the control group, at the thalamus to LA synapses.

Thus, mGluR4 and mGluR7 are functionally expressed in the LA and they control both spontaneous neuronal activity and synaptic transmission from the thalamus. Also, the two receptors have the potential to modulate long term synaptic plasticity which likely impacts learning in the lateral amygdala. This may explain the previously described anxiolytic effects of

ADX88178 and ADX71743 in systemically treated animals. The opposite effects of the compounds at cellular level, suggest that their activity is mediated by different neurons, likely part of parallel networks. Our data suggest a mechanism trough which mGluR4 and mGluR7 modulate pyramidal neurons in the LA with implications for fear and anxiety disorders.

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Poster

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

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Topic: B.03. G-Protein Coupled Receptors

Support: NRF-2016R1D1A1B03930951

Title: Activity-dependent mGluR7 ubiquitination regulates receptor stability and trafficking

Authors: S. LEE, H. LEE, J. SONG, *Y. SUH Dept. of Biomed. Sci., Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: The metabotropic glutamate receptor 7 (mGluR7) is a predominant group III mGluR in the presynaptic active zone, where it regulates the excitatory neurotransmitter release via G-protein signaling cascade. Although it has been reported that many different GPCRs are regulated by post-translational ubiquitination that dynamically regulates protein turnover and trafficking, it remains elusive that mGluR7 undergoes ubiquitination in response to synaptic activity. In this study, using biochemical approaches coupled with confocal imaging, we have explored whether mGluR7 is a target of ubiquitination. We found that mGluR7 is ubiquitinated at the cytoplasmic loop 2 region and the C-terminal tail by the treatment of agonist in HEK 293T cells and primary cortical neurons. In addition, we found the ubiquitination of mGluR7 increases the endocytosis and degradation of mGluR7. Given the importance of the ubiquitin-proteasome system in neurodegenerative and neuropsychiatric disorders, mechanisms underlying mGluR7 ubiquitination will provide insights into the fundamental role of mGluR7 in neurological diseases as well as in physiological processes.

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287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

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Topic: B.03. G-Protein Coupled Receptors

Support: Swiss National Science Foundation Grants 31003A_121963 and 31003A_138382 to Dr.Dietmar Benke

Title: Phosphorylation of $GABA_{B1}$ by CaMKII β triggers lysosomal degradation of $GABA_B$ receptors by regulating MIB2-mediated Lys63-linked ubiquitination

Authors: *K. BALAKRISHNAN¹, K. ZEMOURA^{1,2}, T. GRAMPP¹, D. BENKE¹ ¹Univ. of Zurich, Zurich, Switzerland; ²Picower Inst. for Learning and Memory, MIT, Cambridge, MA

Abstract: G protein-coupled GABA_B receptors consist of GABA_{B1} and GABA_{B2} subunits and control neuronal excitability by mediating slow and prolonged inhibitory neurotransmission. An important factor governing the signaling strength of GABA_B receptors is their availability at the plasma membrane, which is tightly regulated by lysosomal degradation of constitutively internalized receptors. Sorting the receptors to the lysosomal degradation pathway requires Lys63-linked ubiquitination of GABA_{B1} by the E3 ubiquitin ligase Mind bomb-2 (MIB2). How MIB2-mediated Lys63-linked ubiquitination of GABA_{B1} is regulated remained unknown. Here we show that Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) promotes MIB2-mediated Lys63-linked ubiquitination of GABA_B receptors under physiological and pathological conditions. We observed an increased expression of cell surface GABA_B receptors after shortterm inhibition of CaMKII and decreased receptor levels upon overexpression of CaMKIIB, but not CaMKIIa. We found that CaMKII-dependent phosphorylation of GABA_{B1} at Ser-867 promoted the Lys63-linked ubiquitination of GABA_{B1} at multiple sites that was mediated by the E3 ligase MIB2. Mutational inactivation of the CaMKII phosphorylation site in GABA_{B1}(GABA_{B1}(S867A)) prevented the Lys63-linked ubiquitination of GABA_{B1} and increased the cell surface expression of GABA_B receptors. Conversely, the phospho-mimetic mutant GABA_{B1}(S867D) strongly increased Lys63-linked ubiquitination of GABA_{B1} and decreased cell surface receptor levels. Finally, sustained activation of glutamate receptors, a hallmark of cerebral ischemia that downregulates GABA_B receptors via lysosomal degradation, was accompanied with strongly increased GABA_{B1}(Ser-867) phosphorylation-dependent Lys63linked ubiquitination of GABA_B receptors. These findings indicate that CaMKIIB regulates Lys63-linked ubiquitination of GABA_{B1} and thereby controls sorting of internalized GABA_B receptors to lysosomal degradation. This mechanism regulates the amount of cell surface GABA_B receptors under physiological as well as pathological conditions.

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Poster

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

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Title: New advances in the knowledge of the 5HT2A / mGlu2/3 receptor-receptor functional cross talk in mammal central nervous system

Authors: A. PITTALUGA¹, G. OLIVERO¹, M. VERGASSOLA¹, T. BONFIGLIO¹, B. GARRONE², F. DI GIORGIO², S. TONGIANI², C. USAI³, *M. GRILLI¹, M. MARCHI¹ ¹Univ. of Genoa, Genova, Italy; ²Angelini S.p.A. R&D Res. Ctr., Roma, Italy; ³Natl. Rese Council, Genova, Italy

Abstract: Data in literature clearly demonstrated the existence of presynaptic-release-regulating mGlu2/3 autoreceptors in selected regions of central nervous system (CNS). Recent evidence showed that mGlu2/3 receptors can form intergroup dimers with co-localized mGluRs belonging to the third group as well as heteromeric complexes with non-glutamatergic metabotropic receptors, increasing the complexity of the scenario. It is the case of the 5HT_{2A} receptors. Studies to elucidate the 5HT_{2A}-mGlu2/3 interaction were carried out in the cortex, where they were mainly dedicated to describe the mGlu2/3-mediated control of serotonergic signalling. We recently provided evidence that spinal cord glutamatergic nerve endings are endowed with both presynaptic $5HT_{2A}$ heteroreceptors and mGlu2/3 autoreceptors, the activation of which inhibits glutamate exocytosis. The two receptors co-localize on the same terminals, where they functionally couple in an antagonist-like manner to control glutamate exocytosis. Actually, when concomitantly activated, the two receptors became unexpectedly inactive. This observation together with the findings that i) mGlu2/3 and 5HT_{2A} receptor immunoreactivities is largely colocalized and ii) mGlu2/3 immunoprecipitates from spinal cord synaptosomes are also 5HT_{2A} immunopositive seem best interpreted by assuming that, in spinal cord synaptosomes, the two receptors co-localize and functionally cross-talk in an antagonist-like manner. Consistently, exposure of synaptosomes to the 5HT_{2A} antagonists (MDL11939, MDL10007, ketanserin and trazodone) reinforced the release-regulating activity of mGlu2/3 autoreceptors, while antagonists acting at presynaptic release-regulating 5HT receptors other than the 5HT_{2A} ones (namely the

5HT1A, 1B and 1D receptors) were inactive. The gain of function of mGu2/3autoreceptors elicited by 5HT_{2A} antagonists was paralleled by an increased expression of mGlu2/3 receptor proteins in synaptosomal plasmamembranes. To the best of our knowledge, these findings provide the first demonstration of the existence of a presynaptic, heterodimeric control of glutamate exocytosis in spinal cord nerve endings involving mGLu2/3 and 5HT_{2A} receptors. On the basis of our observations, we propose that 5HT_{2A} antagonists could act as *"indirect positive allosteric modulator"* (*IPAM*) of mGlu2/3 receptors. Our results envisage a therapeutic alternative to the use of mGlu2/3 *direct* modulators for the cure of spinal diseases typified by hyperglutamatergicity.

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287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

Location: Halls A-C

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Topic: F.10. Food Intake and Energy Balance

Support: PSC/CUNY 68136-00 46

PSC/CUNY 60102-00 48

Title: Murine genetic variance in muscarinic cholinergic receptor antagonism of sucrose and saccharin solution intakes in three inbred mouse strains

Authors: K. OLSSON¹, F. BOURIE¹, B. ISKHAKOV¹, A. BURAS¹, G. FAZILOV¹, M. SHENOUDA¹, J. ZHEZHERYA¹, *R. J. BODNAR² ¹Psychology, Queens College, CUNY, Flushing, NY; ²Psychology- Neuropsychology, Queens Col., Flushing, NY

Abstract: Solutions containing nutritive (sucrose) and non-nutritive (saccharin) sweeteners stimulate intake in inbred mouse strains. Marked genetic variance has been observed for sucrose and saccharin intake in pharmacological analyses of inbred mouse strains with BALB/c, SWR

and C57BL/6 mice differing in their response to dopamine (DA) and opioid receptor antagonism of different forms of sweet intake. Whereas DA D1 receptor antagonism (SCH23390) reduced sucrose (16%) intake in all three strains, opioid receptor antagonism (naltrexone) reduced sucrose intake maximally in C57/BL/6 mice, in intermediate fashion in BALB/c mice, but not in SWR mice. Saccharin (0.2%) intake was potently reduced by DA D1 receptor antagonism in all three strains, but was more potently reduced by opioid receptor antagonism in SWR and BALB/c relative to C57BL/6 mice. Scopolamine (SCOP), a muscarinic cholinergic receptor antagonist, reduced sweet intake and sugar-conditioned flavor preferences in outbred rats. The present study examined the systemic dose-dependent effects of SCOP (0.1-10 mg/kg) on saccharin and sucrose intakes over a 2 h time course in BALB/c, SWR and C57BL/6 mice. Sucrose intake was significantly reduced following all five doses across the entire time course in C57BL/6 mice, and following the 2.5, 5 and 10 mg/kg doses across the time course in BALB/c mice. In contrast, SWR mice displayed only transient (15 min) reductions in sucrose intake following the three higher SCOP doses. Saccharin intake was significantly reduced following all five doses across the entire time course in C57BL/6 mice, and following the 2.5, 5 and 10 mg/kg doses across the time course in BALB/c mice. In contrast, SWR mice displayed significant though smaller reductions in saccharin intake following the two higher SCOP doses. These data indicate that although both nutritive and non-nutritive sweet intakes are governed by muscarinic cholinergic receptor signaling, this process is subject to murine genetic variance with rank-order sensitivity observed in C57BL/6 > BALB/c > SWR inbred mouse strains.

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Poster

287. GPCRs: Metabotropic Glutamate Receptors and Muscarinic Acetylcholine Receptors

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Topic: F.10. Food Intake and Energy Balance

Support: PSC/CUNY 68136-00 46

PSC/CUNY 60102-00 48

Title: Murine genetic variance in muscarinic receptor antagonism of sucrose conditioned flavor preferences in three inbred mouse strains

Authors: *F. R. BOURIE¹, B. ISKHAKOV³, G. FAZILOV³, M. SHENOUDA³, J. ZHEZHERYA³, A. BURAS³, R. J. BODNAR² ²Psychology- Neuropsychology, ¹Queens Col., Flushing, NY; ³Psychology, Queens College, CUNY, Flushing, NY

Abstract: In addition to innate factors, learning modulates sugar intake by producing conditioned flavor preferences (CFP) in rodents. Genetic variance has been observed in the pharmacological substrates controlling the magnitude and persistence of sucrose-CFP; BALB/c and SWR mice exhibited stronger CFP responses than C57BL/6 mice. Systemic dopamine (DA) D1 and opioid, but not NMDA receptor antagonists attenuated the expression (maintenance) of an already-learned sucrose-CFP in BALB/c and SWR mice. DA D1 and NMDA receptor antagonism also impaired acquisition (learning) of sucrose-CFP in both strains, whereas opioid receptor antagonism impaired sucrose-CFP acquisition only in BALB/c mice, indicating dissociable genetic variance. Muscarinic (scopolamine: SCOP), but not nicotinic cholinergic receptor antagonism eliminated acquisition of sugar-CFP in rats. The present study examined whether SCOP blocked acquisition or expression of sucrose-CFP in BALB/c, C57BL/6 and SWR inbred mouse strains. In expression studies, food-restricted mice were trained in 10 onebottle-trials (1 h) to drink a sucrose (16%) solution mixed with one flavor (CS+, e.g., cherry) on odd-numbered days, and a less-preferred saccharin (0.05%) solution mixed with another flavor (CS-, e.g., grape) on even-numbered days. Two-bottle tests (CS+, CS-, 1 h) with the two flavors mixed in 0.2% saccharin were assessed over three subsequent pairs of tests in which the three strains received SCOP (0, 1, 5 mg/kg). Magnitude differences in sucrose-CFP were observed for BALB/c (77%), C57BL/6 (68%) and SWR (84%) mice. Whereas SCOP dose-dependently reduced expression of sucrose-CFP in BALB/c and C57BL/6 mice, it minimally altered expression of sucrose-CFP in SWR mice. In acquisition studies, separate groups of foodrestricted mice of the three strains received 10 one-bottle training trials (1 h) with CS+ and CSsolutions following vehicle, and SCOP at doses of 1, 2.5 and 5 mg/kg in daily treatment. Six 2bottle tests (CS+, CS-, 1 h) with the two flavors mixed in 0.2% saccharin were assessed over three subsequent pairs of tests without injections. SCOP dose-dependently reduced acquisition of sucrose-CFP in BALB/c mice with the highest dose producing indifference. SCOP's inhibitory effects on acquisition of sucrose-CFP were less pronounced in C57BL/6 and SWR strains. These data implicate muscarinic cholinergic receptor signaling in the maintenance of sucrose-CFP in BALB/c and C57BL/6, but not SWR mice, and in acquisition (learning) of sucrose-CFP in the three strains, but especially BALB/c mice, thereby indicating strong genetic variance in sweetdriven learning.

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Poster

288. Sodium Channels

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Title: Symbiotic bacteria underlie neurotoxin production and evolution of toxin resistance in voltage-gated sodium channels of rough-skinned newts (Taricha granulosa)

Authors: *P. VAELLI^{1,2,3}, K. R. THEIS^{4,3}, J. A. FOSTER^{5,3}, H. L. EISTHEN^{1,2,3} ¹Integrative Biol., Michigan State Univ., East Lansing, MI; ²Ecology, Evolutionary Biology, and Behavior Grad. Program, East Lansing, MI; ³BEACON Ctr. for the Study of Evolution in Action, East Lansing, MI; ⁴Dept. of Microbiology, Immunol. and Biochem., Wayne State Univ., Detroit, MI; ⁵Dept. of Biol., Univ. of Idaho, Moscow, ID

Abstract: Rough-skinned newts (Taricha granulosa) are poisonous salamanders that can possess high concentrations of tetrodotoxin (TTX), a potent neurotoxin that blocks voltage-gated sodium channel (VGSC) conductance in neurons and muscle cells. TTX is present in all species of the genus Taricha, but some populations of T. granulosa (hereafter "newts") possess extreme quantities not seen in any other TTX-bearing species, including puffer fishes, blue-ringed octopuses, and many diverse marine invertebrates. Geographic variation in TTX toxicity across different newt populations is thought to be driven by ecological interactions with predators. Despite the central role of TTX in the physiology and evolution of newts, the mechanisms of TTX production and neurophysiological resistance are unknown. Because of the polyphyletic distribution of TTX among animals, we explored the hypothesis that TTX is produced by symbiotic skin bacteria in newts. We conducted 16S rRNA gene-based sequencing surveys to characterize skin-associated bacterial communities of newts from toxic and non-toxic populations. From here, we employed ecologically-guided cultivation strategies to target skinassociated symbionts and produce pure cultures. We screened cultures for TTX production using a customized HILIC-MS/MS method and confirmed TTX production in multiple isolated bacterial strains. Furthermore, we investigated the molecular adaptations underlying apparent TTX resistance in the VGSCs of newts. We cloned and sequenced the TTX binding site, the S5-S6 pore loop regions, of all six VGSC genes present in this species and compared sequences from toxic and non-toxic populations, as well as from other vertebrates. As a result, we identified several mutations present in the S5-S6 pore loops of all six genes, indicating a remarkable parallel evolution of TTX resistance across the VGSC gene family. Taken together, our results indicate that TTX is derived from the skin microbiome in the extremely toxic rough-skinned newt and that multiple adaptations in VGSCs were required for the newt nervous system to adapt to TTX toxicity. Overall, this research contributes to a growing understanding that symbiotic microbes can affect the physiology of nervous systems, and that evolution by natural selection may target genetic variation across both host and symbiont genomes, collectively termed the 'hologenome'.

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288. Sodium Channels

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Program#/Poster#: 288.02/D23

Topic: B.04. Ion Channels

Title: Role of CaMKIIy in Associative Conditioning and GLR-1 expression in C. elegans

Authors: *A. H. BLACK¹, S. LIN², M. R. PRIBIC³, N. D. JOHNSON², A. R. LOFTUS², L. K. ALFILER², J. K. ROSE² ²Behavioral Neurosci. Program, ³Biol., ¹Western Washington Univ., Bellingham, WA

Abstract: Recent work has shown that the calcium/calmodulin-kinase II (CaMKII) y-isoform plays a role in neuronal mechanisms associated with learning and memory. In C. elegans, the unc-43 gene codes for an ortholog of CaMKII and previous studies report that UNC-43 regulates glutamate receptor expression (specifically GLR-1) in neurons. The current study examined the *unc-43*(gk452) strain (ortholog to CaMKII_{γ}) in associative conditioning and glutamate receptor expression to begin to elucidate the role of this particular isoform in learning. A previous report indicates *unc-43*(gk452) worms show a memory deficit for an associative appetitive chemotaxis assay. Research from our lab has shown unc-43(gk452) worms exhibit a learning deficit for an associative avoidance chemotaxis assay; a procedure that requires prolonged stimulus pairing (>1 hour). An associative conditioning assay whereby increased responsiveness to a discrete stimulus following brief paired presentations with another stimulus has been shown in C. elegans. Our lab has reported that pairing delivery of two distinct stimuli (blue light ~480 nm and a mild mechanosensory vibration; Vib) results in increased responsiveness to either the blue light or Vib stimulus alone. The unc-43(gk452) strain was also found to show a deficit in this light-vibration associative conditioning. The effects of the unc-43(gk452) mutation on glr-1 expression levels were examined with qRT-PCR following light-vibration associative conditioning and in vivo GLR-1::GFP expression patterns were assessed. By observing both behavior and receptor expression in intact animals, these results begin to elucidate the role of this unique CaMKII isoform in associative forms of learning and memory.

Disclosures: A.H. Black: None. S. Lin: None. M.R. Pribic: None. N.D. Johnson: None. A.R. Loftus: None. L.K. Alfiler: None. J.K. Rose: None.

288. Sodium Channels

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Program#/Poster#: 288.03/D24

Topic: B.04. Ion Channels

Support: AHA grant 13SDG16990083

Title: Calcium regulation of voltage-gated sodium channels in serotonergic raphe neurons

Authors: *M. A. NAVARRO, M. MILESCU, L. S. MILESCU Biol. Sci., Univ. of Missouri, Columbia, MO

Abstract: We have previously shown that voltage-gated sodium (Nav) channels in spontaneously firing medullary raphé neurons inactivate with a slow component. This long term inactivation (LTI) modulates neuronal firing and is potentially due to an interaction with fibroblast growth factor - homologous factors (FHF). There is some evidence that the Nav-FHF interaction may also include calmodulin, and thus it may depend on $[Ca^{2+}]$. Here, we test whether intracellular calcium concentration has any effect on LTI in these neurons. We used whole-cell patch-clamp to record Na⁺ currents in neonatal (P0-P3) rat brainstem slices, under two $[Ca^{2+}]_i$: 60 nM and 6 μ M. Under voltage-clamp, we found that the Nav steady-state activation and inactivation curves did not shift significantly with $[Ca^{2+}]_i$. However, the kinetics of recovery from inactivation to perturb the Nav-FHF interaction, and observed a change in the recovery from inactivation, but only at low $[Ca^{2+}]_i$. Under current clamp, neurons loaded with high $[Ca^{2+}]_i$ fired action potentials with greater amplitude and lower threshold than neurons with low $[Ca^{2+}]_i$.

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Poster

288. Sodium Channels

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Topic: B.04. Ion Channels

Support: NIH-R01HL122228

Title: NaV subtypes in itch versus pain C-fibers in mouse skin

Authors: D. JURCAKOVA, M. KOLLARIK, F. RU, *B. J. UNDEM Dept Med., Johns Hopkins Asthma Ctr., Baltimore, MD

Abstract: Sodium channels represent a promising drug target to relieve pain and itch. We have evaluated the sodium channel subtypes in C-fibers in the skin using a unique, recently developed innervated isolated dorsal skin preparation in which action potential discharge in single afferent C-fibers (CV <1 m/s) nerve terminating in the skin is evaluated with extracellular electrode positioned in the dorsal root ganglia. The dissected section of skin also includes the main arterial supply through which chemical stimuli is delivered directly to the receptive field (see J. Physiol, 2017). We have evaluated the pharmacology of the NaVs most responsible for action potential discharge in three C-fiber subtypes that innervate the skin, namely itch fibers (chloroquine (CQ) and capsaicin sensitive, high mechanical thresholds), pain fibers (CQ-insensitive, capsaicinsensitive, high mechanical thresholds); and low threshold mechanosensitive C-fibers (LTMCs) (CQ and capsaicin-insensitive, very low mechanical thresholds).We evaluated the effect of NaV blocking drugs added directly to the receptive field on AP discharge in response to mechanical stimulation. In 100% of the pain fibers (n=65) and 100% of the LTMCs (n= 8) blocking TTXsensitive NaVs abolished AP discharge. In contrast, 20% of itch fibers still responded normally when TTX-sensitive channels were blocked (n=19). In these fibers adding a NaV1.8 blocker (A803467) abolished the responses. We evaluated two selective NaV1.7 blockers (PF05089771 and Cmpd 13), the results were similar so we pooled the data. Blocking NaV 1.7 abolished AP discharge in 63% of pain C-fibers, 40% of itch C-fibers, but only 20% of LTCMs. The selective NaV1.1, 1.2, 1.3 blocker ICA121431 alone did not inhibit responses in any of the three C-fiber phenotypes. In the presence of NaV1.7 blockade ICA abolished the response in 3 of 7 pain fibers and 2 of 2 LTCMs, but had no effect on itch fibers (0/4). Using single cell RT-PCR we evaluated the expression in individual pain (MRGPRA3-negative, TRPV1-positive) vs itch fiber neurons (MRGPRA3 and TRPV1 positive) retrogradely labeled from the region of the dorsal skin that corresponded to our functional studies. The % of pain neurons that expressed NaV 1.1, 1.2,1.3. 1.6. 1.7, 1.8, and 1.9 was 15%, 5%, 5%, 29%, 86%, 95% and 100% (n=21), respectively. In the itch fibers the corresponding % of each NaV was 0%, 0% 8%, 8%, 85%, 100%, 100% (n=13). We conclude that NaV1.7 blockers can inhibit about 40-60% of pain and itch fibers in mouse skin, but is less effective in inhibiting LTCMs. Those C- fibers that respond in the presence of NaV1.7 blockade it is likely due to the activity of 1.8 in itch fibers, 1.6, 1.1 in pain fibers.

Disclosures: D. Jurcakova: None. M. Kollarik: None. F. Ru: None. B.J. Undem: None.

Poster

288. Sodium Channels

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Topic: B.04. Ion Channels

Support: NS078184

Title: The scaffold protein Magi-1 regulates voltage-dependent sodium channels in dorsal rott ganglion neurons

Authors: *K. D. PRYCE¹, R. POWELL², A. BHATTACHARJEE¹

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Abstract: The voltage-gated sodium channel Nav1.8 is preferentially expressed in small- and medium- sized DRG neurons specialized for the detection of noxious stimuli. The underlying molecular machinery responsible for the trafficking and membrane expression of Nav1.8 in dorsal root ganglion (DRG) remains to be fully elucidated. In this study, we have identified the Membrane-associated Guanylate Kinase with Inverted Orientation -1 (Magi-1), a widely expressed multi-PDZ (PSD-95/Dlg-A/ZO-1) and WW domain containing protein as a channel partner that binds directly to NaV 1.8 and plays an essential role in the functional expression of Nav1.8. Here, we used immunohistochemistry, electrophysiology, and membrane biotinylation assays to characterize the physiological significance of the Magi-1/Nav1.8 interaction in DRG neurons. We find that like Nav1.8, Magi-1 was preferentially expressed in small- and mediumsized DRG neurons. We used small interfering RNAs to knockdown Magi-1 in DRG neuronal cultures and observed a profound decrease in the ability for DRG neurons to fire action potentials and a concomitant decrease in the total sodium current as compared to a negative control siRNA. To further elucidate the contribution of Nav1.8 after Magi-1 knockdown, we use tetrodotoxin (TTX) to block all TTX-sensitive sodium currents and verified that Magi-1 knockdown caused a significant and substantial decrease in both TTX-sensitive and TTXresistant sodium currents. To further determine how Magi-1 regulates Nav1.8, we conducted membrane biotinylation assays and demonstrated that after Magi-1 knockdown there was a 50% reduction in the surface expression of Nav1.8. Heterologous expression of Nav1.8 is notoriously difficult requiring accessory proteins, however, co-expression of Nav1.8 with Magi-1 lead to observable currents in heterologous cells. These findings demonstrate that Magi-1 controls the membrane expression of Nav1.8 in DRG neurons and that a downregulation of Magi-1 results in neuronal hypo-excitability. These data suggest that Magi-1 plays a central role in the functional expression of Nav1.8 and that Magi-1 is essential in regulating neuronal excitability through their interaction with sodium channels.

Disclosures: K.D. Pryce: None. R. Powell: None. A. Bhattacharjee: None.

288. Sodium Channels

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Topic: B.04. Ion Channels

Support: Dept. Veterans Affairs

Title: Dual action of CBZ as a blocker and modulator of activation of mutant $Na_v 1.7$ channels that cause inherited erythromelalgia

Authors: *T. ADI^{1,2}, M. ESTACION^{1,2}, Y. YANG^{1,2}, S. D. DIB-HAJJ^{1,2}, S. G. WAXMAN^{1,2} ¹Dept. of Neurol., Yale Univ. Sch. of Med., New Haven, CT; ²Ctr. for Neurosci. and Regeneration Research, Rehabil. Res. Ctr., VA Connecticut Healthcare Syst., West Haven, CT

Abstract: Inherited erythromelalgia (IEM), a severe pain disorder characterized by intense burning pain and redness of the extremities, is caused by gain-of-function dominant mutations to voltage-gated sodium channel Na_v1.7. In the past, pharmacotherapy for IEM has generally been reported to be ineffective. We have previously shown that carbamazepine (CBZ) is effective for two IEM mutants (S241T and V400M) *in vitro* as well as *in vivo*. In principle, CBZ might act via either of two mechanisms: 1) the classical mechanism of use-dependent block, and/or 2) by correcting hyperpolarized shifts in activation, returning activation voltage-dependence towards more wild-type Na_v1.7 values. We have shown that CBZ restores wild-type-like activation in the S241T and V400M Na_v1.7 IEM mutations.

Here we report another IEM mutant, Nav1.7-I234T (I234T), that responds to CBZ pretreatment with a depolarization of activation. As with V400M and S241T, CBZ normalizes the hyperpolarized voltage-dependence of activation and reduces hyperexcitability of DRG neurons expressing I234T. Use-dependent current reduction of I234T is not enhanced by CBZ, suggesting that the classical mechanism of use-dependent block is not a large contributing factor to this reduction in firing. We then implemented a moderate throughput automated patch-clamp system (Nanion Patchliner) to perform voltage-clamp on HEK293 cell lines. We successfully recapitulated the CBZ effect on activation of the S241T and V400M mutant channels as observed using manual patch-clamp, and went on to investigate the effects of additional drugs (CBZ derivatives oxcarbazepine and licarbazepine, and other local anesthetic and antiepileptic drugs). A principal concern was to utilize clinically relevant concentrations of clinically-used drugs equivalent to achievable therapeutic ranges *in vivo*. We assessed drug effects on multiple modes of channel gating (activation, fast-inactivation, and slow-inactivation) as well as effects on use-dependent block and dose-response, thereby providing a more detailed insight into both mechanism of drug action as well as inherent biophysical properties of the mutants. We predict

that this *in vitro* pharmacology approach may mitigate the trial-and-error treatment of IEM patients and promote a more personalized approach to pain pharmacotherapy.

Disclosures: T. Adi: None. M. Estacion: None. Y. Yang: None. S.D. Dib-Hajj: None. S.G. Waxman: None.

Poster

288. Sodium Channels

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Support: Grant from the Medical Research Service and Rehabilitation Research Service, Dept. of Veterans Affairs

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

Title: A gain-of-function Nav1.9 mutation from patients with peripheral neuropathy is rescued by low temperature

Authors: *S. D. DIB-HAJJ^{1,2,3}, C. HAN^{1,2,3}, M. SOPACUA⁴, J. HOEIJMAKERS⁴, F. DIB-HAJJ^{1,2,3}, P. SHAH^{1,2,3}, R. ALMOMANI⁵, M. GERRITS⁵, L. MACALA^{1,2,3}, G. LAURIA⁶, I. MERKIES^{4,7}, C. FABER^{4,5}, S. G. WAXMAN^{1,2,3}

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Abstract: Sodium channel Nav1.9 is preferentially expressed in peripheral nociceptive somatosensory neurons and visceral afferents, and myenteric neurons. Gain-of-function mutations of Nav1.9 have been linked to human painful disorders, including painful peripheral neuropathy and familial episodic pain, and painless channelopathies. Identification and functional assessment of new Nav1.9 mutations will help to elucidate the phenotypic spectrum of Nav1.9 channelopathies. Here we report a novel Nav1.9 mutation, isoleucine 807 substitution by valine (I807V) in the domain II S6 segment, which was identified from two 59 year old unrelated patients. Both patients displayed painful symptoms including tingling in their hands and feet. One patient experienced dysautonomic symptoms including hyperhidrosis, palpitations, diarrhea and constipation. They both were diagnosed with small fiber neuropathy based on their clinical

symptoms and abnormal quantitative sensory testing results. Our initial attempts to characterize this mutation after incubation of transfected neurons at 37°C were hampered because of the small amplitude of the Nav1.9 current. The mutant channel current was rescued by incubating transfected neurons at 30°C. Voltage-clamp analysis shows a 5.3 mV hyperpolarizing shift of activation albeit with a reduced current density compared with wild-type channels. There was no effect on channel steady-state fast inactivation. The hyperpolarized activation combined with unaffected fast inactivation indicate an increased window current within the physiological voltage domain close to the resting membrane potential of neurons. Since Nav1.9 channel plays an important role in regulating resting potential and prolonging the depolarizing response to subthreshold stimuli, the increased window current predicts that the expression of Nav1.9 mutant channels would render DRG neurons hyperexctiable, consistent with pain symptoms observed from the two patients carrying this Nav1.9-I807V mutation. The fact that lowering temperature rescues the current amplitude suggests that this mutant channel may play a more important role in peripheral terminals, close to a skin temperature of 32-33°C, rather than at the soma or central terminals which are exposed to a physiological temperature of 37°C.

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Poster

288. Sodium Channels

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Topic: B.04. Ion Channels

Support: Grant from Medical Research Service and Rehabilitation Research Service, Dept. of Veterans Affairs

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

Title: From Nav1.7 to Nav1.8: A case for precision medicine

Authors: *C. HAN^{1,2,3}, A. THEMISTOCLEOUS⁴, L. MACALA^{1,2,3}, I. BLESNEAC⁴, C. FRATTER⁵, D. L. BENNETT⁴, S. D. DIB-HAJJ^{1,2,3}, S. G. WAXMAN^{1,2,3} ¹Dept. of Neurology, Yale Uni. Sch. of Med., New Haven, CT; ²Ctr. for Neurosci. & Regeneration Research, Yale Univ. Sch. of Med., New Haven, CT; ³Rehabil. Res. Center, VA Connecticut Healthcare Syst., West Haven, CT; ⁴Nuffield Dept. of Clin. Neurosci., Oxford, United Kingdom; ⁵Oxford Med. Genet. Laboratories, Oxford Univ. Hosp. NHS Fndn. Trust, Oxford, United Kingdom
Abstract: Voltage-gated sodium channel Nav1.7 and Nav1.8 are preferentially expressed in peripheral sensory neurons and are important contributors to pain signaling. Gain-of-function mutations in Nav1.7 and Nav1.8 have also been identified in patients with painful peripheral neuropathies. Using atomic structure modeling, mutant cycle analysis and pharmacological testing, we predicted the responsiveness of the Nav1.7 mutation S241T mutation to carbamazepine (CBZ), based on a similar behavior of a "seed" mutation V400M that was known to confirm responsiveness to CBZ. With pre-treatment of cells expressing either the S241T or the V400M with clinically-relevant concentrations of CBZ (30 uM), the mutation-induced hyperpolarizing shift of activation of Nav1.7 channels was corrected. Based on these in vitro data, a double-blind placebo-controlled clinical trial on two patients carrying the S241T mutation demonstrated clinical efficacy of CBZ treatment. Here we present a study on a novel Nav1.8 mutation which suggests that this precision medicine approach can be extended to another channel. A 67-year old male patient with type 2 diabetes and neuropathic pain showed a total loss of intra epidermal nerve fiber density in a skin biopsy (0.0 fibers/mm), and profoundly and bilaterally reduced thermal and mechanical thresholds in QST testing. Genetic analysis identified a missense mutation in Nav1.8, S242T, which corresponds to the Nav1.7-S241T mutation. Voltage-clamp analysis of Nav1.8-S242T mutation demonstrated a hyperpolarized and accelerated activation, slowed deactivation as well as enhanced slow-inactivation, gain-offunction features that are shared with the Nav1.7-S241T channels. However, unlike the Nav1.7-S241T mutation, Nav1.8-S242T channels show enhanced fast-inactivation. Current-clamp analysis showed that like the Nav1.7-S241T mutation, Nav1.8-S242T mutant channels render DRG neurons hyperexcitable, manifested as reduced current threshold, hyperpolarized voltage threshold and increased evoked firing. The increased excitability of DRG neurons is consistent with pain symptoms reported by the patient carrying this Nav1.8-S242T mutation. Based on the location of this mutation, we hypothesized that Nav1.8-S242T channels should respond to preincubation with CBZ. Voltage-clamp recordings confirmed that 30 µM carbamazepine depolarizes activation of Nav1.8-S242T, similar to the CBZ effect on the Nav1.7-S241T mutant channels. Our data show that the DI/S4-S5 linker plays an important role in the gating of Nav1.8, and suggest that treatment of patients carrying this mutation may be effective to treat pain.

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Poster

288. Sodium Channels

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Topic: B.04. Ion Channels

Support: PROPANE 602273

Dept. Veterans Affairs

Title: Gain-of-function sodium channel beta2 mutation in idiopathic small nerve fiber neuropathy

Authors: *M. R. ESTACION^{1,2}, M. ALSALOUM^{1,2}, C. G. FABER³, G. LAURIA⁴, M. SOPACUA³, M. M. GERRITS⁵, S. DIB-HAJJ^{1,2}, I. S. J. MERKIES^{3,6}, S. G. WAXMAN^{1,2} ¹Neurol, Yale Univ. Sch. of Med., New Haven, CT; ²Ctr. for Neurosci. and Regeneration Res., Veterans Affairs Med. Ctr., West Haven, CT; ³Neurol., Univ. Med. Ctr. Maastricht, Maastricht, Netherlands; ⁴Neuromuscular Dis. Unit, IRCCS Fndn., Milan, Italy; ⁵Dept of Clin. Genomics, Univ. Med. Ctr. Maastricht, Maastricht, Netherlands; ⁶Neurol., Spaarne Hosp., Hoofddorp, Netherlands

Abstract: Small nerve fiber neuropathy (SFN) often occurs without identifiable causes, but recent targeted genetic studies have been performed in patients with idiopathic SFN (I-SFN). We sought to identify a genetic basis for I-SFN by screening patients with idiopathic SFN for novel mutations in the sodium channel genes which are preferentially and abundantly expressed in peripheral axons.

A patient referred with possible I-SFN who met criteria of ≥ 2 SFN-related symptoms, normal strength, tendon reflexes, vibration sense and nerve conduction studies, and reduced intraepidermal nerve-fiber density (IENFD) plus abnormal quantitative sensory testing (QST) and no underlying etiology for SFN, was assessed clinically, and screened for mutations in SCN9A, SCN10A, SCN11A but no mutations were found in those genes. Further analysis of sodium channel beta subunit genes revealed a mutation upon which we have performed functional analyses. Functional analysis using current-clamp revealed that heterologous expression of the mutant beta2 subunit rendered dorsal root ganglion neurons hyperexcitable compared wild-type beta2 subunit.

Sodium channel beta subunits have been reported to modulate the properties of voltage-gated sodium channels in a number of ways. Beta subunits can act to increase current density, alter inactivation kinetics, and modulate subcellular localization. In our recordings from small DRG neurons transfected with either WT or mutant beta2 subunits, an increase of stimulated excitability to expression of the mutant beta2 subunit suggests that the mutant beta2 subunit has altered regulation of one or more voltage-gated sodium channels. Since the difference in firing was largest at the stronger current injections that cause higher firing rates, we evaluated the relative fractions of fast (TTX-sensitive) vs the kinetically slower (TTX-resistant) sodium currents. In addition, we compared whether cumulative inactivation of either component was altered by the presence of the mutant bata2 subunit. Results of this analysis will be presented.

Disclosures: M.R. Estacion: None. M. Alsaloum: None. C.G. Faber: None. G. Lauria: None. M. Sopacua: None. M.M. Gerrits: None. S. Dib-Hajj: None. I.S.J. Merkies: None. S.G. Waxman: None.

288. Sodium Channels

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Topic: B.04. Ion Channels

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

Erythermalgia Association

Paralyzed Veterans of America

Title: Atypical changes in DRG neuron excitability and complex pain phenotype associated with a Nav1.7 mutation that massively hyperpolarizes activation

Authors: ***J. HUANG**¹, B. TANAKA¹, M. ESTACION¹, S. LIU¹, S. M. WALKER², S. D. DIB-HAJJ¹, S. G. WAXMAN¹

¹Dept. of Neurol. and Ctr. for Neurosci. and Regeneration Res., Yale Univ. Sch. of Med., West Haven, CT; ²Developmental Neurosci., UCL Great Ormond Street Inst. of Child Hlth., London, United Kingdom

Abstract: Sodium channel Na_v1.7 is known to play a central role in human pain signaling, with dominant gain-of-function mutations causing severe pain syndromes including inherited erythromelagia (IEM) and recessive loss-of-function mutations of Na_v1.7 producing congenital insensitivity to pain. IEM is caused by mutations of Na_v1.7 that hyperpolarize activation, with most of them shifting activation of by 5-12 mV. When expressed within dorsal root ganglion (DRG) neurons, these mutant channels depolarize resting membrane potential (RMP) by 4-6 mV and produce DRG neuron hyperexcitability that underlies a relatively stereotyped clinical phenotype of severe distal limb pain triggered by mild warmth. The I234T Na_v1.7 mutation hyperpolarizes activation by 18 mV and is associated with a more complex clinical phenotype that can include IEM-like distal pain, proximal pain, corneal anesthesia, and absence of pain in response to some injuries. How a mutation that produces a gain-of-function in Na_v1.7 causes loss of pain sensibility has remained enigmatic. Here we document additional painless injuries (painless fractures, painless venipuncture and injections) associated with the I234T mutation, and elucidate a possible mechanism by which expression of I234T mutant channels result in loss of excitability in a subpopulation of DRG neurons.

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288. Sodium Channels

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Topic: B.04. Ion Channels

Support: Medical Research Service and Rehabilitation Research Service, Dept. of Veterans Affairs

Title: Myosins interact with voltage gated sodium channels, sodium calcium exchangers and sodium potassium ATPases

Authors: *B. DASH, C. HAN, E. J. AKIN, S. DIB-HAJJ, S. G. WAXMAN Neurol., Yale Univ. Dept. of Neurol., West Haven, CT

Abstract: The distinct electrical properties of axonal and dendritic membranes, and the ionic properties of other cellular membranes are largely a result of the ionic balance maintained by a multitude of transmembrane (TM) proteins such as ion channels (e.g., voltage gated sodium channels/Na_v), ion exchangers (e.g., sodium calcium exchangers/NCX), ion pumps (sodium potassium ATPase/NKA); etc. The synthesis, transport, sorting to different cellular compartments, and insertion of these TM proteins into neuronal or other cellular membranes is highly regulated. The mechanisms by which these TM proteins reach their destinations are not well understood. We hypothesized that the actin based motor proteins, myosins known to transport various cellular cargoes, might play an important role in the trafficking of Nav, NCX, and NKA to the cellular membranes. To this end we used co-immunoprecipitation (Co-IP) and western blot (WB) assays, patch clamp electrophysiological recording and total internal reflection fluorescence (TIRF) microscopy using multiple substrates such as young or adult animal (mouse or rat) brain samples, rat dorsal root ganglion (DRG) or hippocampal neurons, ND7/23 cells and/or transiently or stably transfected HEK cells. Our results indicate that one or more isoforms of the class II non-muscle myosins such as myosin heavy chain 9 (myh9), myosin heavy chain 10 (myh10) or myosin heavy chain 14 (myh14) interact with Nav, NCX2 and NKA al. Additionally, we found that other myosins such as Myo5a, Myo6 or Myo19 also interact with NKA a1. Voltage clamp recordings from transfected ND7/23 cells show that myh10 regulates current density and gating properties of Nav1.8. Overexpression of myh10 in rat DRGs also led to broadening of action potentials. Based on these findings we hypothesize that these novel interactions of myosins with TM proteins (Na_v, NCX2 and NKA α 1) may play an important role in maintaining cellular sodium, calcium and/or potassium homeostasis which might be exploited for developing therapeutics for pain or other neuronal or non-neuronal diseases.

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288. Sodium Channels

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Program#/Poster#: 288.12/D33

Topic: B.04. Ion Channels

Title: Targeting pain pathways by inhibition of voltage-gated sodium channels

Authors: *J. L. COSTANTIN¹, A. OBERGRUSSBERGER², S. STÖLZLE-FEIX², N. BECKER², C. HAARMANN², M. RAPEDIUS², T. GOETZE², I. RINKE-WEIß², E. DRAGICEVIC², C. BOT¹, R. HAEDO¹, M. GEORGE², A. BRÜGGEMANN², N. FERTIG² ¹Nanion Technologies, Livingston, NJ; ²Nanion Technologies, Munich, Germany

Abstract: Chronic and neuropathic pain is a significant health problem affecting millions of people worldwide each year, with 1 in 5 individuals experiencing moderate to severe chronic or persistent pain. Neuropathic pain serves no protective or healing purpose and appears to arise due to an increase in intrinsic nerve excitability. Voltage-gated Na channels (NaV) are attractive targets for the treatment of these conditions due to their physiological role in action potential generation and propagation and, thus, neuronal excitability. Most of the clinically available pain therapeutics targeting NaV channels are rather non-selective and are associated with cardiotoxic and CNS side effects. Nav1.7 is found primarily in the peripheral nervous system and is thought to play a role in nociception and pain sensing. Recently, venom isolated from different species including spider and centipede have been shown to selectively block Nav1.7 and are powerful analgesics in animal models of pain. Similarly, mutations in the SCN9A gene have been shown to result in loss of function of the Nav1.7 channel in patients with congenital indifference to pain (CIP). The Nav1.8 gene (originally named PN3 or SNS; gene symbol SCN10A) encodes a NaV channel, and is selectively expressed in dorsal root ganglion (DRG) neurons. In contrast to the fast and rapidly inactivating TTX-sensitive channels, Nav1.8 is TTX-resistant, with slower kinetics, and a depolarized voltage-dependence of activation and inactivation. We present data of Nav1.7 and Nav1.8 on a novel high throughput screening patch clamp platform. Nav1.7 was expressed in CHO cells and the current voltage relationship recorded was consistent with Nav1.7 obtained using other methods. Vhalf of activation was -24 mV (n = 275). Using a double step voltage protocol we were able to investigate whether compounds, such as tetracaine, exhibit state dependence. We show that tetracaine exhibited a lower IC₅₀ on the second pulse, i.e. the inactivated state of the receptor, compared with the resting state. Nav1.8 expressed in CHO cells started to activate at approximately -40 mV, peaking at between 10 mV and 20 mV with a Vhalf of activation of -2.7 mV (n = 380). In order to study NaV channels involved in pain pathways in a more physiological environment, we used stem cell-derived neurons, more specifically with an overexpression of Nav1.8. In these cells, endogenous NaV-mediated currents were recorded with activation parameters consistent with Nav1.7 (i.e. blocked by TTX in the nM range). Moreover,

information about the role of NaV channels in neuronal signalling in intact neuronal networks was gleaned using microelectrode array (MEA) technology.

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Poster

288. Sodium Channels

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 288.13/D34

Topic: B.04. Ion Channels

Title: A small molecule activator of $Na_v 1.1$ channels increases fast-spiking interneuron excitability and GABAergic transmission *In vitro* and has an anti-convulsive effect *In vivo*

Authors: *C. HOUGAARD¹, K. FREDERIKSEN², D. LU³, J. YANG³, H. S. JENSEN², J. F. BASTLUND², C. YANG³, M. GRUNNET² ¹Synaptic Transmission In Vitro, ²H Lundbeck A/S, Valby, Denmark; ³Shanghai ChemPartner Co, Shanghai, China

Abstract: Fast-spiking parvalbumin-positive GABAergic interneurons mediate the fast rhythmic synaptic inhibition of pyramidal cells associated with gamma oscillations - a mechanism for neuronal processing of information related to memory and cognitive performance. Inappropriate function of fast-spiking interneurons is associated to decreased cognitive capabilities. Nav1.1 (SCN1A) channels, located in GABAergic fast-spiking interneurons are pivotal for action potential generation and propagation in these neurons. Impaired Nav1.1 channel function is linked to various diseases in the central nervous system, including seizure disorders, autistic behavior and intellectual disability.In the present study we have identified a small molecule activator of Nav1.1 channels (3-Amino-5-(4-methoxyphenyl)thiophene-2-carboxamide, AA43279). In whole-cell voltage-clamp recordings using HEK-293 cells expressing human

Na_v1.1 channels, AA43279 induced a concentration-dependent increase in the Na_v1.1 current mainly by impairing the fast inactivation kinetics of the channels. In hippocampal brain slices from Sprague Dawley rats, AA43279 augmented action potential firing recorded from fast-spiking interneurons and increased the frequency of spontaneous inhibitory post-synaptic currents (sIPSCs) recorded in pyramidal neurons. On the other hand, AA43279 had no effect on action potential firing in pyramidal neurons. When tested *in vivo*, AA43279 had anti-convulsive properties in the maximal electroshock seizure threshold test. AA43279 was tested for off-target effects on 72 different proteins, including other Na_v1.X channels and exhibited reasonable selectivity towards Na_v1.1 channels.Taken together, AA43279 could constitute a valuable tool for revealing physiological and pathophysiological functions of Na_v1.1 channels.

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Poster

288. Sodium Channels

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Title: Hyperexcitability of GABAergic neurons triggers cortical spreading depression: New mechanism of migraine?

Authors: O. CHEVER¹, S. ZERIMECH¹, M. AYRAULT¹, F. DUPRAT¹, *M. A. MANTEGAZZA^{2,1} ¹CNRS, Inst. of Mol. and Cell. Pharmacol., Valbonne, France; ²Inst. of Mol. and Cell. Pharmacol., Valbonne, France

Abstract: Migraine is a common episodic brain disorder; 30% of patients have transient neurological disturbances before the headache, called aura. A mendelian form of migraine with aura, Familial Hemiplegic Migraine (FHM), has allowed to disclose some molecular/cellular pathological mechanisms. In FHM1 and 2 (Ca_V2.1 Ca²⁺ channel and Na⁺K⁺ATPase mutations, respectively), different dysfunctions lead to excessive glutamatergic transmission that facilitates CSD. FHM3 is caused by mutations of Na_V1.1 (*SCN1A*) Na⁺ channel, which is particularly important for GABAergic neurons' excitability. Epileptogenic loss-of-function Na_V1.1 mutations lead to hypoexcitability of GABAergic neurons, reduced inhibition and network hyperexcitability. Conversely, we have shown that FHM3 mutations can cause Na_V1.1 gain-of-

function and GABAergic neurons' hyperexcitability in transfected cells, suggesting a novel migraine mechanism. However, it is not clear how this could lead to CSD/migraine and there is no causal link yet between GABAergic neurons' hyperexcitability and CSD.

We addressed this issue performing selective optogenetic stimulation of GABAergic neurons in cre/lox mice and studying the neurophysiological correlate of aura: cortical spreading depression (CSD): a wave of transient intense neuronal firing followed by a long lasting depolarization that could lead to headache. We found that increased activity of GABAergic neurons can by itself trigger CSD. The use of selective pharmacological blockers showed that activation of GABAergic or glutamatergic ionotropic receptors is not necessary for CSD initiation, whereas efflux of K⁺ ions caused by interneurons' firing can induce $[K^+]_{out}$ build-up, leading to initiation of CSD. Notably, the optogenetic stimulation of GABAergic neurons was able to ignite CSD also in vivo.

Thus, we have provided a causal relationship between initial hyperactivity of GABAergic neurons, progressive increase of $[K^+]_{out}$ at the site of CSD initiation, and CSD ignition. Neuronal firing-induced K^+ build-up is the key factor for CSD ignition in this optogenetic model of FHM-3, differently than in models of FHM-1/-2. This is a novel mechanism that may be at play in FHM-3 and possibly also in other types of migraine.

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Poster

288. Sodium Channels

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Title: Akt regulates neuronal excitability by modulating Nav1.6 sodium channel

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Abstract: Protein kinase B, also known as Akt, is a serine/threonine kinase that receives converging signals from multiple transmembrane receptors. Activation of Akt is critical in providing protection against neuronal hyperexcitability and synaptic dysfunction. Yet, the molecular mechanisms underlying this pathway are still poorly understood. Phosphorylation plays an essential role in regulating function of voltage-gated sodium (Nav) channels with profound effects on intrinsic excitability and activity-dependent plasticity. Here, we hypothesized that some of the known modulatory effects of Akt on excitability could arise from direct regulation of the Nav1.6 channel isoform that is enriched at the axonal initial segment (AIS) and mediates repetitive firing in neurons throughout the cortico-limbic circuit. To test this hypothesis, we determined the effect of triciribine, a pan Akt inhibitor, on Na⁺ currents in HEK293 cells stably expressing the Nav1.6 channel isoform using whole-cell patch-clamp. We found that 1 hour exposure to triciribine significantly potentiates Nav1.6 peak current density (at voltage step of -10 mV was -47.8±9.4 pA/pF in control, n=11 versus -99.4±14.6 pA/pF in triciribine group, n=11; p<0.01 with Student *t*-test) with no changes in voltage-dependence of activation and steady-state inactivation. To provide correlative evidence for this effect, we performed patch-clamp recordings in hippocampal slices and found that triciribine increases repetitive firing of CA1 pyramidal neurons (number of spikes was 15.1±3.3 in control, n=10 versus 22.9 \pm 2.4 in the triciribine group, n=10; p<0.05 with Mann-Whitney test), and decreased action potential current threshold (124±17.1 pA in control, n=10 versus 75±13.4 in triciribine group, n=10; p<0.05 with Student *t*-test). To determine whether inhibition of Akt resulted in changes in Nav1.6 expression and pattern distribution that might explain increase in excitability, we studied the effect of triciribine on Nav1.6 expression level at the AIS in primary hippocampal cultures. Akt inhibition over a prolonged period of time (12 hour) resulted in upregulation of Nav1.6 channel expression at the AIS (ratio of Nav1.6 to PanNav was 0.5±0.2 in control, n=12 versus 1.1±0.2, n=13 in triciribine group; p<0.05 with Student *t*-test) along with a shift in Nav immunoreactivity toward the distal, more excitable segment of the AIS (4.1±0.5 µm in control, n=15 versus 5.8±0.5 µm in triciribine group, n=14; p<0.05 with Student *t*-test). In summary, we provide a novel mechanism by which Akt might regulate neuronal excitability through a direct effect on Nav1.6 channel function and sub-cellular distribution.

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Poster

288. Sodium Channels

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Title: Disease-specific mechanism of action of voltage-gated sodium channel allosteric modulators in a mood disorder mouse model

Authors: *K. E. WINTERS

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Abstract: Protein: protein interactions (PPI) offer unexploited opportunities for probe development in the CNS. The voltage-gated Na+ (Nav) channel complex comprises of a matrix of interacting proteins including fibroblast growth factor 14 (FGF14), a regulator of neuronal excitability in the cortico-mesolimbic circuit. The FGF14:Nav1.6 complex is also known to be part of the glycogen synthase kinase 3 (GSK3) pathway, which is implicated in various neuropsychiatric disorders, including mood disorder such as anxiety and depression. However, little is known about how the interaction between the FGF14, Nav1.6 and GSK3 might contribute to changes in excitability underlying any of these disorders. Here, we present evidence for a disease-specific mechanism of action of a novel peptidomimetic probe, ZL181, which targets the PPI interface of the FGF14:Nav1.6 channel complex. Using whole-cell patch clamp electrophysiology in the acute brain slice preparation, we show that 1 hour incubation with 50 µM of ZL181 increases intrinsic excitability in medium spiny neurons (MSN) of the nucleus accumbens (NAc) in the GSK3 knock-in mouse model of mood disorder. Specifically, compared to DMSO controls, ZL181 treated MSNs exhibited significantly increased number of spikes $(6.3\pm3.5 \text{ spikes in control}, n=3 \text{ versus } 22\pm4.4 \text{ spikes in the ZL181 group}, n=5, \text{ values taken at}$ current step of 120 pA; p<0.05 with Student *t*-test), increased instantaneous firing (9.6±5.2 Hz in control, n=3 versus 30.9±5.8 Hz in the Zl181 group, n=3, values taken at current step of 120 pA; p<0.05 with Student *t*-test), and lower action potential current threshold (116.7±23.3 pA in control, n=3 versus 44±5.1 pA in the Zl181 group, n=5; p<0.01 with Student *t*-test) These data can be contrasted with supporting ongoing studies which demonstrate that ZL181 decreases excitability in MSNs of wild-type mice. Overall, these results suggest disease-specific remodeling of the Nav channel complex that might be of interest for mood disorder therapeutic development.

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288. Sodium Channels

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Topic: B.04. Ion Channels

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Title: Mechanism of chemical induced peripheral neuropathy (cipn) by vincristine

Authors: *H. LEE¹, M. JOUNG², Y. CHOI², H. SUH⁴, S. JUNG³ ¹Physiol., Hanyang Univ. Col. of Med., Seoul, Korea, Republic of; ³Physiol., ²Hanyang Univ., Seoul, Korea, Republic of; ⁴Phamacology, Hallym Univ., Chooncheon, Korea, Republic of

Abstract: The possible roles of NaV1.8 channels located in the dorsal root ganglia (DRG) and the spinal cord in the regulation of chemotherapy-induced peripheral neuropathy were studied in ICR mice. We found in the present study that repeated intraperitoneal (i.p.) injections with vincristine (0.1 mg/kg per day for 7 consecutive days), caused an elevation of the hargreaves latency and reduction of pain threshold in von-frey test. The NaV 1.8 channel expression was increased in DRG vincristine-induced peripheral neuropathy animal model. Next, the NaV1.8 channels activities in DRG cells were magnified in vincristine-induced peripheral neuropathy animal models. Moreover, The electrical activities of NaV1.8 channels of nerves were far sensitive in all vincristine-induced peripheral neuropathy animal models. In addition, A803467, a selective NaV1.8 channel blocker, inhibited NaV1.8 channel activity in DRG, revealing IC50 value of A803467 is 150±20 nmole.

Furthermore, the i.p. administration with A803467 (from 20 to 80 mg/kg) attenuated vincristineinduced neuropathy as manifested in both hargreaves and Von-frey tests in a dose-dependent manner. Our results suggest that NaV1.8 channels located in DRG and the spinal cord play important roles for the production of vincristine-induced peripheral neuropathy.

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Poster

288. Sodium Channels

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Title: Strong G-protein-mediated voltage-dependent inhibition of voltage-gated sodium channels

Authors: *G. B. MATTHEISEN¹, T. TSINTSADZE², S. M. SMITH³ ¹PCCM, OHSU, Portland, OR; ³PCCM, ²VAPORHCS/OHSU, Portland, OR

Abstract: Voltage-gated sodium channels (VGSCs) are strategically positioned to mediate cellular plasticity due to their influence on action potential waveform. VGSC function may be strongly inhibited by local anesthetic and antiepileptic drugs and modestly modulated via second messenger pathways. Here we report that allosteric modulators of the calcium-sensing receptor (CaSR), cinacalcet, calindol, calhex, and NPS 2143, completely inhibit VGSC current in cultured and acutely isolated mouse neocortical neurons. This inhibition of VGSC current persisted in CaSR deficient neurons, indicating a CaSR-independent mechanism. Cinacalcet-mediated blockade of VGSCs was prevented by the GDP analog GDP β s, indicating G-proteins mediated this effect. However, unlike other reports of VGSC modulation, cinacalcet-mediated inhibition was independent of protein kinase A or C activation. Cinacalcet negatively shifted steady-state inactivation of VGCs and inhibition was reversed by prolonged hyperpolarization. These data identify a dynamic signaling pathway by which G-proteins regulate VGSC current to indirectly modulate neuronal excitability.

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Poster

288. Sodium Channels

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Topic: B.04. Ion Channels

Support: National Natural Sciences Foundation of China (81503056 and 81470163),

Title: Loperamide inhibits sodium channels to alleviate inflammatory hyperalgesia

Authors: *H. YU, Y. WU, M. LI, X. WANG Pharmacol., Inst. of Materia Medica, Chinese Acad. of M, Beijing City, China

Abstract: Previous studies demonstrated that Loperamide, originally known as an anti-diarrheal drug, is a promising analgesic agent primarily targeting mu-opioid receptors. However some evidences suggested that non-opioid mechanisms may be contributing to its analgesic effect. In the present study, Loperamide was identified as a Nav1.7 blocker in a pilot screen. In HEK293 cells expressing Nav1.7 sodium channels, Loperamide blocked the resting state of Nav1.7 channels (IC50=1.86±0.11 µM) dose-dependently and reversibly. Loperamide produced a 10.4 mV of hyperpolarizing shift for the steady-state inactivation of Nav1.7 channels without apparent effect on the voltage-dependent activation. And the drug displayed a mild use- and state-dependent inhibition on Nav1.7 channels, which was removed by the local anestheticinsensitive construct Nav1.7-F1737A. But inhibition of Nav1.7 at resting state was not altered significantly by the F1737A mutation. Compared to its effects on Nav1.7, Loperamide exhibited higher potency on recombinant Nav1.8 channels in ND7/23 cells (IC50=0.60±0.10 µM) and weaker potency on Nav1.9 channels (3.48±0.33µM). Notably more pronounced inhibition was observed in the native Nav1.8 channels (0.11±0.08 µM) in DRG neurons. Once mu-opioid receptor was antagonized by Naloxone in DRG neurons, potency of Loperamide on Nav1.8 was identical to that of recombinant Nav1.8 channels. The inhibition on Nav channels might be the possible mechanism of Loperamide for pain relief beyond mu-opioid receptor. In the meanwhile, the opioid receptor pathway may also influence the blocking effect of Loperamide on sodium channels, implying a cross-talk between sodium channels and opioid receptors in pain processing.

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Poster

288. Sodium Channels

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Topic: B.04. Ion Channels

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Title: Site-directed mRNA editing of sodium channels has potential to alter neuronal firing and network dynamics: Computer models

Authors: *W. W. LYTTON¹, ***W. W. LYTTON**¹, ***W.** W. LYTTON², A. KNOX³, J. J. C. ROSENTHAL⁴ ¹SUNY Downstate, Brooklyn, NY; ²Neurol., Kings County Hosp., Brooklyn, NY; ³Neurol., Univ. of Wisconsin, Madison, Madison, WI; ⁴Marine Biol. Lab., Woods Hole, MA

Abstract: mRNA editing offers the potential for targeting transcripts in the living animal or patient, providing the possibility of treatment of underlying genetic diseases. Adenosine deamination in mRNA has was used to alter the conductance of mammalian fast sodiums (Naf) channels (NaV1.4), changing a lysine to an arginine residue in the region of the peptide that determines selectivity -- the aspartate-glutamate-lysine-alanine motif DEKA is altered to DERA. The removal of channel selectivity allowed passage of both potassium and sodium through the channel, altering the effective reversal potential of a proportion of these channels. The proportion of wild type (WT) versus edited channels (EC) can be controlled experimentally and will be controllable in vivo. We therefore looked at the effects of changes in the EC/WT proportion in a set of single cell models and in a cortical network model. In the Hodgkin-Huxley model, we demonstrated the expected reduction in action potential amplitude, with increase in EC/WT. At a point related to the size of the modeled axon (input impedance), and to the density of potassium channel, the action potential failed as the sodium channels could no longer source sufficient current to oppose the outward currents. Speed of conduction was also affected by the proportion of mutated channel. This was primarily noticeable at locations far from the axon terminal where a baseline velocity of 2 m/s was reduced by 20% before conduction failure occurred. By contrast, conductance speed near the axon terminal was faster, 10 m/s, and was preserved with increased EC/WT, as current build-up near the high-impedence boundary allowed charge to build up fast enough to compensate for the reduced drive. After compensating for the faster kinetics by reducing overall channel density, similar models could be run at mammalian temperatures as were run in the origianl models at 6.3 C. Modification of sodium channels has the potential for future clinical use for the epilepsies and for pain syndromes.

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Poster

288. Sodium Channels

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Title: Specific interaction of amphiphiles with sodium channels

Authors: Z. WANG¹, S. R. LEVINSON², *T. HEINBOCKEL¹ ¹Dept. of Anat., Howard Univ. Col. of Med., Washington, DC; ²Dept. of Physiol. and Biophysics, Univ. of Colorado Denver Sch. of Med., Denver, CO

Abstract: Amphiphiles are chemical compounds possessing both hydrophilic and lipophilic properties. They are widely used in pharmaceuticals, food, cosmetics and for drug delivery. Their effects include ameliorating symptoms of neurological pain, increasing blood brain barrier, enhancing transdermal drug delivery, and prolonging sensory-selective nerve blockade of local anesthetics. Their effects are thought to result from non-specific lipid-protein interactions, namely, a bilayer-protein hydrophobic coupling mechanism. Little is known about the molecular interaction between amphiphiles and target proteins. Here, we hypothesize that some amphiphiles specifically interact with sodium channels to exert their pharmacological action. Two groups of chemical analogues, Polysorbates and Spans, were selected to test the direct action on sodium channels using whole-cell patch-clamp recordings from transfected CHO cells that stably expressed the Nav1.2 sodium channel isoform. Polysorbate 20 and Polysorbate 80 display tonic inhibition of sodium currents, modulation of voltage-dependent availability and larger use-dependent block. However, Spans, which are chemically analogous to Polysorbates but lack the poly(-O-CH2Ch3) group in their hydrophilic head, fail to modulate the function of sodium channels. The results indicate that 1) not all amphiphiles modulate sodium channel function through a bilayer-protein hydrophobic coupling mechanism; 2) Polysorbates specifically interact with sodium channels to inhibit sodium channel function. The specific interaction with sodium channels provides direct evidence that the prolonged sensory-selective nerve blockade of anesthetics by amphiphiles is pharmacodynamic rather than pharmacokinetic, and will lead to the development of novel prolonged-duration local anesthetics and a novel therapeutic strategy for treatment of neuropathic pain.

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Poster

288. Sodium Channels

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Title: Gene therapy for Dravet syndrome: a proof of concept

Authors: *A. RICOBARAZA¹, M. VALENCIA¹, M. GONZÁLEZ-APARICIO¹, M. NICOLÁS¹, S. ARRIETA¹, M. BUÑUALES¹, E. PUERTA², R. SÁNCHEZ-CARPINTERO^{3,4}, G. GONZÁLEZ-ASEGUINOLAZA¹, J. ARTIEDA³, R. HERNÁNDEZ-ALCOCEBA¹

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Abstract: Dravet syndrome (DS) is a severe epileptic encephalopathy with infantile onset, characterized by refractory seizures, increased risk of sudden death, as well as mental, behavioral and motor comorbidities. In most cases, the genetic basis is a haploinsufficiency caused by mutations in SCN1A, which encodes the alpha subunit of a voltage-dependent Na⁺ channel (Nav1.1). Due to the complex physiopathology of DS, etiological approaches such as gene therapy have unique chances to obtain a global improvement in the life of these patients. We aim to deliver a functional copy of the SCN1A gene to the brain using High-Capacity adenoviral vectors (HC-Ad). To provide proof of concept about the feasibility of this approach we generated a preliminary vector prototype carrying a codon-optimized SCN1A cDNA under the control of a ubiquitous promoter sequence (CAG). The expression cassette inserted into the vector genome was stable in E. Coli and gave rise to viable HC-Ad particles following a standard rescue and amplification protocol. The resulting HCA-CAG-SCN1A vector was able to infect neurons and increase the amount of Nav1.1 in a dose-dependent manner. Biodistribution analysis using HC-Ad vectors encoding GFP demonstrated efficient transduction of neurons upon intracerebral administration. Finally, in vitro luciferase reporter assays were performed to select a regulatory sequence with preferential activity in GABAergic/paravalbumin-expressing inhibitory neurons. In summary, the results obtained so far indicate that gene therapy based on HC-Ad vectors is a viable option for the treatment of DS.

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Poster

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Title: The mechanism of propofol action on voltage-gated sodium channels

Authors: ***E. YANG**¹, D. GRANATA², Y. WANG³, R. G. ECKENHOFF⁵, P. TANG⁴, Y. XU⁶, V. CARNEVALE², M. COVARRUBIAS¹

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Abstract: Voltage-gated Na⁺ channels (Navs), which play a pivotal role in the electrical excitability of the central nervous system, are inhibited by clinically relevant concentrations of many general anesthetics and are thus highly relevant anesthetic targets. The molecular mechanisms of this inhibition, however, remain unclear. Here, we investigated the electrophysiological response of NaChBac and NaVMs, two anesthetic-sensitive bacterial homologs of eukaryotic Navs, to the intravenous anesthetic propofol at 2, 5, and 10 µM. In both NaChBac and NaVMs, propofol induced hyperpolarizing shifts of the pre-pulse inactivation and conductance-voltage (G-V) relationships, reduced the time constant of inactivation, and increased the time constant of deactivation in a dose dependent manner without significant effects on recovery from inactivation. Previous investigations suggested that general anesthetics might inhibit NaChBac by open channel block in a manner resembling local anesthetics. Contrary to predictions based on this mechanism, however, propofol induced hyperpolarizing shifts in the G-V curve with minimal effects on current amplitude and the rate of current decay in a non-inactivating NaChBac mutant at both 2 and 5 µM. Propofol may bind to the channel to stabilize the open state, ultimately facilitating activation-coupled inactivation, a mechanism that is supported by kinetic modeling. Guided by molecular dynamics simulations and ¹⁹F-NMR, we have evaluated putative propofol binding sites in the pore and voltage-sensing domains with electrophysiology and mutational analysis to identify structural determinants of Nav gating involved in modulation by propofol. With the photoactive propofol analog *m*-Azipropofol, we will use photoaffinity labeling and mass spectrometry to identify propofol binding sites in these Navs.

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Poster

288. Sodium Channels

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Title: Peptides mapping of the FGF14:Nav1.6 complex interface

Authors: *A. K. SINGH¹, P. A. WADSWORTH², O. O. FOLORUNSO³, S. R. ALI⁴, F. LAEZZA⁵

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Abstract: Voltage-gated sodium (Nav) channels provide the basis for electrical excitability in the brain. Nav channels are comprised of nine isoforms (Nav1.1-1.9), which are specifically regulated by accessory proteins such as intracellular fibroblast growth factor 14 (FGF14). FGF14 binds directly to the cytosolic domain of the Nav1.6 channel, and regulates its biophysical properties and expression, leading to significant effects on intrinsic excitability in neurons. In pursuit of probes that could modulate the FGF14:Nav1.6 complex, we designed and synthesized three peptides (FLPK, PLEV, EYYV) based on homology modeling studies of the FGF14:FGF14 homodimer and the FGF14:Nav1.6 channel complex. To evaluate the potency and mechanism of action of each peptide against the FGF14:Nav1.6 channel complex, we combined computational modeling, molecular dynamics, split-luciferase complementation assay (LCA), surface plasmon resonance (SPR) and whole cell patch-clamp electrophysiology. First, in silico peptide docking revealed that FLPK likely interacts with residues (V160 and Y158) at the interface of the FGF14:Nav1.6 homology model, and subsequent LCA validation supported this hypothesis. Our LCA results show that FLPK disrupts the FGF14:Nav1.6 channel complex interaction and the effect is abolished in the presence FGF14^{Y158N/V160N} mutations. Furthermore, SPR showed that FLPK has a high binding affinity for FGF14 (K_D=6.4µM) and no measurable affinity for Nav1.6 C-tail. Lastly, using whole-cell patch clamp electrophysiology, we show that FLPK prevents FGF14-dependent regulation of Nav1.6 currents, voltage-dependent activation and steady-state inactivation of Nav1.6 channels. In summary, these results show the FLPK disrupts the FGF14:Nav1.6 complex which could be important for therapeutic development against Nav channel dysfunctions.

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Poster

288. Sodium Channels

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MD-PhD Training Program in Health Disparities and Aging T32 (PW)

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Title: Functional modulation of voltage-gated sodium channel Nav1.6 by peptidomimetics

Authors: *O. FOLORUNSO¹, L. BOURNER², P. A. WADSWORTH³, S. R. ALI², Z. LIU², H. CEHN², J. ZHOU², F. LAEZZA²

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Abstract: Voltage-gated sodium (Nav) channels interact with auxiliary proteins, including intracellular fibroblast growth factor 13 (FGF13), which modulate their biophysical properties in different regions within the CNS and PNS. These protein-protein interactions (PPI) are necessary to maintain neuronal excitability, and FGF13 dysfunction is associated with epilepsy and neuropathic pain. The physiologically relevant Nav1.6 C-terminal tail binds to FGF13, and the functional specificity of this interaction provides an opportunity for the development of novel Nav isoform-specific probes. Here, we combined the split-luciferase complementation assay (LCA) with molecular modeling to screen various peptidomimetics to target intracellular FGF:Nav channel interfaces. We identified ZL192 as a potential regulator of the FGF13:Nav1.6 complex. We show that ZL192 is specific to the FGF13:Nav1.6 complex, and has no effect on other Nav1.6 interactors such as FGF14. Furthermore, our dose response experiments show that ZL192 action is specific to the FGF13-1b ($IC_{50} = 24.4 \mu M$) and not the FGF13-1a isoform. Next, we investigated the functional impact of ZL192 on HEK293 cells stably expressing Nav1.6 and transiently transfected with FGF13-1b-GFP using whole-cell patch clamp recordings. Our initial studies show that there is a significant decrease in the peak current density at multiple voltage steps (at -25mV, p= 0.022, ANOVA, Fisher's LSD) in FGF13-1b-GFP plus ZL192 compared to GFP-Nav1.6 cells, and a hyperpolarizing shift in voltage dependence of steady-state inactivation of the Nav1.6 channel in FGF13-1b-GFP plus ZL192, (p= 0.038, ANOVA post-hoc Bonferroni) which was not observed with FGF13-1b-GFP alone. Therefore, ZL192 might selectively modulate a pool of Nav1.6 channels associated with FGF13-1b leading to a directed effect on neuronal excitability, and a potential applicability for drug discovery in the epilepsy and pain fields.

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Poster

288. Sodium Channels

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Title: GSK3 β is a new molecular determinant of neuronal excitability

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Abstract: Glycogen synthase kinase 3 (GSK3) is a multifaceted enzyme with ubiquitous expression in the central nervous system (CNS). Increased levels of GSK3 trigger a cascade of serine/threonine (S/T) phosphorylation events that correlates with maladaptive plasticity of neuronal circuitries in neuropsychiatric disorders spurring an interest in identifying new molecular targets within this pathway for drug discovery purposes. In previous studies we have shown that changes in GSK3 levels modify neuronal excitability by regulating protein:protein interactions (PPI) between fibroblast growth factor 14 (FGF14) and the voltage-gated Na+ channel. Suppression of GSK3 leads to loss in neuronal excitability in hippocampal neurons with dispersion of the FGF14:Nav channel complex from the axonal initial segment. Based on in vitro phosphorylation studies showing that GSK3β phosphorylates both FGF14 and the Nav1.6 channel, we hypothesized that GSK3ß may form a stable complex with either FGF14 and Nav1.6. To test this hypothesis, we first applied the split-luciferase complementation assay (LCA) to screen the interactions between each of these proteins, as well as to show how complex formation is dependent upon regulation by cellular signaling pathways. Furthermore, Surface Plasmon Resonance (SPR) on E.coli purified proteins was used to assess putative binding affinity of GSK3^β to either FGF14 or Nav1.6, as well as to map respective interaction sites. SPR studies revealed strong binding affinity (K_D=0.159 μM) between FGF14 and GSK3β pointing for the C-tail of FGF14 as a necessary structural element mediating PPI. NMR studies using 15Nlabeled FGF14 further confirmed FGF14 binding to Nav1.6. Altogether, these results reveal

FGF14, Nav1.6 and GSK3 β as potential new structural determinants of neuronal excitability in the brain circuit.

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Poster

288. Sodium Channels

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(BRFSF_2015-05

Title: Sodium channel beta2 subunits prevent action potential propagation failures at axonal branch points

Authors: *M. B. HOPPA¹, I. CHO², L. PANZERA³, M. CHIN¹ ¹Biol. Sci., ³Biol., ²Dartmouth Col., Hanover, NH

Abstract: The arrival of an action potential (AP) at a presynaptic terminal dictates both calcium channel opening and the driving force for calcium entry, which are powerful levers modulating synaptic strength. Excitatory hippocampal neurons contain highly ramified axons (~200 branch points) that form a large number of en passant boutons (~8,000). Unmyelinated sections of axon are very diverse structures encompassing branch points and numerous presynaptic terminals with undefined molecular partners of sodium channels. At present, we have little idea about how cells regulate the density and subcellular distribution of ion channels to ensure AP delivery and sculpt the propagating waveform. This question has been particularly difficult to study in hippocampal neurons due to the fine structures of distal axonal branches that preclude detailed electrophysiological analysis. Here we have used a combination of optogenetic indicators to make subcellular measurements of both membrane potential and calcium influx at nerve terminals to study sodium channel distribution across the axon of cultured hippocampal neurons. Using these probes, we demonstrate that large variations in AP shape exist between branches of a single axonal arborization as well as disparate sensitivity to propagation blockade by TTX. We go on to show that these properties are independent of morphology and likely related to local molecular repertoires. We go on to demonstrate that Na^+ channel $\beta 2$ subunits ($Na_y\beta 2$) are required to prevent AP propagation failures across the axonal arborization of cultured rat hippocampal neurons. In the absence of Na_v β 2s, we identify two specific phenotypes: (1) membrane excitability and AP-evoked Ca^{2+} entry are reduced at synapses, and (2) AP

propagation is severely compromised with over 40% of axonal branches no longer responding to AP-stimulation. Thus, $Na_v\beta 2$ is a critical regulator of axonal excitability and synaptic function in unmyelinated axons. Given the fact that synaptic transmission is highly sensitive to changes Ca^{2+} influx controlled by the biophysical properties of the AP, this finding reveals a new important role for $Na_v\beta 2$ subunits to control the spread of excitability in the nervous system.

Disclosures: M.B. Hoppa: None. I. Cho: None. L. Panzera: None. M. Chin: None.

Poster

288. Sodium Channels

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Topic: B.04. Ion Channels

Support: BRFSG-2015-05

Title: Neurofascin-186 modulates axon initial segment morphpology and function

Authors: *S. ALPIZAR, M. B. HOPPA Biol. Sci., Dartmouth Col., Hanover, NH

Abstract: Voltage gated sodium channel (VGSC) types 1.2 and 1.6 are recruited to the axon initial segment (AIS) following the enrichment of the scaffolding protein AnkyrinG during development. The location and distribution of these VGSCs has a strong impact on neuronal excitability. Also uniquely localized at the AIS are cellular adhesion molecules (CAMs) such as Neuronal Cellular Adhesion Molecule and Neurofascin-186 (NF-186). While these molecules are known for their functions in neurite extension, axon formation, and neuron-glial interactions, they have recently been implicated in modulating AnkyrinG stability at the AIS, although mechanisms in which this occurs remain elusive. Our aim is to identify a potential role of NF-186 in stabilizing critical AIS components and therefore influencing structural parameters of the AIS. We also aim to elucidate the functional consequences of NF-186 loss on action potential (AP) firing. Here, using primary rat hippocampal cultures, we combined localization measurements of fluorescently tagged VGSCs with genetic ablation of Neurofascin-186 using CRISPR. These localization experiments were combined with optical AP measurements that allow for waveform analysis using QuasAr, a genetically encoded voltage indicator that provides accurate detection of single action potentials as well as high-frequency trains. Additionally, the ability to measure voltage changes at both the soma and distal axon allowed us to observe NF-186's influence on both VGSC types enriched at the AIS. The loss of NF-186 altered the localization of AnkyrinG within the axon. Here we show the impact of NF-186 ablation on VGSC location and dynamics at the AIS. NF-186 may play a direct role in AnkyrinG localization which influences VGSC distribution and dynamics at the AIS.

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Poster

289. Monamine Transporters

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Topic: B.05. Transporters

Support: DA035714

DA041932

Title: Allosteric modulatory effects of SRI-30827 on HIV-1 Tat protein-induced inhibition of human dopamine transporter function and cocaine condition place preference in inducible Tat transgenic mice

Authors: *J. ZHU

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Abstract: The inducible HIV-1 Tat transgenic (iTat) mouse model recapitulates many aspects of neurocognitive impairments observed in HIV infected individuals. Tat and cocaine synergistically increase synaptic dopamine (DA) levels by directly inhibiting DA transporter (DAT) activity, ultimately leading to dopaminergic neuron damage. This study determined allosteric modulatory effects of SRI-30827 on HIV-1 Tat protein-mediated regulation of human DAT and cocaine condition place preference (CPP) in iTat mice. Results show that SRI-30827 attenuated Tat-induced inhibition of [³H]DA uptake and [³H]WIN35,428 binding in PC12 cells expressing human DAT. After a 7-d doxycycline (Dox) treatment, HPLC analysis revealed that DA content in the prefrontal cortex (PFC) and nucleus accumbens (NAc) of iTat-Tg mice were increased by 92% and 37%, respectively, compared to control mice. Consistently, DA/DOPAC in the PFC and NAc of iTat-Tg mice was increased by 44% and 26%, respectively. We performed the patch clamp recording to measure medium spine neurons (MSN) firing in brain NAc slices of iTat mice in the presence of DA and cocaine. Results show that that action potential frequency of NAc shell MSN was significantly increased in iTat mice compared to control mice. Further, action potential frequency of NAc shell neurons was decreased in response to 5 μ M cocaine, and further decreased when cocaine and 5 μ M were applied together, which were completely attenuated in iTat mice. Finally, we found that ICV infusion of SRI-30827, a novel allosteric modulator, partially attenuated the potentiated cocaine-CPP in iTat mice. These findings suggest the hypothesis that Tat potentiates cocaine rewarding effect and allosteric modulator has potential for treatment of Tat-induced drug reward

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289. Monamine Transporters

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Danish Research Counsil

Title: Dominant negative variant of DAT associate with early-onset parkinsonism and psychiatric disease

Authors: *F. H. HANSEN¹, N. V. ARENDS¹, S. TOLSTOY¹, K. L. JENSEN¹, S. APARNA², J. AGUILAR², T. SKJØRRINGE³, L. FRIBERG⁴, L. MØLLER⁵, A. GALLI⁷, L. E. HJERMIND⁶, U. GETHER¹

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Abstract: The dopamine transporter (DAT) exerts a critical function in dopamine homeostasis by mediating reuptake of dopamine. An increasing number of missense mutations in the dopamine transporter (DAT) have been identified in patients suffering from psychiatric disorders. Moreover, complete 'loss of function' mutations in DAT have been identified a rare cause of hereditable infantile/childhood onset parkinsonism-dystonia. We recently described the first patient, carrying DAT missense mutations, who suffered from both early-onset parkinsonism and ADHD. Here, we further expand the clinical spectrum of DAT associated disease by presenting an additional patient that present with the unique combination of earlyonset parkinsonism and concurrent psychiatric disorder. This patient is heterozygote for a missense mutation in the C-terminal PDZ binding domain of DAT. Two SPECT scans of the patient performed seven years apart, uncovered reduced DAT binding along with a mild progressive worsening. A characterization of the mutant in heterologous cells revealed reduced dopamine uptake capacity (60% of WT DAT), attenuated amphetamine induced efflux, and reduced expression of DAT-K619N. Importantly, the K619N mutation has a pronounced effect in vivo. Drosophila Melanogaster that express the hDAT-K619N mutant show reduced dopamine uptake and 90% reduction in amphetamine induced dopamine efflux, compared to flies expressing WT hDAT. Moreover, we demonstrate that DAT-K619N has dominant negative actions on endogenous DAT, as viral expression of DAT-K619N in mice significantly reduces striatal dopamine uptake. Our identification of yet another patient with DAT associated

parkinsonism and neuropsychiatric disorder further supports that abnormal DAT function may constitute a risk factor for both psychiatric disorders and parkinsonism.

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Poster

289. Monamine Transporters

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The Faculty of Health and Medical Sciences, University of Copenhagen

the Novo Nordisk Foundation

Title: Cholesterol- and neuronal activity-dependent dopamine transporter nanodomains revealed by super-resolution microscopy

Authors: *M. D. LYCAS¹, T. RAHBEK-CLEMMENSEN¹, S. ERLENDSSON¹, J. ERIKSEN¹, M. APUSCHKIN¹, F. VILHARDT², T. N. JØRGENSEN¹, F. H. HANSEN¹, U. GETHER¹ ¹Dept. of Neurosci. and Pharmacol., ²Dept. of Cell. and Mol. Med., Univ. of Copenhagen, Kobenhavn N, Denmark

Abstract: Dopamine regulates reward, cognition and locomotor functions. By mediating rapid reuptake of extracellular dopamine, the dopamine transporter (DAT) is critical for spatiotemporal control of dopaminergic neurotransmission. Here, we show with super-resolution imaging that DAT is regulated by dynamic sequestration into cholesterol-dependent nanodomains in the plasma membrane of presynaptic varicosities and neuronal projections of dopaminergic neurons. Stochastic optical reconstruction microscopy (STORM) revealed irregular DAT nanodomains (~70 nm mean diameter) that were virtually dissolved by cholesterol-depletion. A similar DAT nanodomain localization was demonstrated in heterologous cells by live, photoactivated localization microscopy (PALM). In the neurons, dual-color dSTORM showed that tyrosine hydroxylase and vesicular monoamine transporter-2 are distinctively localized adjacent to, but

not overlapping with, the DAT nanodomains. In addition, dual-color dSTORM images of DAT and syntaxin-1 indicate a functional role of the clustered expression of DAT. The molecular organization of DAT in nanodomains was reversibly reduced by short-term activation of NMDA-type ionotropic glutamate receptors, implicating DAT nanodomain distribution as a potential mechanism to modulate dopaminergic neurotransmission in response to excitatory input. Through super resolution microscopy, the functional characteristics of DAT clusters are elucidated in the context of the dopaminergic presynapse.



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Poster

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Title: Phenotypic characterization of aging dopamine transporter DAT-AAA knock-in mice

Authors: A. B. KØNIG¹, C. FITZPATRICK¹, M. RICKHAG¹, *U. GETHER² ¹Ctr. for Neurosci., ²Univ. Copenhagen, Koebenhavn N, Denmark

Abstract: Being part of a multi-protein complex situated in the presynaptic compartment, the dopamine transporter (DAT) re-uptakes released dopamine from the extracellular space, thus maintaining synaptic dopamine homeostasis. We have developed a novel knock-in (KI) mouse strain with a disrupted DAT C-terminal PSD-95/Dlg1/ZO-1 homology (PDZ)-domain by substituting the C-terminal PDZ-target sequence (-LLV) with three alanines (-AAA) (DAT-AAA mice). This resulted in abolished PDZ-domain interaction and dramatic loss of functional DAT in striatal synaptic terminals. Interestingly, DAT mutations have recently been identified in patients with neuropsychiatric disorder, such as ADHD and autism, as well as in patients with juvenile and early-onset neurodegenerative parkinsonism. Some of these mutations display functional impairment similar to that observed for DAT in DAT-AAA mice. We therefore set out to phenotypically characterize aging DAT-AAA mice to assess their validity as a possible model for dopamine pathologies. Applying an extensive battery of motor tests at ages 9, 12, 15, 18 and 21 months, we found that DAT-AAA mice showed a steady and substantial hyperactive locomotive behavior in the open field test when compared to wild type (WT) mice. DAT-AAA mice furthermore showed increased amounts of rearings at 21 months consistent with a preserved hyperdopaminergic motor phenotype. We found no differences in fine motor skills, motor coordination and balance when testing the mice in the pole test, the adhesive removal test, the hanging wire test and on the rotarod, nor were there signs of pathological clasping behavior or kyphosis. There were no differences in anxiety/exploratory behavior in the elevated plus maze at 12 months. Our findings in the DAT-AAA mice describe a hyperactive locomotive phenotype that persists with age and displays no apparent dysfunctions of fine motor skills, motor coordination and balance. A further exploration of this dopaminergic phenotype should be relevant for understanding the physiological consequences of disrupting C-terminal PDZ interactions in vivo and for evaluating the mice as models for dopaminergic dysfunction and dopamine related diseases.

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289. Monamine Transporters

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Support: NIMH Intramural Research

Title: The trace amine receptor 1 (TAAR1) is coupled to G_s and G_{13} G-protein subunits in distinct subcellular compartments

Authors: *S. M. UNDERHILL¹, S. H. MILLAN³, P. D. HULLIHEN⁴, J. CHEN³, S. G. AMARA²

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Abstract: Amphetamine (AMPH) and its derivatives are useful therapeutic agents, but can also have a negative impact as a consequence of their addictive properties. Acute AMPH exposure elevates extracellular dopamine and glutamate by a variety of mechanisms including an increase in the rate of internalization of the plasma membrane dopamine transporter (DAT) and of the excitatory amino acid transporter 3 (EAAT3). AMPH-mediated trafficking of these carriers requires activation of the small GTPase RhoA that is negatively regulated by direct phosphorylation by protein kinase A (PKA). The trace amine receptor 1 (TAAR1) is a G-protein coupled receptor (GPCR) known to couple through $G_{s\alpha}$ and activate PKA signaling. TAAR1 also has been shown previously to contribute to the actions of psychostimulants in dopamine neurons and thus these observations led us to examine the potential role of TAAR1 in RhoA and PKA activation as well as in transporter trafficking. We used CRISPR-Cas9 gene editing technology to disrupt the TAAR1 gene in HEK293 cells and then measured AMPH-stimulated trafficking of DAT and EAAT3, as well as activation of Rho and PKA. In cells lacking TAAR1, AMPH did not induce internalization of DAT or EAAT3. We could not detect Rho or PKA activation in the knockout cells despite robust responses in the parallel wildtype TAAR1(+) cultures. AMPH-induced internalization of DAT and EAAT3 could be restored in the knockout cell line by transfecting with a modified, nuclease-resistant TAAR1. We hypothesized that TAAR1 was coupled to more than one subtype of G-protein alpha-subunit that mediated these diverse actions. To test this, we used mini-genes that interfere with various alpha-subunits and we determined that TAAR1 also couples to a $G_{13}\alpha$, an activator of RhoA signaling. By using short targeting domains to direct FRET sensors to various intracellular compartments, we found that the TAAR1 receptors that couple to $G_{13\alpha}$ subunits display signaling that is restricted to regions around the ER. In contrast, TAAR1- activated PKA signaling was detected in non-raft membranes. These observations show that TAAR1 serves as the intracellular target that mediates the effects of AMPH on RhoA and cAMP signaling and suggest new pathways to consider in order to better understand the mechanisms of action of AMPH.

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Poster

289. Monamine Transporters

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Title: Biogenic amine transporter activity of cathinone analogues

Authors: *A. M. DECKER¹, A. LANDAVAZO¹, J. S. PARTILLA², B. E. BLOUGH¹, M. H. BAUMANN², R. B. ROTHMAN² ¹RTI Intl., Rtp, NC; ²IRP, NIDA, NIH, Baltimore, MD

Abstract: Psychostimulants are highly addictive drugs that stimulate central and peripheral nervous system function and cause a variety of harmful physiological effects in humans. "Bath salts" or "legal highs" are synthetic stimulants that have recently become a serious abuse problem. The major components of many of these "bath salts" are synthetic cathinones, such as mephedrone, methylone, and methcathinone, which produce powerful stimulant, hallucinogenic, and addictive effects, all of which are similar to the effects experienced by cocaine, MDMA (ecstasy), and amphetamine users. Like many psychostimulants, synthetic cathinones are biogenic amine transporter (BAT) ligands and act as either transporter reuptake inhibitors or substrate type releasers. Given the detrimental effects of synthetic cathinones, it is becoming increasingly important to understand the pharmacology of these drugs so that new treatments for their abuse can be developed.

The current study set out to synthesize a series of methcathinone analogues with altered phenyl ring substitutions. All analogs were evaluated for BAT reuptake inhibition and release activity in freshly prepared rat brain synaptosomes. Our results show that several analogues are more potent dopamine and serotonin transporter releasers compared to methcathinone.







Mephedrone

Methylone

Methcathinone

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Poster

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Topic: B.05. Transporters

Support: 4TR32GM007628-29

5R01DA035263-04

Title: Structural, functional and behavioral characterization of an in-frame deletion associated with autism in the dopamine transporter gene

Authors: J. I. AGUILAR^{1,2}, N. CAMPBELL^{3,2}, A. SHEKAR^{1,2}, D. PENG⁴, A. MORLEY⁸, A. DURAN⁵, G. GALLI⁹, B. O'GRADY⁶, L. BELLAN⁷, R. RAMACHANDRAN¹⁰, J. S. SUTCLIFFE⁴, H. H. SITTE¹¹, K. ERREGER^{4,2}, J. MEILER⁵, T. STOCKNER⁸, H. MATTHIES^{4,2}, H. MCHAOURAB⁴, *A. GALLI^{4,2,1}

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Abstract: Dopamine (DA) plays an important role in the central nervous system by regulating a variety of functions, including cognition and motivation. While DA dysfunction is known to be involved in several neuropsychiatric disorders, its role in autism spectrum disorder (ASD) is largely unknown. We have recently identified several single nucleotide variants in the *SLC6A3* gene in individuals with ASD. The *SLC6A3* gene encodes the DA transporter (DAT), a presynaptic membrane protein critical to DA neurotransmission. Specifically, DAT mediates the active high affinity re-uptake of DA from the synapse into the synaptic bouton, maintaining DA

homeostasis. We report a novel SLC6A3 variant identified in an ASD proband from whole exome sequencing. This variant encodes an in-frame deletion of three nucleotides, resulting in the deletion of an asparagine at position 336 ($\Delta N336$). In vitro experiments demonstrate that $\Delta N336$ results in nearly absent DAT-dependent DA uptake despite normal surface expression. We utilize Drosophila melanogaster as an animal model to determine functionally and behaviorally whether expression of hDATAN336 in DA neurons results in DA dysfunction. Drosophila has enabled rapid progress in neuroscience research due to its cost-effectiveness, genetic tractability, rapid life cycle and conserved mechanisms of neurotransmission. Notably, mechanisms mediating DA neurotransmission including transport, synthesis, and secretion are largely conserved. Expression of $\Delta N336$ in *Drosophila* leads to impaired brain DA uptake and reverse transport of DA. Moreover, flies expressing hDAT Δ N336 display increased basal locomotion and grooming. This data is consistent with increased levels of extracellular DA. We evaluated further whether impairments in DA clearance affect defensive responses (i.e. freezing or fleeing) and/or social interactions, behaviors which are altered in individuals with ASD. Flies expressing $\Delta N336$ display aberrant freezing, fleeing, as well as, social behaviors. These results add to the growing body of literature associating altered regulation of DA homeostasis to complications in ASD.

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Poster

289. Monamine Transporters

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Topic: B.05. Transporters

Support: NIH_NIMH IRP

Title: Conformational changes induced by $G\beta \gamma$ subunits regulate dopamine transporter function

Authors: *J. GARCIA-OLIVARES¹, ***J. GARCIA-OLIVARES**¹, S. A. WASSERMAN¹, D. TORRES-SALAZAR¹, W. C. HONG², S. G. AMARA¹ ¹Natl. Inst. of Mental Hlth., Bethesda, MD; ²Buttler Univ., Indianapolis, IN

Abstract: The dopamine transporter (DAT) is regulated by cellular mechanisms such as phosphorylation, ubiquitination, and interactions with other proteins. We have reported a mechanism for regulation of the DAT by $G\beta\gamma$ subunits of heterotrimeric G-proteins. We found that after G-protein activation the $G\beta\gamma$ subunit binds directly to the C-terminus of the DAT and

decreases dopamine (DA) accumulation. We later found that this decrease in DA accumulation does not involve a change in uptake, but instead results from an increase in DA efflux through DAT. To further understand the mechanism of $G\beta\gamma$ regulation of DAT function, we examined whether the functional effects were linked to conformational changes in the DAT that facilitate efflux in HEK293 cells. An extracellularly-oriented cysteine residue (C306) in the DAT was used to probe conformational changes induced by the G_β activator peptide, mSIRK, and by ligands such as cocaine and other modulators of DAT function. The accessibility of C306 to an impermeant thiol-reactive biotin reagent was significantly reduced in cells treated with mSIRK. It has previously been shown that cocaine increases the accessibility of C306 by stabilizing an outward facing conformation of the DAT. However, pretreatment of cells with mSIRK reduced the cocaine-induced increase in C306 reactivity, suggesting that G_β activation limits the available binding sites for cocaine, perhaps by stabilizing an inward conformation. We also use a set of Cys-substitution mutants (I159C; K264C; S262C) in combination with permeant and impermeant thiol-reactive reagents to examine the DAT induced conformational changes that facilitate a shift in DAT conformation towards an inwardly-oriented efflux mode. Finally, we wanted to test if the observed conformational changes were result of the GBy binding or if the binding of $G\beta\gamma$ is conformation-dependent. For that we used the proximity-ligation assay to study how the interaction DAT-GBy is modified in the presence of different conformationspecific DAT-ligands. Our results provide a basis for further studies to establish the structural determinants for dopamine efflux and the relevance of GBy modulation for the actions of psychostimulants such as cocaine and amphetamines

Disclosures: J. Garcia-Olivares: None. **S.A. Wasserman:** None. **D. Torres-Salazar:** None. **W.C. Hong:** None. **S.G. Amara:** None.

Poster

289. Monamine Transporters

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Topic: B.05. Transporters

Support: NIH Grant DA021213

Title: Regulation of amphetamine actions by the dopamine transporter and G protein beta gamma subunits interaction

Authors: *S. S. HARRIS¹, J. A. PINO¹, J. C. MAUNA², E. THIELS², G. E. TORRES¹ ¹Pharmacol. and Therapeut., Univ. of Florida, Gainesville, FL; ²Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The dopamine transporter (DAT) plays an important role in regulating dopamine (DA) neurotransmission by transporting extracellularly released dopamine back into dopaminergic neurons, thereby limiting the duration and intensity of dopamine signaling. In addition, DAT can also function to release DA, a process known as reverse transport or efflux. This reverse transport of DA through DAT is the mechanism by which amphetamine and methamphetamine produce their effects. Amphetamine is used clinically in the treatment of conditions such as attention deficit hyperactivity disorder (ADHD) and narcolepsy, but is also abused due to its rewarding and addictive properties. However, the molecular details associated with the actions of amphetamine are not well understood. Our lab reported a novel interaction between G protein $\beta\gamma$ subunits and DAT, whereby $G\beta\gamma$ subunits bind directly to the C-terminus of DAT. Our previous findings showed that activation of GBy subunits by mSIRK down-regulate DAT-induced DA uptake. We recently provided additional evidence using heterologous systems and primary DA neurons in culture that mSIRK also increases DA efflux through DAT and this effect is blocked by inhibition of G_β subunit activation by gallein or a peptide that binds within the C-terminal region where GBy binds to DAT. Moreover, we also show, *in vivo*, that inhibition of GBy activation by gallein reduces amphetamine-induced increases in locomotor activity and extracellular DA levels in the nucleus accumbens in rats. Findings from these studies suggest that GBy subunits interact directly with DAT to regulate DA efflux and this interaction is involved in amphetamine's actions. Therefore, the present study sought to further characterized the role of G_β subunits in the actions of amphetamine using *ex vivo* and *in vivo* approaches. Rat striatal tissue was used to determine the effects of activation of GBy subunits by mSIRK on amphetamine-induced DA release using a superfusion assay. We also determined the effects of mSIRK on amphetamine-induced extracellular DA levels in the nucleus accumbens using in vivo microdialysis. Our preliminary results provide further support for the role of GBy subunits in modulating the actions of amphetamine in the brain.

Disclosures: S.S. Harris: None. J.A. Pino: None. J.C. Mauna: None. E. Thiels: None. G.E. Torres: None.

Poster

289. Monamine Transporters

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Topic: B.05. Transporters

Support: DA036420

MHD007593

CA163069

MHD007586

GM059994

Title: Comparative proteomics analysis of dopamine transporter interactome upon methamphetamine and cocaine treatment

Authors: *S. M. INGAM¹, T. RANA¹, S. ELUHU¹, N. BERRYMAN², B. OGHIDE², S. PRATAP¹, J. GOODWIN¹

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Abstract: The dopamine transporter (DAT) is a neurotransmitter transporter that regulates the extra-cellular concentrations of dopamine (DA) by re-uptaking DA into dopaminergic neurons. Psychostimulants disrupt DA homeostasis by competitively blocking DAT (cocaine) or inhibiting DA reuptake and reverse transport activity, resulting in increased synaptic DA levels and efflux of DA via DAT (methamphetamine). We investigated the proteomic differences between these psychostimulants by tandem mass spectrometry to examine the interacting proteome of DAT when exposed to methamphetamine (Meth), a DAT substrate and cocaine (Coc), a DAT blocker. We used Perseus software platform to interpret our proteomics data. Analysis of the data revealed distinct protein repertoires for Coc and Meth treated cells. We confirmed some known DAT interacting proteins at various time points. Post spectroscopy analysis revealed novel protein interactions including redox, actin-binding, calcium binding/regulating, and voltage-dependent proteins based on drug treatment. Here we provide a comprehensive, qualitative analysis of proteins detected in response to psychostimulant treatment. Co-immunoprecipitation and confocal microscopy experiments confirm novel protein interactions with DAT when exposed to Meth or Coc. Lastly, comparative analyses of the Meth and Coc interactome have brought to light several interesting molecular pathways, offering the possibility to explore psychostimulant-specific protein interactions in the cell.

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Poster

289. Monamine Transporters

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Support: NIH DA15169 (H.E.M.)

NIH NS076991 (G.G.)

F31 DA039592 (C.G.S.)

Title: Behavioral impact of Rin GTPase and dopamine transporter trafficking: AAV studies using novel conditional and inducible shRNA

Authors: *C. G. SWEENEY¹, J. XIE², G. GAO², H. E. MELIKIAN¹ ¹Brudnick Neuropsychiatric Res. Inst., ²Gene Therapy Ctr., Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: Dopamine (DA) signaling is critical for movement, motivation, and addictive behavior. Following synaptic release, DA is rapidly taken up into the presynaptic neurons by the DA transporter (DAT), thereby spatiotemporally restricting downstream DA signaling. DAT plasma membrane presentation is requisite to regulate extracellular dopamine levels. Neuronal DAT surface expression is acutely regulated via PKC activation, which drives rapid DAT loss from the cell surface via endocytic trafficking. We previously reported that this mechanism requires the small, neuronal GTPase, Rin (Rit2). Although PKC-stimulated DAT downregulation has been well described for over a decade, the physiological importance of regulated DAT trafficking, and Rin's role in DAT downregulation in vivo, are poorly defined. Here, we tested whether Rin-dependent, PKC-stimulated DAT trafficking is required for DA-related behaviors. To accomplish this objective, we developed an rAAV (TET-OFF/ON) vector that uses a tetracycline responsive element (TRE) to drive Rin-targeted shRNA expression selectively in cells expressing the Tet transactivator (tTA). When injected into Pitx3^{IRES-tTA/+} mouse midbrain, we observed selective reporter expression and Rin knockdown in midbrain DAergic neurons, which was suppressed when mice were maintained on a doxycycline diet. Rin knockdown in DAergic neurons resulted in an anxiolytic phenotype, as determined by elevated plus maze and open field behavioral assays. Ongoing experiments are testing whether Rin expression in DA neurons plays a role in psychostimulant reward, and whether Rin is required for PKC-stimulated DAT trafficking in ex vivo striatal slices. These studies are among the first to achieve both conditional and inducible gene knockdown in vivo and, importantly, provide evidence that regulated DAT trafficking may exert a robust influence on DA-dependent behaviors.

Disclosures: C.G. Sweeney: None. J. Xie: None. G. Gao: None. H.E. Melikian: None.

Poster

289. Monamine Transporters

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Topic: B.05. Transporters

Support: NIH Grant DA15169

Title: The impact of regulated dopamine transporter endocytosis on dopamine-dependent behaviors: *In vivo Drosophila melanogaster* studies

Authors: *R. R. FAGAN^{1,3}, C. G. SWEENEY^{1,3}, P. EMERY², H. E. MELIKIAN^{1,3} ²Dept. of Neurobio., ¹Univ. of Massachusetts Med. Sch., Worcester, MA; ³Brudnick Neuropsychiatric Res. Inst., Worcester, MA

Abstract: The neurotransmitter dopamine (DA) critically controls movement, sleep, learning, and reward. The plasma membrane dopamine transporter (DAT) clears extracellular DA following release, thereby spatially and temporally limiting synaptic DA availability. Recent reports have identified multiple DAT coding variants in attention-deficit/hyperactivity disorder, infantile parkinsonism-dystonia, and autism spectrum disorder probands, illustrating DAT's critical role in DA homeostasis and DA-related behaviors. Both DAT activity and surface expression are acutely regulated by endocytic trafficking. PKC activation leads to diminished DAT surface levels, and studies from our lab have demonstrated that the small, neuronal GTPase, Rin (RIT2) and the non-receptor tyrosine kinase, Ack1, are critical factors in controlling DAT surface stability and endocytic response to PKC stimulation. However, it is unknown whether regulated DAT endocytosis impacts DA-related behaviors such as locomotion, sleep, or reward. Moreover, it is not known whether trafficking-dysregulated DAT coding variants influence DA homeostasis. For example, the DATR615C mutation, found in an autism spectrum proband, exhibits significantly enhanced basal endocytosis, however whether this mutation deleteriously affects DA-dependent behavior is unknown. The Drosophila melanogaster model system has provided novel insights into the impact of DAT dysfunction on DA-dependent behaviors. In the current study, we capitalized on the tractability of *Drosophila* to test whether perturbing DAT trafficking impacts DA-related behaviors. Overexpressing constitutively active Ric, the Drosophila Rin homolog, or knocking down Ric via RNAi inhibited PKC-mediated DAT downregulation in *Drosophila* cell lines, suggesting that dDAT and hDAT are similarly subject to Rin (or Ric)-dependent downregulation. In vivo Drosophila studies revealed decreased locomotion and decreased daytime, but not nighttime, sleep in response to either overexpressing constitutively active Ric or knocking down Ric in DAergic neurons using the TH-Gal4 driver. Ongoing and future experiments will test whether dDAT is subject to PKC-dependent surface loss, and whether this process requires Ric and/or Ric activity, in both cultured cells and ex vivo, intact Drosophila brains. Furthermore, we will investigate whether DAT trafficking dysfunction affects DA-dependent behaviors by expressing trafficking-dysregulated hDATs in a dDAT null background. Taken together, these studies comprise one of the first *in vivo* investigations testing the physiological impact of regulated DAT trafficking on DA-related behaviors.

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Poster

289. Monamine Transporters

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Topic: B.05. Transporters

Support: NIH Grant DA007606

Title: Roles for cholesterol and the isoprenylation pathway in serotonin transporter regulation

Authors: *C. MITCHELL¹, A. SCHROERING², B. K. YAMAMOTO³

¹Dept. of Pharmacol. and Toxicology, Indiana Univ., Indianapolis, IN; ²Univ. of Toledo, Toledo, OH; ³Neurosciences, Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: The serotonin transporter (SERT) localizes in cholesterol-rich microdomains within the plasma membrane. Although the role of these microdomains serves as a scaffold for phosphorylating kinases and protein-protein interactions, the role of cholesterol within these microdomains on serotonin transporter (SERT) function remains to be understood. To investigate the general role of cholesterol on SERT function, serotonergic RN46A-B14 cells were stably overexpressed with myc-tagged SERT. Cholesterol within lipid rafts were disrupted by simvastatin, an inhibitor of the rate-limiting enzyme in cholesterol synthesis, 3-hydroxy-3methyl-glutaryl-coenzyme A (HMGCoA) reductase. Serotonin (5-HT) transport activity was assayed by measurement of intracellular 5-HT content after incubation of cells with 5-HT. Simvastatin significantly increased 5-HT uptake in a dose and time-dependent manner at 24hr, 48hr, and 72hr of simvastatin exposure. The SERT inhibitor, fluoxetine blocked the enhanced uptake at each time point, indicating that simvastatin enhances 5-HT uptake in a SERTdependent manner. Cholesterol precursors downstream of HMGCoA reductase were added back into the media to determine the pathway necessary for the enhanced 5-HT uptake. Mevalonate completely blocked the enhanced uptake, and geranylgeranyl pyrophosphate partially blocked the enhanced uptake. However, the more immediate precursor to cholesterol, squalene, did not block the enhanced uptake. Results indicate that simvastatin enhances 5-HT uptake via SERT in a manner *independent* of cholesterol. The involvement of the isoprenylation pathway downstream of HMGCoA reductase is currently under investigation.

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Poster

289. Monamine Transporters

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Title: Dopamine transporter activation regulates Kv2.1 cluster size and activation potential

Authors: *J. J. LEBOWITZ¹, J. A. PINO², D. O. SAMBO¹, S. STREIT¹, K. DIVITA¹, C. HENCKEL¹, M. LIN¹, G. E. TORRES², H. KHOSHBOUEI¹ ¹Dept. of Neurosci., ²Dept. of Pharmacol. and Therapeut., Univ. of Florida, Gainesville, FL

Abstract: Selective neuronal vulnerability is a pervasive phenomenon in which distinct neuronal subtypes are differently affected by a pathological burden. In the case of Parkinson's disease, the vast axonal field and high level of tonic firing activity intrinsic to dopaminergic (DAergic) neurons are thought to contribute to their degeneration. Interestingly, within the ventral midbrain, substantia nigra (SNc) DAergic neurons degenerate in the presence of Lewy bodies, while the neighboring DAergic neurons of the ventral tegmental area (VTA) are relatively spared. Because many of the data that corroborate this are from postmortem human studies, however, it is difficult to know which subtle differences between the two DAergic populations lead to the difference in vulnerability. Previous work has focused on intracellular Ca²⁺ flux and oxidative stress as important differences between the two regions, but many additional therapeutic targets remain unexplored. Altered dopamine transporter (DAT) activity is implicated in Parkinson's disease, where missense DAT mutations are directly associated with adult early-onset Parkinsonism and progressive dopaminergic neurodegeneration. Kv2.1 is a voltage gated potassium channel that has also been shown to be dysregulated in a mouse model of neurodegeneration. Both modulators of neuronal excitability, Kv2.1 and DAT were shown to co-immunoprecipitate and the association was confirmed via FRET microscopy. Importantly, we found that the degree of colocalization is cell region specific in the SNc, but not the VTA. Functional analysis found that methamphetamine-induced DAT activation reduced the interaction between the two proteins and decreased Kv2.1 cluster size which is directly coupled to activation potential changes and thus neuronal excitability. Recent data from our lab and the literature suggest the sigma-1 receptor (SIG-1R), which is a chaperone protein that reduces oxidative stress, not only decreases DAT activity, but also interacts with Kv2.1 and DAT. Ongoing experiments are exploring the nature and mechanism of DAT regulation of Kv2.1

activation potential changes in the SNc vs.VTA and SIG-1R as a novel therapeutic target to confer protection from degeneration in the SNc.

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Poster

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Topic: B.05. Transporters

Support: U54 MD007593

- G12 MD007586
- U54 CA163069

R24 DA036420

DA036420

MHD007593

CA163069

Title: Dopamine transporter interactome reveals differential interaction with DJ-1 when exposed to METH

Authors: *N. BERRYMAN¹, S. INGRAM², S. ELUHU², B. OGHIDE¹, T. RANA², H. KHOSHBOUEI³, J. S. GOODWIN²

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Abstract: Extracellular dopamine (DA) concentrations are tightly regulated by reuptake through the dopamine transporter (DAT). Methamphetamine (METH) - a widely abused psychostimulant - is a substrate for DAT, disrupting dopamine homeostasis by inhibiting DA reuptake and by triggering the reverse transport of DA into the synaptic cleft. DA dysregulation can be detrimental to proper brain function and has been implicated in several disease states. We postulated that DAT-interacting proteins are altered in the presence of METH, compared to control conditions, leading to the dysregulation of DAT. A proteomic-based study that employed liquid chromatography-tandem mass spectrometry (LC-MS/MS), immunoprecipitation, immunostaining and other complementary techniques, examined the interactome of DAT and

revealed an association between DJ-1 and DAT in control conditions, but a dissociation of DJ-1 and DAT in METH conditions. DJ-1, a multifunctional protein also known as PARK7, inhibits the aggregation of α -synuclein and is widely studied for its involvement in Parkinson's Disease. Recently, it was reported that DJ-1 interacts with DAT to increase DA uptake; overexpression led to enhanced uptake of DA and knockdown lead to decreased uptake. Based on this evidence, we examined how DJ-1 affects DAT function in the presence of METH, contributing to the dysregulation of the transporter. We found that METH decreased DJ-1 interaction with DAT and altered DAT function.

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Poster

289. Monamine Transporters

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Support: NIH Grant DA041673

NIH Grant HD069238

Title: Differential site-specific serotonin transporter phosphorylation governs kappa-opioid receptor mediated biphasic serotonin clearance

Authors: *S. RAMAMOORTHY, S. SUNDARAMURTHY, L. D. JAYANTHI Pharmacol. and Toxicology, Virginia Commonwealth Univ., Richmond, VA

Abstract: Upregulation of dynorphin/kappa-opioid receptor (KOR) systems has been implicated in the pathogenesis of depression and the mood dysregulation that charactizes withdrawal from cocaine and other drugs of abuse. Serotonin (5-HT) plays an important role in regulating the KOR systems impotantly, KOR proteins are expressed on serotonergic neurons and terminals. Presynaptic 5-HT transporter (SERT) activity controls serotonergic neurotransmission by rapid reuptake of released 5-HT, thus playing an important role in several behavioral and physiological functions. We previously demonstrated that KOR activation down-regulates SERT activity through decreasing active surface SERT, enhanced SERT internalization, decreased SERT delivery, and increased SERT phosphorylation via Akt and CaMKII dependent siganling pathways (PMC5148672). Our current studies showed that depending on Thr276-Ser277-SERT phosphorylation, KOR activaton differentially affects SERT function and trafficking. KOR-Akt induced decreased SERT function via Thr276 phosphorylation, while KOR-CaMKII signaling increased SERT function via Ser277 phosphorylation. Collectively, our studies revealed a novel mechanism of KOR-mediated biphasic regulation of SERT functions associated with differential SERT phosphorylation of Thr276 and Ser277- sites via Akt and CaMKII respectively. Moreover, these findings identify a novel mechanism by which KOR regulates 5-HT neurotransmission via phosphorylation dependent SERT regulation, and suggesting that SERT dysregulation/phosphorylation may be one mechanism underlying dynorphin/KOR-mediated alterations in mood and affect. Findings from these studies will enhance our understanding of the neural substrates upon which dynorphin/KOR act to regulate behaviors, aid in the development of effective pharmacological agents for the treatment of addiction, depression and provide new insights into the role of SERT phosphorylation in regulating synaptic 5-HT clearance and behavior.

Disclosures: S. Ramamoorthy: None. S. Sundaramurthy: None. L.D. Jayanthi: None.

Poster

289. Monamine Transporters

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Topic: B.05. Transporters

Support: R21DA039451

Title: Interaction of neurokinin signaling and norepinephrine transport in amphetamine behavior

Authors: *L. D. JAYANTHI, P. MANNANGATTI, S. RAMAMOORTHY Pharmacol. & Toxicology, Virginia Commonwealth Univ., Richmond, VA

Abstract: A close anatomical and physiological association exists between neurokinin and noradrenergic systems in the brain. The neurokinin 1 receptor (NK1R) and its endogenous ligand substance P have been linked to stress and drug-abuse. Animal studies indicate therapeutic benefits of NK1R antagonists in treating stimulant abuse and thus, the utility of NK1 directed therapeutics is an area of interest. Substance P is released in the rat ventral striatum following amphetamine (AMPH) and is known to enhance acute stimulatory effects of AMPH. On the other hand, NK1R antagonists decrease psychostimulant-induced locomotor activation. Our recent studies have shown that aprepitant, a clinically used NK1R antagonist, attenuates conditioned place preference (CPP) and locomotor activation induced by AMPH and cocaine (PMC5266628). The psychostimulants cocaine and AMPH target all three monoamine transporters and AMPH has nearly equal affinity for the norepinephrine and dopamine transporters (NET & DAT). We and other investigators have shown that AMPH as well as NK1R activation downregulate both NET and DAT. Here we examined whether NK1R-mediated NET or DAT regulation is involved in AMPH-induced behaviors. Our results show that while aprepitant attenuated AMPH-induced CPP in the wild type and DAT knockout (KO)

mice, it failed to affect AMPH-CPP in the NET KO mice. Our previous work demonstrated that substance P and AMPH mediated NET downregulation is dependent on the transporter T258/S259 trafficking motif (PMID16740633; PMC2939970) and that NET regulation by NK1R is facilitated by NET-NK1R protein-protein interactions (PMC3789959). A single bilateral intraaccumbal microinjection of TAT-T258/S259-WT peptide but not the T258A/S259A-mutant peptide prior to post-conditioned test significantly attenuated AMPH-induced CPP expression and CPP reinstatement in rats. Collectively, our data indicate a role for NK1R-mediated T258/S259-dependent NET regulation in AMPH-induced behaviors. Since the sequence surrounding T258/S259 motif is identical in NET and DAT, ongoing studies using NET and DAT KO mice are exploring the utility of TAT-T258/S259 peptide strategy to delineate the role of T258/S259-dependent NET regulation in AMPH mediated behaviors.

Disclosures: L.D. Jayanthi: None. P. Mannangatti: None. S. Ramamoorthy: None.

Poster

289. Monamine Transporters

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Topic: B.05. Transporters

Support: Butler University faculty startup fund

Holcomb research award

Title: Regulation of the sigma-1 receptor multimerization

Authors: *W. C. HONG

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Abstract: Following the initial proposal of sigma receptors in 1976 and subsequent molecular cloning of the sigmal receptor ($\sigma_1 R$) in 1996, extensive studies have shown that $\sigma_1 R$ is a unique membrane protein that may serve as a molecular chaperone. In 2016 a breakthrough on the crystal structure of $\sigma_1 R$ revealed a homotrimer, with each protomer containing a single transmembrane domain, a cytoplasmic ligand-binding domain and trimerization interface. Because oligomerization of membrane proteins may regulate their function, we devised a novel biochemical method to examine $\sigma_1 R$ multimerization. Epitope-tagged $\sigma_1 R$ from transfected cells was solubilized using mild detergents, separated in non-denaturing gels, and detected by immunoblotting. Immuno-reactive signals were displayed as three bands, likely corresponding to $\sigma_1 R$ monomer, dimer, and multimer based on their apparent electrophoretic mobility. Multimerization was abolished in a splice variant of $\sigma_1 R$ lacking amino acid residues 119 - 149. Further, pH values in the lysis buffer substantially affected $\sigma_1 R$ multimerization, suggesting that

side chain protonation of certain amino acid residues may impact on $\sigma_1 R$ protomer interaction. Importantly, $\sigma_1 R$ multimers were significantly decreased by treatment with (+)-pentazocine (agonist) but increased by BD1063 (antagonist), suggesting that oligomerization of $\sigma_1 R$ is regulated by binding of agonists or antagonists in a distinct manner. Ligand binding also appeared to alter multimerization of $\sigma_1 R$ on the cell surface, as detected by surface biotinylation assays. Since $\sigma_1 R$ has been shown to modulate the function of various membrane proteins, we hypothesize that $\sigma_1 R$ ligands differentially regulate the formation of $\sigma_1 R$ monomers and affect their dynamic interactions with client proteins.

Disclosures: W.C. Hong: None.

Poster

289. Monamine Transporters

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Topic: F.02. Behavioral Neuroendocrinology

Title: Slc22a3, a potential second presynaptic serotonin transporter

Authors: *M. ARNOLD¹, A. O. WILLIAMS², A. AGRAWAL², H. E. DAY², J. TALBOOM⁴, M. ORCHINIK⁵, C. A. LOWRY³

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Abstract: Brain serotonergic systems play a role in cognitive and affective function and have been implicated in the pathophysiology of trauma-related, anxiety, and affective disorders. Drugs that block serotonin reuptake into presynaptic terminals are used widely in the treatment of these disorders, and therefore understanding mechanisms underlying presynaptic clearance of serotonin is of major clinical interest. The neurotransmitter serotonin is cleared from brain synapses by the presynaptic sodium-dependent, high-affinity, low-capacity serotonin transporter, solute carrier family 6 (neurotransmitter transporter) member 4 (Slc6a4). However, evidence suggests possible expression of a second serotonin transporter, the sodium-independent, lowaffinity, high-capacity serotonin transporter, organic cation transporter 3 (Oct3; Slc22a3), in serotonergic neurons. However, it is unclear if Slc22a3 localizes to the presynaptic serotonergic terminal, or to other cellular compartments within these cells, and, furthermore, if it co-localizes to the same presynaptic terminals as Slc6a4. For example, recent studies at the electron microscope level have identified Slc22a3 expression on neuronal and glial endomembranes, including Golgi, mitochondrial and nuclear membranes. Using a dual label fluorescence in situ hybridization histochemistry approach we demonstrated overlapping expression patterns of tryptophan hydroxylase 2 (Tph2), the rate-limiting enzyme in the biosynthesis of brain serotonin, and Slc22a3 mRNA expression within the rat dorsal raphe nucleus. To understand Slc22a3

protein location on the presynaptic terminals we used a quadruple label immunofluorescence for Slc6a4, Slc22a3, postsynaptic density protein 95 (Psd-95) and DAPI (4',6-diamidino-2-phenylindole), using n-super-resolution microscope (n-SIM) imaging. We were able to demonstrate that Slc22a3 and Slc6a4 co-localize in the same presynaptic terminals within the rat dorsal raphe nucleus, the main source of serotonergic projections to forebrain limbic structures. These findings highlight the need to understand the dynamic interactions between Slc6a4 and Slc22a3 in controlling serotonin reuptake and serotonergic signaling, physiology, and behavior in health and disease.

Disclosures: M. Arnold: None. A.O. Williams: A. Employment/Salary (full or part-time):; Double Helix LLC, Boulder, CO 80302, USA. A. Agrawal: A. Employment/Salary (full or parttime):; Double Helix LLC, Boulder, CO 80302, USA. H.E. Day: None. J. Talboom: None. M. Orchinik: None. C.A. Lowry: None.

Poster

289. Monamine Transporters

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Topic: A.07. Developmental Disorders

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NARSAD from the Brain and Behavioral Research Foundation (NLB, MJR)

Title: Investigation of IL-1 β activation of serotonin transporter-mediated serotonin clearance *In vivo*

Authors: *N. L. BAGANZ¹, M. J. ROBSON¹, W. A. OWENS², L. C. DAWS², R. D. BLAKELY¹

¹Biomed. Science, Charles E. Schmidt Col. of Med. and Brain Inst., Florida Atlantic Univ., Jupiter, FL; ²Cell. and Integrative Physiol., Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: Central serotonin (5-HT) neurotransmission and immune system activation have been linked to the etiology of multiple psychiatric disorders, including depression and autism. Previously, we showed that peripheral immune system activation in mice using the endotoxin lipopolysaccharide (LPS) rapidly (30-60 min) increases 5-HT clearance, and produces depressive-like effects on behavior. These effects are lost in constitutive IL-1R1 knockout mice and in wild-type mice pre-treated with systemic injections of the p38 MAPK inhibitor SB203580. Moreover, we found that *in vitro* incubations of synaptosomes with IL-1β rapidly increases activity of the antidepressant-sensitive 5-HT transporter (SERT), effects absent in

synaptosomes of IL-1R1 KO mice or synaptosomes pre-treated with SB203580. These findings suggest that IL-1 β , via p38 MAPK signaling to SERT, mediates the ability of peripheral LPS to enhance rates of CNS 5-HT clearance. To validate this hypothesis, we performed *in vivo* chronoamperometry in the dorsal hippocampus (CA3) of mice, evaluating the impact of locally applied p38 MAPK activator anisomycin, as well as IL-1 β , to enhance clearance of 5-HT. As predicted, anisomycin rapidly (2 minutes) enhanced 5-HT clearance, with effects blocked by prior application of SB203580. Similarly, local IL-1 β rapidly (10 minutes) enhanced 5-HT clearance. Ongoing studies seek to test the actions of both systemic LPS and local IL-1 β to modulate 5-HT clearance in mice generated that allow for 5-HT neuron-specific elimination of IL-1R1. Together, these experiments will further specify pathways by which peripheral and CNS inflammatory signaling can rapidly alter brain extracellular 5-HT homeostasis.

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Poster

289. Monamine Transporters

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Support: NIH Awards MH094527 (RDB)

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BBRF YI Award (MJR)

Title: Structural, biochemical and functional evidence of a biased conformation of serotonin transporters imposed by an autism-associated mutation

Authors: *M. A. QUINLAN^{1,2}, D. KROUT³, M. J. ROBSON², L. K. HENRY³, R. D. BLAKELY²

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Abstract: The serotonin (5-HT) transporter (*Slc6a4*, SERT) plays a crucial role in regulating the extracellular availability of 5-HT. Current models suggest that SERT, like other solute carriers (SLC), translocate substrate across the membrane through a multi-state process that results in alternating exposure of the 5-HT binding site to the extracellular and cytoplasmic spaces. We hypothesize that population of these states can be biased by natural regulatory stimuli as well as disease-associate mutations, leading to a change in 5-HT affinity, 5-HT uptake velocity and/or

efflux capacity, independent of SERT surface trafficking. Here we report our efforts to determine if these hypotheses can explain the change in transport function associated with the autismassociated SERT coding variant Gly56Ala, using both transfected cells and the SERT Ala56 mouse model. In one effort we are using the substituted cysteine accessibility method to compare Cys109 and Cys277 accessibility in WT hSERT and hSERT Ala56, expressed in HEK-MSR cells. Our data reveal that the $t_{1/2}$ of methanethiosulfonate reagent inactivation of hSERT Ala56 is increased for Cys109 and decreased for Cys277 compared to WT hSERT, consistent with stabilization of hSERT Ala56 in an outward facing conformation. We are also examining the sensitivity of the N-terminus to digestion by trypsin. Previous studies show that when SERT is stabilized in an outward facing conformation, the N-terminus of the transporter is less sensitive to trypsin digestion, compared to inward facing conformations. Our findings indicate that the Nterminus of hSERT Ala56 is less sensitive to tryptic cleavage compared to WT hSERT. These findings are consistent with hSERT Ala56 as biased in an outward facing conformation. To examine conformational bias with native SERT Ala56, we are assessing fenfluramine-induced 5-HT efflux from [³H]5-HT preloaded brain slices, prepared from mice expressing WT SERT or SERT Ala56. Our findings indicate that slices from SERT Ala56 mice demonstrate a dosedependent sensitivity for fenfluramine-mediated [³H]5-HT efflux. Since 5-HT efflux is believed to be favored by an inward facing conformation, a decreased efflux sensitivity to fenfluramine adds further support or the hypothesis that SERT Ala56 is stabilized in an outward facing conformation. These studies are being complemented by candidate and proteomic analyses of SIPs to evaluate the contribution of altered protein associations in stabilizing SERT Ala56 conformations, as well as by studies of IL-1R1/p38A MAPK signaling, which like SERT Ala56, enhances SERT 5-HT affinity and increases transport function at low substrate concentrations.

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Poster

290. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant T32 GM007377

Title: Transgenic mice carrying a serine to alanine mutation at residue 1928 of the voltage-gated calcium channel $Ca_V 1.2$ display strong, sex-dependent differences in behavior related to learning, memory, and stress

Authors: *K. E. IRETON¹, J. W. HELL² ¹Neurosci., ²Dept. of Pharmacol., UC Davis, Davis, CA

Abstract: The voltage-gated calcium channel (VGCC) Cav1.2 is the most abundant VGCC in the brain as well as the heart, and influences cellular excitability, gene expression, and synaptic plasticity in neurons. Manipulations of its activity and expression affect learning-, stress-, and reward-seeking-related behaviors in rodents, and genome wide association studies in humans have identified mutations in its gene CACNA1C as risk factors for bipolar disease and schizophrenia, besides the known role of a specific mutation in Timothy syndrome, a fatal condition causing severe mental and cardiac dysfunction. Cav1.2 channel activity is known to be upregulated by norepinephrine via β -adrenergic receptors (β -AR). A mutant mouse line with a mutation at serine 1928 to alanine (S1928A), the most important site of phosphorylation by PKA downstream of β-AR signaling, was previously found to display no apparent cardiac or obvious neurological deficits, indicating S1928 phosphorylation was not needed to regulate CaV1.2 activity in the heart. However, our lab has recently published that strong differences exist in channel activity regulation and induction of theta tetanus LTP in mutant hippocampal neurons, in culture and brain slice, highlighting a surprising dichotomy between regulation of Cav1.2 between brain and heart. We hypothesized that due to the mutant deficit in LTP induction, they ought to display at least a mild deficit in some long-term memory-dependent behaviors compared to wildtypes. Much to our surprise, we found strong, but sex-dependent, enhancements in mutant short- and long-term learning behavior, compared to litter-matched controls. The magnitude of the changes in behavior roughly correlated with the stress of the behavioral assay, indicating enhanced stress resilience in mice lacking the S1928 phosphorylation site for β-AR regulation. We continue to test the mice in further behavioral assays, including fear conditioning for a direct test of stress-related learning. Our initial results indicate a profound physiological role of CaV1.2 in regulating rodent learning, memory, and stress-response behaviors, and may add to the growing body of evidence that CaV1.2 could be a promising target for pharmaceutical intervention in certain human neurological disorders.

Disclosures: K.E. Ireton: None. J.W. Hell: None.

Poster

290. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

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Topic: B.08. Synaptic Plasticity

Support: R01 grants DA017392

MH081935

Fondation pour la Recherche Médicale

Fondation Bettencourt Schueller

Title: Theta-burst firing of a single dentate granule cell induces presynaptic LTP at mossy celldentate granule cell synapse

Authors: *K. NASRALLAH¹, P. E. CASTILLO²

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Abstract: The dentate gyrus, a major input area of the hippocampus, contains two types of excitatory neurons: dentate granule cells (GCs) and hilar mossy cells (MCs). MCs receive inputs from GCs and project back to GCs locally (intralamellar), contralaterally, and along the longitudinal (dorsoventral) axis of the hippocampus, thereby establishing an associative positivefeedback loop (GC-MC-GC). Such projections may be important to connect the dorsal hippocampus, primarily involved in spatial memory, with the ventral hippocampus, primarily involved in emotional memory. However, how MCs contribute to dentate gyrus function remains poorly understood. Here, we studied the dynamic properties of MC-GC synapses using rodent hippocampal slices. We found that theta-burst firing (TBF) of a single GC by direct depolarization is sufficient to induce LTP at MC-GC synapses (TBF-LTP), but not at medial perforant path excitatory inputs onto GCs. While the induction of TBF-LTP requires postsynaptic calcium rise in an NMDA receptor-independent manner, this plasticity is expressed presynaptically as indicated by a decrease in paired-pulse ratio, coefficient of variation and failure rate. Taken together, these observations strongly suggest the involvement of retrograde signaling in TBF-LTP. Moreover, TBF-LTP was abolished by the TrkB antagonist ANA-12 (15 µM) and conditional TrkB deletion from GCs, as well as by bath application of the PKA inhibitors H89 (10 μ M) and myristoylated PKI₁₄₋₂₂ amide (1 μ M), but not by intracellularly loading GCs with PKI₆₋₂₂ amide (2.5 µM). Thus, TBF-LTP requires postsynaptic BDNF/TrkB and presynaptic cAMP/PKA signaling. By increasing the strength of MC inputs onto GCs, TBF-LTP could facilitate information transfer between dorsal and ventral hippocampus, and contribute to memory formation and epilepsy.

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Poster

290. LTP: Pre- and Postsynaptic Mechanisms II

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Topic: B.08. Synaptic Plasticity

Support: Huntington Society of Canada

Research and Development Corporation of Newfoundland and Labrador

NSERC Discovery Grant

Title: The effect of extracellular glutamate on synaptic plasticity and its relevance to neurodegenerative disease

Authors: J. R. BARNES, *M. P. PARSONS

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Abstract: Recent literature on the mechanisms of synaptic plasticity has highlighted a dichotomous role of synaptic and extrasynaptic NMDA receptor (exNMDAR) activation, with the former promoting long-term potentiation (LTP) and the latter inhibiting it. Glutamate transporters rapidly clear glutamate from the extracellular space, thereby preventing exNMDAR overactivation, and many lines of evidence suggest a dysfunction of the glutamate transporter proteins in various neurodegenerative diseases associated with cognitive decline. Thus, an attractive and well-cited hypothesis states that glutamate transporter dysfunction underlies the LTP and cognitive impairments in disease states including Alzheimer Disease (AD). However, the precise relationship between the spatiotemporal dynamics of extracellular glutamate and synaptic plasticity has yet to be investigated. Here, we use a novel optogenetic sensor of glutamate, termed iGluSnFR, to monitor extracellular glutamate dynamics in real-time (200 Hz) in the mouse hippocampus during standard LTP-inducing protocols. A range of concentrations of various glutamate transporter blockers were used to impair glutamate clearance during LTP induction, allowing us to characterize the relationship between glutamate uptake and LTP magnitude; these results were compared to those obtained for amyloid beta, a toxic protein in the AD brain that is thought to impair LTP in part by reducing glutamate transporter function. Interestingly, we found concentrations of the GLT-1 glutamate transporter inhibitor DHK that dramatically impaired glutamate clearance rates yet had no effect whatsoever on LTP magnitude. Surprisingly, LTP was not impaired until glutamate clearance rates were slowed well over twofold with TBOA, a nonselective glutamate transporter blocker. Lastly, Aβ had no significant effect on glutamate clearance rates, yet potently suppressed LTP. Overall, these data indicate that synaptic plasticity is not as sensitive to glutamate spillover as previously thought, and that the degree of transporter impairment required to inhibit LTP is likely not reached in physiological or even pathological conditions.

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Poster

290. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

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Program#/Poster#: 290.04/E11

Topic: B.08. Synaptic Plasticity

Support: CIHR

FRQS

Title: Activity-dependent netrin-1 secretion unmasks silent synapses in the adult hippocampus

Authors: *S. D. GLASGOW¹, I. V. BEAMISH³, S. LABRECQUE⁴, E. WONG³, L. J. TRIGIANI³, J. GIBON³, D. HAN³, S. N. HARRIS³, F. GUIDO², E. HAMEL³, R. A. MCKINNEY², P. DE KONINCK⁵, P. A. SEGUELA³, E. S. RUTHAZER³, T. E. KENNEDY³ ¹Dept. of Neurol. & Neurosurg., ²McGill Univ., Montreal, QC, Canada; ³Neurol. & Neurosurg., Montreal Neurolog. Institute, McGill Univ., Montreal, QC, Canada; ⁴Ctr. De Recherche Univ. Laval Robert-Giffard, Quebec, QC, Canada; ⁵Cell. Neurobio., Univ. Laval, Quebec, QC, Canada

Abstract: Hippocampal neurons in the adult brain express the secreted chemotropic guidance cue netrin-1. We show that membrane depolarization and long-term potentiation (LTP) induction triggers dendritic post-synaptic secretion of netrin-1. Using selective genetic deletion, we demonstrate that neuronal expression of netrin-1 is required for activity-dependent LTP and hippocampal-dependent spatial memory. We show that application of exogenous netrin-1, in the absence of high frequency stimulation, is sufficient to potentiate excitatory glutamatergic transmission at hippocampal Schaffer collateral synapses. Netrin-1 functions downstream of NMDAR activation to engage DCC-dependent synaptic recruitment of GluA1 receptors via activation of CaMKII. Further, netrin-1 increases the volume of thin-type dendritic spines and the frequency of miniature AMPAR-mediated excitatory postsynaptic currents with no change in presynaptic function. Using minimal-intensity stimulation, we demonstrate that netrin-1 unmasks silent glutamatergic synapses. These findings indicate that activity-dependent netrin-1 release recruits GluA1 AMPA receptors to unmask silent synapses in the adult brain.

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Poster

290. LTP: Pre- and Postsynaptic Mechanisms II

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Topic: B.08. Synaptic Plasticity

Support: Swedish Research Council 2014-3254

The Swedish foundation for International Cooperation in Research and Higher Education (STINT)

Title: Ketamine induces lasting alterations of AMPA receptors and synaptic plasticity in the mesolimbic circuit

Authors: *O. SKITEVA¹, N. YAO¹, X. ZHANG², P. SVENNINGSSON², K. CHERGUI¹ ¹Physiol. and Pharmacol., ²Clin. Neuroscience, Section of Translational Neuropharmacology, and Ctr. for Mol. Med., Karolinska Institutet, Stockholm, Sweden

Abstract: Low doses of ketamine trigger rapid and lasting antidepressant effects after one injection in treatment-resistant patients with major depressive disorder. Modulation of AMPA receptors (AMPARs) in the hippocampus and prefrontal cortex is suggested to mediate the antidepressant action of ketamine and one of its metabolites (2R, 6R)-hydroxynorketamine ((2R,6R)-HNK). We have examined if ketamine and (2R,6R)-HNK affect glutamatergic transmission and plasticity in the mesolimbic system, brain regions known to play key roles in reward-motivated behaviors, mood and hedonic drive. We found that one day after the injection of a low dose of ketamine, long-term potentiation (LTP) in the nucleus accumbens was impaired. Loss of LTP was maintained for 7 days and was not associated with an altered basal synaptic transmission mediated by AMPARs and N-methyl-D-aspartate receptors (NMDARs). Inhibition of mTOR signaling with rapamycin did not prevent the ketamine-induced loss of LTP but inhibited LTP in saline-treated mice. However, ketamine blunted the increased phosphorylation of the GluA1 subunit of AMPARs at a CaMKII/PKC site induced by an LTP induction protocol. Moreover, ketamine caused a persistent increase in the phosphorylation of GluA1 at a PKA site. (2R,6R)-HNK also impaired LTP in the NAc. In dopaminergic neurons of the ventral tegmental area from ketamine- and (2R,6R)-HNK-treated mice, AMPAR-mediated responses were depressed while those mediated by NMDARs were unaltered, which resulted in a reduced AMPA/NMDA ratio, a measure of long-term synaptic depression. These results demonstrate that a single injection of ketamine or (2R, 6R)-HNK induces enduring alterations in the function of AMPARs and synaptic plasticity in brain regions involved in reward-related behaviors.

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Poster

290. LTP: Pre- and Postsynaptic Mechanisms II

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Topic: B.08. Synaptic Plasticity

Support: Supported by Basic Science Research Program through the NRF funded by the Ministry of Education, Science and Technology (2016R1A2B2016533).

Title: NMDA receptors are specifically involved in LTP induction in late-spiking neurons of the rat visual cortex

Authors: K. JOO¹, K.-H. CHO², H.-J. JANG³, *D.-J. RHIE⁴ ¹The Catholic Univ. of Korea, Seoul, Korea, Republic of; ²The Catholic university of Korea, Seoul, Korea, Republic of; ³Catholic Univ. Korea, Seoul, Korea, Republic of; ⁴Coll Med. Catholic Univ. Korea, Seoul, Korea, Republic of

Abstract: Cortical inhibitory interneurons play important roles in development, information processing, circuit dynamics and plasticity. These inhibitory interneurons are also driven by excitatory connections and thus plasticity of the excitatory synaptic transmission on interneurons is critical to understand the cortical functions. However, subtype-specific long-term synaptic plasticity remains largely unknown. Therefore, using intracellular recordings and confocal reconstruction, we investigated the mechanism of long-term synaptic plasticity in each subtypes of inhibitory interneurons, especially in late-spiking (LS) neurons of layer 2/3 of the primary visual cortex in rats. Inhibitory interneurons were classified into four subtypes according to the electrophysiological and morphological characteristics: fast-spiking (FS), LS, burst-spiking (BS) and regular-spiking non-pyramidal (RSNP) neurons. Excitatory postsynaptic potentials were recorded by stimulation of underlying layer 4. We used different conditioning stimulation to induce long-term depression (LTD) and potentiation (LTP). All the inhibitory neurons showed LTD with low-frequency stimulation (1 Hz, 15 min) except FS neurons. LTD induced in LS neurons was blocked by intracellular application of BAPTA (10 mM) and bath application of nimodipine (10 µM), but not by bath application of D-AP5 (50 µM). Theta-burst stimulation (5 shocks at 100 Hz, 5 times at 5 Hz) did not change the synaptic efficacy in LS neurons. Tetanic stimulation (5 x 20 Hz trains for 1s at 0.5 Hz, 5 times at 2 min interval) reliably induced LTP in LS neurons as well as pyramidal neurons. LTP in LS neurons was blocked by intracellular application BAPTA and bath application of D-AP5 or nimodipine. These results suggest that LS neurons in layer 2/3 of the visual cortex exhibit bidirectional long-term changes in synaptic strength, which are dependent on the increase of intracellular calcium. NMDA receptors are specifically involved in the induction of LTP, while L-type calcium channels are involved in both types of plasticity. The bidirectional modification of synaptic transmission in LS neurons may be important in visual information processing and postnatal development. Supported by Basic Science Research Program through the NRF funded by the Ministry of Education, Science and Technology (2016R1A2B2016533).

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Poster

290. LTP: Pre- and Postsynaptic Mechanisms II

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Topic: B.08. Synaptic Plasticity

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NIMH R15MH101703

Title: PKA and Ube3a synergistically regulate SK2 synaptic expression and LTP

Authors: *J. SUN, G. ZHU, Y. LIU, R. ADHIKARI, X. HAO, C. CATO, L. HERRERA, M. BAUDRY, X. BI Western Univ. of Hlth. Sci., Pomona, CA

Abstract: The ubiquitin ligase, UBE3A, plays important roles in both brain development and function, since its deficiency results in Angelman Syndrome (AS) while its over-expression increases the risk for autism. We previously showed that Ube3a ubiquitinates SK2, a Ca^{2+} activated small conductance potassium channel, and facilitates its removal from excitatory synapses. Ube3a deficiency results in increased postsynaptic SK2 levels, thereby effectively inhibiting NMDAR activation and LTP induction. SK2 levels in cell membranes are also regulated by protein kinase A (PKA); PKA phosphorylates SK2 in its C-terminal domain, facilitating its endocytosis. Here, we report that activation of PKA by forskolin (FSK) in hippocampal slices from AS mice significantly improves theta burst stimulation (TBS)-induced LTP by removing synaptic SK2 from synaptic membranes. FSK treatment also prevented the LTP enhancement of apamin, a SK2 blocker, in AS mice. Western blot analyses indicated that PKA activation reduced SK2 levels in hippocampal membrane fractions from AS mice. While TBS was unable to induce LTP in hippocampal slices from AS mice, a second TBS (TBS2) applied 15 min after the first TBS (TBS1) successfully induced LTP; immunofluorescence staining results showed that TBS1 induced a significant reduction in the percentage of SK2immunopositive synapses in both WT and AS mice 15 min later. Furthermore, incubation of hippocampal slices with KT5720, a cell-permeable specific PKA inhibitor, blocked TBS2induced LTP in AS mice, indicating that PKA-mediated SK2 removal could compensate for the lack of Ube3a-mediated SK2 removal. Subsequent experiments showed that FSK treatment not only increased SK2 phosphorylation but also its ubiquitination, suggesting that PKA and Ube3a interact to regulate SK2. Mutation of the PKA sites Ser568-570 to alanine (non-phosphorylatable 3S/A-SK2) abolished the effect of FSK on SK2 ubiquitination in COS-1 cells, while mutation of Ser568-570 to aspartate (phosphomimetic 3S/D-SK2) enhanced SK2 ubiquitination. Finally, in *vitro* ubiquitination experiments with GST-3S/D-SK2 showed that phosphomimetic mutations significantly increased SK2 ubiquitination. Our results indicate that both Ube3a-mediated ubiquitination and PKA-induced phosphorylation stimulate SK2 endocytosis, and that PKA and Ube3a synergistically regulate synaptic SK2 levels. The complex interactions between PKA and Ube3a in the regulation of SK2 synaptic levels might provide new tools to for developing new treatments for AS patients as well as various forms of autism.

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Poster

290. LTP: Pre- and Postsynaptic Mechanisms II

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Topic: B.08. Synaptic Plasticity

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Title: The Octopus vulgaris LTP expression and maintenance is mediated by activity dependent long-term elevation in NO concentration

Authors: *B. HOCHNER¹, N. STERN-MENTCH^{1,2}, N. NESHER², T. SHOMRAT², A. L. TURCHETTI-MAIA¹

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Abstract: The Octopus vulgaris vertical lobe (VL) is organized as a fan-out fan-in network. The fan-out glutamatergic synaptic connection possess a robust presynaptic, NMDA-independent, activity-dependent LTP. The VL LTP is important for the acquisition of long-term memory outside the VL (Shomrat T. et al 2008). We have previously shown that the presynaptic expression of LTP involves activation of nitric oxide synthase (NOS), and that a nitric oxide (NO)-dependent 'molecular switch' is responsible for the very long LTP maintenance (>10h) (Turchetti-Maia et al SFN 2016). This molecular switch seems to replace the common protein synthesis dependent LTP maintenance as 20µM anisomycin, which effectively blocks protein synthesis in octopus brain, neither blocked LTP induction nor its long-term expression, while NOS inhibitors reversibly blocked LTP expression. The presence of NOS in the VL is further supported by the intense positive NADPH-d labeling in the VL neuropil. Unexpectedly, our current results do not support the involvement of the common NO dependent cGMP cascade in the VL LTP, as none of the treatments that aim at intervening with this cascade, like inhibitors of guanylyl cyclase, cGMP analogs and phosphodiesterase inhibitors, had an effect on LTP expression. Moreover, NO-donors and NO-scavengers that can modulate low concentration ranges of NO (<nM), and thus are effective in the regulation of the cGMP cascade, had no effect on LTP expression. These negative results suggest that NO might function at higher concentrations, similar to those needed for the direct non-enzymatic proteins s-nitrosylation. To test the feasibility of such mechanism we adapted Dias C. et al (2016) electrochemical method to measure NO amperometrically in the VL neuropil. The results showed that induction of LTP either by tetanization or by phorbol ester are accompanied by an increase in the amperometric

signals that correspond with the oxidation peak potential of NO (750 mV), an increase which is indeed at the μ M range. These findings support our previous conclusion that LTP expression is mediated by a constitutive elevation in NO concentration and further suggest that this elevation is to a relatively higher μ M range. In summary, our amperometric results, together with the abundant NADPH-d staining of the VL neuropil, suggest that LTP is mediated by a higher concentration range of NO that takes place at synaptic regions of the VL. We therefore hypothesize that a process such as s-nitrosylation, which is activated by a relatively high NO concentration, mechanistically could serve as an effective local retrograde message for ensuring specificity in presynaptic Hebbian plasticity.

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Poster

290. LTP: Pre- and Postsynaptic Mechanisms II

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Topic: B.08. Synaptic Plasticity

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Title: Activity-dependent plasticity of synaptic zinc signaling in the dorsal cochlear nucleus - A novel synaptic plasticity mechanism

Authors: *N. W. VOGLER, T. TZOUNOPOULOS

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Abstract: In many brain areas, such as the neocortex, limbic structures, and the auditory brainstem, glutamatergic nerve terminals also contain zinc in their synaptic vesicles (synaptic zinc). In the dorsal cochlear nucleus (DCN), an auditory brainstem nucleus, synaptic zinc is released from glutamatergic parallel fiber synapses. Synaptically released zinc inhibits presynaptic glutamate release by recruiting endocannabinoid signaling, and it also inhibits postsynaptic NMDA and AMPA receptors (NMDARs/AMPARs), thus functioning as a major inhibitory modulator of excitatory synaptic strength (Perez-Rosello et al., 2013; Anderson et al., 2015; Kalappa et al., 2015). This inhibition is activity-dependent, because vesicular zinc levels are reduced following sound exposure (Kalappa et al., 2015). However, the signaling mechanisms underlying this plasticity remain unknown. To study these mechanisms, we employed *in vitro* electrophysiological recordings in DCN brain slices. Application of the extracellular zinc chelator ZX1 (100μM) potentiates AMPAR and NMDAR EPSCs evoked by

stimulation of zinc-rich parallel fibers, demonstrating AMPAR/NMDAR inhibition by synaptic zinc. High-frequency stimulation (HFS, 3 x 100 Hz) of parallel fibers eliminates potentiation by ZX1, indicating activity-dependent plasticity of zinc-mediated inhibition (zinc plasticity). Zinc plasticity is NMDAR-independent, because it is unaffected by the NMDAR antagonist APV (50μ M). However, zinc plasticity is blocked by the metabotropic glutamate receptor (mGluR) antagonist MCPG (500μ M), and the Type 1-specific mGluR antaonists MPEP (4μ M) and LY367385 (100μ M). Additionally, it is blocked by the intracellular calcium buffer BAPTA (10mM). Therefore, zinc plasticity requires activation of Type 1 mGluRs and increases in postsynaptic calcium. Furthermore, the application of CPA (20μ M), an inhibitor of SERCA ATPase which depletes calcium from intracellular stores, is sufficient to induce zinc plasticity. Our results demonstrate activity-dependent plasticity of zinc-mediated inhibition at DCN parallel fiber synapses. Zinc plasticity is NMDAR-independent, but involves activation of Type 1 mGluRs and release of calcium from intracellular stores. Together, these results reveal a novel synaptic plasticity mechanism that modulates zinc-mediated inhibition of glutamatergic neurotransmission.

Disclosures: N.W. Vogler: None. T. Tzounopoulos: None.

Poster

290. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 290.10/F5

Topic: B.08. Synaptic Plasticity

Support: NINDS Grant NS045260

NINDS Grant NS085709

NICHD Grant HD089491

Title: Projection-specific bias in CB₁R signaling and function in hippocampus: Implications for memory encoding

Authors: *C. D. COX¹, W. WANG², Y. JIA⁴, L. C. PALMER⁴, K.-M. JUNG³, D. PIOMELLI⁵, C. M. GALL⁴, G. LYNCH⁴

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Abstract: Recent studies have shown that the lateral perforant path (LPP) afferents to hippocampus exhibit an unusual form of LTP: in contrast to potentiation in hippocampal field CA1, LPP LTP is dependent upon postsynaptic generation of the endocannabinoid 2-

archadonolglycerol (2-AG), presynaptic CB_1 receptor (CB_1R) signaling to actin, and increased neurotransmitter release. These findings raise questions as to the mechanisms through which 2-AG and CB1 predominantly suppress excitatory transmission in the CA1 Schaffer-commissural (S-C) system but increase neurotransmitter release in LPP innervation of the dentate gyrus. As recent work has shown that CB₁R signaling to the synaptic vesicle protein Munc18-1 is critical for CB₁-mediated release suppression, we tested effects of CB₁R agonist WIN55212-1 (WIN) in the two hippocampal subfields. WIN infusion (in the presence of picrotoxin) depressed S-C synaptic responses, blocked LTP and increased presynaptic phospho (p) Munc18-1 levels in field CA1. In contrast, WIN infusion increased the amplitude of *lpp*LTP without effect on LPP baseline responses or presynaptic pMunc18-1 levels in the LPP field. CB₁ has been reported to work cooperatively with integrins to signal through focal adhesion kinase (FAK) and RhoA kinase (ROCK) and influence the actin cytoskeleton. In line with this, we found WIN infusion increased presynaptic pFAK and pROCK levels in the LPP field. Similarly, high frequency stimulation used to induce *lpp*LTP caused a CB₁R-dependent increase in pFAK and pROCK in LPP terminals. These findings indicate that there is projection-specific bias in CB₁R signaling and function in hippocampus with predominant signaling (i) through the ERK1/2 - Munc18-1 pathway, leading to direct suppression of excitatory synaptic transmission, in field CA1 and (ii) through a FAK - ROCK cascade leading to enhanced neurotransmitter release in the LPP. These results further suggest that forms of learning that depend upon the LPP (cue identity, semantic information) may engage and depend upon the FAK/ROCK cascade. This was tested in rats trained in a two-odor discrimination paradigm known to depend on the LPP. We found that odor discrimination learning caused a CB₁R-dependent increase in presynaptic pROCK in the LPP field. Together these findings show sharp differences in the bias of CB₁R signaling at two major excitatory relays in hippocampal circuitry and suggest a neurobiological basis for discrete cannabinoid effects on learning.

Disclosures: C.D. Cox: None. W. Wang: None. Y. Jia: None. L.C. Palmer: None. K. Jung: None. D. Piomelli: None. C.M. Gall: None. G. Lynch: None.

Poster

290. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

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Program#/Poster#: 290.11/F6

Topic: B.08. Synaptic Plasticity

Support: NINDS Grant NS045260

NICHD Grant HD089491

Title: Facilitating endocannabinoid signaling restores a component of episodic memory in Fmr1-KO mice

Authors: *C. M. GALL, W. WANG, B. M. COX, Y. JIA, A. A. LE, C. D. COX, K. M. JUNG, D. PIOMELLI, G. LYNCH Anat. and Neurobio., Univ. of California, Irvine, CA

Abstract: Autism spectrum disorders frequently entail disturbances in episodic memory but the neurobiological basis of this intellectual disability is not understood. The hippocampus is critical for the formation and use of episodic memory, with cue identity ('what') information relayed to hippocampus via the lateral perforant path (LPP). We have shown that in normal rats and mice the LPP afferents of the dentate gyrus exhibit an unusual form of long-term potentiation (*lpp*LTP) that depends on the CB₁ receptor and signaling by the endocannabinoid 2archadonolglycerol (2-AG) at excitatory synapses (Wang et al., 2016). The present electrophysiological analyses of acute hippocampal slices show that *lpp*LTP is profoundly impaired in Fmr1-KOs relative to wild type mice. The plasticity impairment was substantially greater than for other major afferents to the dentate gyrus granule cells or in hippocampal field CA1. Behavioral studies demonstrated that learning to recognize odors presented in a series depends on the LPP and that such serial odor learning is severely impaired in the KOs. Acquisition of odor identity outside of the sequence was not affected by the mutation. Finally, we show that multiple strategies for enhancing 2-AG signaling in Fmr1-KO mice, rescues both *lpp*LTP and serial odor learning. Thus, manipulations that target endocannabinoid transmission in hippocampus restore plasticity in a pathway critical to the encoding of the semantic element of an episode and thereby rescue an important component of cognition in Fragile X model mice.

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Poster

290. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

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Program#/Poster#: 290.12/F7

Topic: B.08. Synaptic Plasticity

Support: NIH Grant R15NS078645

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Institutional Mentoring Environment Grants

Title: Long-term potentiation of inhibitory inputs onto VTA GABA neurons

Authors: *T. M. NUFER¹, J. G. EDWARDS² ¹Physiol. and Developmental Biol., ²Brigham Young Univ., Provo, UT

Abstract: The mesolimbic dopamine circuit, and in particular the ventral tegmental area (VTA), processes reward and motivational behaviors. The VTA contains dopaminergic cells thought to mediate reward as well as GABAergic inhibitory cells that regulate dopamine cell activity. In response to drug exposure, synaptic connections of this circuit can be rewired via synaptic plasticity—a phenomenon thought be responsible for the pathology of addiction. Therefore, an understanding of normal and pathologic synaptic plasticity in VTA neural circuitry is critical if we are to better understand and treat addiction. While much is known about dopamine neuron plasticity, less is known regarding the potential for and type of plasticity exhibited by VTA GABA cells. VTA GABAs receive inhibitory input from outside the VTA, however we know little about these inputs and their synaptic plasticity. A recent study demonstrated a novel form of GABAergic long-term potentiation (LTP) onto VTA GABA cells, which was presynaptic and dependent on voltage-gated calcium channels and D1 dopamine receptors (Bocklisch et al., 2013, Science). We have confirmed this GABA LTP in VTA GABA cells from GAD67/GFPpositive mice using whole cell electrophysiology. Paired pulse ratios suggest a presynaptic mechanism, as noted previously (baseline, 1.20 ± 0.19 ; after stimulation, 0.79 ± 0.14 ; n=9, p=0.068). Therefore, we examined several presynaptic mediators of plasticity, including cannabinoid receptor 1 (CB1) and nitric oxide. In CB1 knock-out animals (n=6) or in the presence of the CB1 antagonist AM251 (n=4), significant LTP was still exhibited (p < 0.05). The nitric oxide synthase antagonist L-NAME also failed to block the observed LTP (n=4, p < 0.05), and the nitric oxide donor SNAP failed to induce plasticity (n=5, p > 0.05). This LTP was also independent of D2 dopamine receptors, as the D2 antagonist eticlopride did not block LTP (n=5). However, surprisingly this LTP appears to be NMDA-dependent, as it was blocked by the NMDA receptor antagonist APV (n=4; p > 0.05). Collectively, our data suggest a presynaptic mechanism of LTP that is yet to be fully determined, but production of a retrograde factor produced from excitatory synaptic activation is likely involved.

Disclosures: T.M. Nufer: None. J.G. Edwards: None.

Poster

290. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 290.13/F8

Topic: B.08. Synaptic Plasticity

Support: CityU Research Grant 9610347

Title: Synaptic plasticity of interlamellar ca1 network of hippocampus

Authors: *H. TETTEH¹, D. SUN², J. SU¹, S. YANG¹, S. YANG² ¹Dept. of Biomed. Sci., City Univ. of Hong Kong, Kowloon, Hong Kong; ²Nano-bioengineering, Incheon Natl. Univ., Incheon, Korea, Democratic People's Republic of **Abstract:** The connection along the longitudinal axis of the CA1 hippocampal cells has not received attention while most studies have looked at the transverse orientations of the CA3 and/or CA1 regions. Recently, our group has shown that the CA1-CA1 pyramidal neurons are well-organized and have a longitudinally projecting synaptic network. This triggered an interest in synaptic plasticity of long-term potentiation (LTP) and long-term depression (LTD) within the longitudinal CA1 hippocampal region. Here we studied long-term synaptic plasticity in CA1 longitudinal plane using *in vitro* and *in vivo* recordings. Animals used were C57BL6 mice. We found that the longitudinal network has N-methyl-D-aspartate receptor (NMDAR)-dependent LTP. Meanwhile, there is no apparent long-term depression (LTD) under the frequently used LTD induction protocols. These results implicate a unique functional property in the longitudinal projection, stimulating a further research regarding how the longitudinal network contributes to information processing.

Disclosures: H. Tetteh: None. D. Sun: None. J. Su: None. S. Yang: None. S. Yang: None.

Poster

290. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

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Program#/Poster#: 290.14/F9

Topic: B.08. Synaptic Plasticity

Support: CDMRP Grant W81XWH-08-2-0136 to MJF

Title: Highly irregularly patterned 10 Hz stimulation, but not regularly patterned 10 Hz stimulation, activates a disinhibition mechanism that induces long-term potentiation following mild traumatic brain injury

Authors: *Q. S. FISCHER, D. KALIKULOV, M. J. FRIEDLANDER Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: Previously, using whole cell (WC) recordings in acute slices of visual cortex, we found that 10 Hz discontinuous highly irregular stimulation patterns (interstimulus intervals with a coefficient of variation = 1, or CV = 1) but not regular stimulation patterns (CV = 0), led to a significant net long-term potentiation (LTP) of postsynaptic responses in rats with mild traumatic brain injury (mTBI rats) but not in control rats. Here we used field potential (FP) recordings and pharmacological manipulations (gama-aminobutyric acid receptor blockade, or GABAblk) to investigate the potential underlying mechanisms for these phenomena. Acute slices of visual cortex were taken from 10 - 13 week old normal rats and rats who had received an mTBI 2 - 3 weeks prior. We applied a defined pattern of stimulation to layer 4 and made FP recordings in layer 2/3. Conditioning stimulation consisted of 900 pulses at 10 Hz (9 epochs x 100 stimuli spaced equally over 900 sec) with a CV of 0 or 1. Recording of evoked FPs were made in

response to 0.1 Hz test stimulation before and after conditioning in artificial cerebral spinal fluid alone or with the addition of GABA receptor blockers (GABAblk). The post- to pre-conditioning ratio of the peak amplitudes of the 0.1 Hz evoked FPs was used to assess plasticity. We defined long-term depression (LTD) as a significant (P < 0.05, t-test) decrease ($\geq 10\%$) in post/pre ratios, LTP as a significant increase in post/pre ratios, or no change (NC) as post/pre ratios that changed < 10% and/or were not significantly different (P > 0.05, t-test). For regular (CV = 0) stimulation, control rats exhibited a net LTD while mTBI rats exhibited a net NC, and this was comparable in WC (0.81 \pm 0.08, N = 17 vs. 0.90 \pm 0.07, N = 17; P = 0.40, t-test) and FP (0.89 \pm 0.04, N = 16 vs. 0.95 ± 0.05 , N = 12; P = 0.44, t-test) recordings. Moreover, GABAblk did not significantly change FP values in controls $(0.89 \pm 0.04, N = 16 \text{ vs. } 0.92 \pm 0.04, N = 5, P = 0.79 \text{ t-test})$ or mTBI rats $(0.95 \pm 0.05, N = 12 \text{ vs. } 0.89 \pm 0.09, N = 8$: P = 0.57 t-test). For highly irregular (CV = 1) stimulation, control rats showed a net LTD or net NC while mTBI rats showed a net LTP which was similar in WC (0.63 ± 0.07 , N = 19 vs. 1.17 ± 0.12 , N = 18; P = 0.001, t-test) and FP $(0.94 \pm 0.05, N = 24 \text{ vs. } 1.15 \pm 0.10, N = 17; P < 0.05, t-test)$ recordings. Finally, GABAblk reduced FP values in mTBI rats (1.15 ± 0.10 , N = 17 vs. 0.99 ± 0.05 , N = 18; albeit not quite significantly P = 0.12, t-test), but not control rats (0.94 \pm 0.05, N = 24 vs. 0.95 \pm 0.06, N = 18; P = 0.90, t-test). These results indicate that highly irregular (CV = 1) 10 Hz stimulation, but not regular (CV = 0) 10 Hz stimulation, activates a disinhibition mechanism that induces LTP following mTBI.

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Poster

290. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 290.15/F10

Topic: B.08. Synaptic Plasticity

Title: Metabolic processes underlying Hebbian hippocampal long term potentiation

Authors: *P. MIRANDA¹, H.-A. PARK¹, S. SACCHETTI¹, K. N. ALAVIAN², H. LI³, H. IMAMURA⁴, H. NOJI⁵, J. D. SHEPHERD⁶, A. E. CHAVEZ⁷, R. ZUKIN⁸, E. A. JONAS¹ ¹Yale Univ. Dept of Intrnl. Med., New Haven, CT; ²Intrnl. Medicine, Endocrinol., Imperial Col. London, London, United Kingdom; ³Yale Univ., New Haven, CT; ⁴Biostudies, Kyoto Univ., Kyoto, Japan; ⁵Applied Chem., Univ. of Tokyo, Tokyo, Japan; ⁶Neurobio. and Anat., Univ. of Utah, Salt Lake City, UT; ⁷Dominick P Purpura Dept Neurosci., Albert Einstein Col. Medici, Bronx, NY; ⁸Dept Neurosci, Albert Einstein Col. Med., Bronx, NY

Abstract: Long-term potentiation (LTP) and depression (LTD) are the mechanisms by which neurons modulate their inherent synaptic plasticity and support the storage and recovery of memories in the mammalian brain. The ability to potentiate a synapse long term declines

significantly in neurodegenerative disorders. In addition to deficiencies in synaptic plasticity, degenerating neurons display acute and chronic mitochondrial dysfunction, suggesting that dysregulated mitochondria play a hand in synaptic dysfunction in addition to their known role in apoptotic death. Our previous work shows that the anti-apoptotic protein Bcl-xL not only prevents somatic cell death, but it also potentiates long-term synaptic responses. Here, we show that Bcl-xL is responsible for dramatic changes of ATP levels at synapse-specific sites in hippocampal neurons during LTP. Using fluorescent imaging of an ATP-FRET (ATeam) construct in living cells, we find that LTP induction in cultured neurons causes a short decrease in ATP levels followed by a persistent long term increase in ATP production. This suggests that after high frequency or intense synaptic stimulation, neurons may become metabolically more efficient; oxygen consumption rates during LTP are now being performed to confirm or refute the proposed change in efficiency. The long-term increase in ATP levels of LTP-stimulated synapses is blocked when we inhibit Bcl-xL and when ATP synthase activity is blocked with oligomycin. Bcl-xL inhibition also prevents a long-term increase in surface glutamate receptor insertion. We are completing studies to see what effect blocking ATP synthase activity has on glutamate receptor insertion. In hippocampal slice recordings, inhibition of Bcl-xL greatly impairs early stage LTP and prevents late stage LTP. We suggest that long term changes in mitochondrial efficiency brought on by activity-dependent translocation of Bcl-xL to mitochondria are required for LTP. These results shed light upon the role of changes in mitochondrial metabolic efficiency in the acute induction and long-term maintenance of learning and memory storage. If such mitochondrial changes fail to occur, synaptic dysfunction associated with neurodegeneration may ensue. Our study places mitochondria and Bcl-xL at the center of synaptic metabolism and synaptic plasticity.

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Poster

290. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 290.16/F11

Topic: B.08. Synaptic Plasticity

Support: CONACYT Grant 286537

Title: Effects of non-selective activation of dopaminergic receptors by apomorphine in the hippocampus

Authors: *L. ARROYO GARCÍA, SR¹, R. A. VAZQUEZ-ROQUE¹, A. DIAZ¹, A. RODRIGUEZ-MORENO², F. DE LA CRUZ³, G. FLORES¹

¹Benemerita Univ. Autonoma De Puebla, Puebla, Mexico; ²Univ. Pablo de Olavide, Sevilla, Spain; ³Inst. Politecnico Nacional, Ciudad de Mexico, Mexico

Abstract: Apomorphine is a dopaminergic agonist used in the treatment of Parkinson's disease (PD), which activates D1-like and D2-like dopaminergic receptors. Currently, the role of apomorphine in non-motor activity is poorly studied, and the effects of dopaminergic activation in brain areas that do not have motor functions are unknown. The aim of this study is to determinate the effect of dopaminergic receptor activation in plasticity and behavior in the hippocampus. Adult mice were chronically administrated with 1 mg/kg apomorphine during 15 days. Memory and learning, synaptic plasticity, oxidative stress response and morphological changes were evaluated. For plasticity experiments, electrophysiological recordings (fEPSPS) were obtained in the CA1 region of hippocampal slices prepared from adult mice and the effect on LTP of apomorphine (20 µM) studied. In this experimental condition LTP was prevented. Also in the presence of apopmorphine, by using the Morris water maze test, a decrease in the learning and the long-term memory capabilities of mice were observed. Additionally, a decrease in the total dendritic length of CA1 neurons, and an increase in the number of astrocytes were observed in the presence of apomorphine by using the Golgi-Cox stain and GFAP immunohistochemistry, respectively. These results suggest that, in the hippocampus, the nonselective activation of dopaminergic receptors by apomorphine prevents LTP, causes neuronal damage and triggers deficiencies in learning and memory.

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Poster

290. LTP: Pre- and Postsynaptic Mechanisms II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 290.17/F12

Topic: B.08. Synaptic Plasticity

Support: HHMI Medical Research Fellows Program

Title: The role of postsynaptic cell-adhesion molecules in the trafficking of AMPA-type glutamate receptors

Authors: *A. RILEY, X. WU, R. C. MALENKA, T. C. SUDHOF Stanford Univ., Stanford, CA

Abstract: Long-term potentiation (LTP), an activity dependent, long-lasting change in synaptic strength, is a critical form of synaptic plasticity underlying learning and memory and has been implicated in various neuropsychiatric disorders. The expression of NMDA receptor-dependent

LTP is dependent on the trafficking of AMPA receptors (AMPARs) to the postsynaptic membrane. The present project is guided by the unexpected role of cell adhesion proteins, which mediate trans-synaptic signaling, in LTP. Specifically, the project focuses on the role of postsynaptic neuroligin 1 and its binding to presynaptic neurexins. This heterophilic interaction is present in mature and developing synapses and its absence has been shown to impair LTP. Here, we generate various neuroligin constructs in which different functional domains of neuroligin 1 are mutated. We used these constructs to determine which specific neuroligin domains are necessary for the AMPAR exocytosis that occurs during LTP. Specifically, we prepared primary dissociated mouse hippocampal cultures from neuroligin 1 (NL1) and neuroligin 1, 2, 3, and 4 (NL1234) conditional knockout mice (cKO) and infected these cultures at DIV7-9 with lentiviruses containing Cre recombinase and either wildtype (WT) NL1 or mutant forms of NL1. At DIV18-20, we induced a form of chemical LTP by bath applying glycine and measured the resultant increases in surface expression of endogenous AMPARs using immunocytochemistry. Our results suggest that the exocytosis of AMPARs during chemical LTP is impaired upon deletion of neuroligin 1; can be rescued solely by the extracellular domain of neuroligin 1; does not require neuroligin dimerization, and is impaired when NL1 binding to neurexin is inhibited. Thus, the trans-synaptic interaction between NL1 and presynaptic neurexins appears to be necessary for the increase in surface expression of endogenous AMPARs following the induction of chemical LTP in dissociated cultures prepared from NL cKO mice. This hypothesis is also being tested using acute hippocampal slices prepared from NL cKO mice (see abstract from Wu et al....).

Disclosures: A. Riley: None. X. Wu: None. R.C. Malenka: None. T.C. Sudhof: None.

Poster

290. LTP: Pre- and Postsynaptic Mechanisms II

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Topic: B.08. Synaptic Plasticity

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Program of Shanghai Subject Chief Scientist (17XD1401700)

Title: Extrasynaptic NMDA receptor dependent long-term potentiation of hippocampal CA1 pyramidal neurons

Authors: *S.-T. LI¹, Q. YANG¹, J. WANG², J. LUO² ¹Bio-X Institutes, Shanghai Jiao Tong Univ., Shanghai, China; ²Zhejiang Univ. Sch. of Med., Zhejiang, China

Abstract: In the adult mouse hippocampus, NMDA receptors (NMDARs) of CA1 neurons play an important role in the synaptic plasticity. The location of NMDARs can determine their roles in the induction of long-term potentiation (LTP). However, the extrasynaptic NMDARs (ES-NMDARs) dependent LTP haven't been reported. Here, through the use of a 5-Hz stimulation and MK-801 (an irreversible antagonist of NMDARs) in the CA1 neurons of adult mice hippocampal slices, synaptic NMDARs were selectively inhibited and NMDAR-mediated excitatory postsynaptic currents were not recovered. We found that a robust LTP was induced by 3-train 100-Hz stimulation when the synaptic NMDARs and extrasynaptic NR2B containing NMDARs were blocked, but not in the any of the following conditions: blocking of all NMDARs (synaptic and extrasynaptic), blocking of the synaptic NMDARs, and blocking of the synaptic NMDARs and extrasynaptic NR2A-containing NMDARs. The results indicate that this LTP is ES-NMDARs dependent, and NR2B-containing ES-NMDARs modulates the threshold of LTP induction.

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Poster

291. Signal Propagation

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 291.01/G2

Topic: B.10. Network Interactions

Support: NSF Grant 1632891

NSF Grant 1547693

Title: Utilization of a newly synthesized degradable biocomposite: Copper-high aspect ratio structure (CuHARS) for dynamic signaling to brain cells

Authors: *K. C. ST MARTHE, A. KARAN, Z. NORCROSS, M. A. DECOSTER Biomed. Engin., Louisiana Tech. Univ., Ruston, LA

Abstract: New materials at the nano- and micro-scale are being engineered for a host of potential neuroscience applications. This includes desired outcomes of drug delivery, cellular guidance, and sensing, for example. Here we have applied a newly synthesized, copper-

containing high aspect ratio structure (CuHARS), for dynamic signalling delivery to brain cells in vitro. CuHARS is a biocomposite discovered by our group, containing the amino acid dimer cystine and the metal copper. CuHARS scales from nanometer to micrometer in diameter, and micrometer in length. Upon synthesis termination, CuHARS is stable in water, but degraded up to 95% as determined by image analysis when incubated for 18 days in microglial brain cell culture media under physiological conditions. Degradation is accelerated by use of metal chelators such as ethylenediaminetetraacetic acid (EDTA), with similar near-complete degradation occurring in 4 days; the effect was concentration dependent (10 mM- 50mM). We applied these findings to the question of whether discrete, biodegradable biocomposites could be used in dynamic signalling networks of brain cells. We engineered two types of brain cell environments: 1) glial depleted, and 2) glial enriched, and then carried out intracellular calcium imaging on both systems. All experiments were carried out on identical platings of cells, with glial depleted cultures developed over time using the anti-mitotic cytosine arabinoside (1mM final concentration). The two cell environments once developed were stimulated with successive, increasing concentrations of glutamate (Glu) and with ionomycin at the end of the scan to cause calcium influx in all cells. Responses to Glu were detected as low as 250 nM in some cells. In contrast, control cultures of pure astrocytes responded consistently at 1,000x greater Glu (high micromolar to millimolar stimulus or to control stimuli such as KCl and ATP). The dynamics of intracellular calcium changes were very different in environments 1 and 2, with glial enriched cultures demonstrating more oscillatory dynamics post Glu compared to glial depleted cultures. The randomness of oscillations in stimulated cells was compared to a discrete logistic model that could be tuned for chaotic behavior. Using this model, it was found that glial enriched cultures were more chaotic. In this same imaging system, CuHARS were fluorescently labeled such that they could be observed simultaneously with cells. This system along with a novel degradable biocomposite (CuHARS) could provide new tools for dynamically stimulating brain cells over longer periods than when using traditional, immediately soluble chemical applications.

Disclosures: K.C. St Marthe: None. A. Karan: None. Z. Norcross: None. M.A. DeCoster: None.

Poster

291. Signal Propagation

Location: Halls A-C

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Topic: B.10. Network Interactions

Support: CNRS

European Community (Human Brain Project, H2020-720270)

ICODE excellence network

Title: Enhanced responsiveness in asynchronous irregular network states as a mechanism for modulating sensory awareness

Authors: *A. DESTEXHE^{1,2}, Z. GIRONES¹, Y. ZERLAUT^{1,3} ¹CNRS, Gif-sur-Yvette, France; ²European Inst. for Theoretical Neurosci., Paris, France; ³Inst. Italiano di Tecnologia, Rovereto, Italy

Abstract: Desynchronized brain states are known to be associated with arousal and increased awareness, but the underlying cellular and network mechanisms are unknown. Here, we explore networks of excitatory and inhibitory neurons displaying asynchronous irregular (AI) states, where the activities of the two populations are balanced. At the single-cell level, it was previously shown that neurons subject to balanced and noisy synaptic inputs can display enhanced responsiveness. We show here that this enhanced responsiveness is also present at the network level, but only when single neurons are in a regime where their conductance state and fluctuation level are consistent with experimental measurements. By scanning a large number of AI states, we show that the network responsiveness can be explained based on the mean conductance state, the mean membrane potential (Vm), and the amplitude of Vm fluctuations in individual neurons. Optimal responsiveness is obtained when these parameters are all consistent with experimental measurements. Multi-layer networks endowed with such "realistic" AI states are capable of transmitting information reliably across layers (Fig. 1). We conclude that when displaying "realistic" AI states, networks can be exquisitely sensitive to afferent inputs, transmit information reliably, and make the information available to the entire network. We propose that these features define a low-level form of sensory awareness, which can be modulated at the millisecond scale.



Figure 1: A. Scheme of successive layers each consisting of a network of excitatory and inhibitory neurons. A Gaussian-distributed pulse packet is given as input to a subset of neurons. B. Propagation of the pulse packet across layers is state dependent. Blue (Active): all layers displayed AI states consistent with intracellular measurements. Brown: quiescent networks (at equivalent Vm) for comparison. The pulse packet was reliably transmitted only in AI states, and the interlayer propagation speed was also faster (modified from Zerlaut and Destexhe, Neuron, 2017; Girones and Destexhe, arXiv 2016).

Disclosures: A. Destexhe: None. Z. Girones: None. Y. Zerlaut: None.

Poster

291. Signal Propagation

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Topic: B.10. Network Interactions

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Title: A study of single-pulse cortical stimulation effects across cortical micro-domains

Authors: *P. KUDELA¹, R. OH³, G. MILSAP², N. E. CRONE⁴, W. S. ANDERSON¹ ¹Neurosurg., Johns Hopkins Univ., Baltimore, MD; ²Johns Hopkins Univ., Biomedical Engineering, MD; ³Johns Hopkins, Whiting School of Engineering, MD; ⁴Neurol., Johns Hopkins Hosp., Baltimore, MD

Abstract: Introduction: Direct cortical stimulation is widely used for functional mapping of eloquent cortex and for interrupting seizures using closed-loop brain stimulators. The neurophysiological effects of cortical stimulation in a volume of cortical tissue are complex and difficult to predict as they may depend on the context of ongoing neural activity and may be affected by many factors including tissue heterogeneity, morphology, and cortical connectivity. To investigate how direct cortical stimulation induces neuronal activity spatially and temporally we combined micro-intracranial electrocorticographic (mECoG) recordings from human subjects with a large scale computational model of neocortex. Using single-pulse electrical stimulation we investigated the dynamics and evolution of stimulus-evoked cortical activity across micro-domains sampled by micro-electrode arrays. Methods: Two adult patients (male and female) were implanted with a subdural grid embedded with 4x4 micro-electrode arrays (superior temporal or precentral/postcentral gyri). Center-to-center distances between the macro and micro electrodes were between 4 and 8 mm. Monopolar stimulation with biphasic pulses were delivered to the macroelectrode elements (pulse width 0.3 ms, stim. current 1-10 mA, 50 trials, 5

s intervals between pulses). Data from the micro-electrodes were sampled at 30 kHz. The cortical model was designed in pGENESIS 2.3 to simulate the effects of monopolar stimulation on cortical neurons modeled as multi-compartmental cells. Stimulus-evoked responses for both recorded and simulated data were derived by trial-averaging. Result: Early (<10ms) and late cortical responses resembling sharp-and-slow waves were observed in mECoG from micro-domains after stimulation. Early responses were difficult to analyze due to stimulation artifacts. Peak latency analyses of the late responses from different micro-electrodes revealed statistically significant differences in the measured latencies, suggesting a consistent pattern of activity propagation across micro-domains. This propagation was not uniform and had saltatory characteristics across micro-domains. Conclusions: Early cortical responses reflect direct neuronal recruitment by the stimulating electrode and possibly early activity propagation through the superficial cortical layers that follows stimulation. The late responses likely reflect interactions with subcortical structures, and the different latencies among late responses across micro-domains may partially result from initial propagation after stimulation. We have also observed similar effect in cortical model simulations.

Disclosures: P. Kudela: None. R. Oh: None. G. Milsap: None. N.E. Crone: None. W.S. Anderson: F. Consulting Fees (e.g., advisory boards); Globus Medical,, Longeviti, LLC.

Poster

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SUNY Albany Research Foundation

Title: Balancing excitation and inhibition shapes the dynamics of a neuronal network for movement and reward

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Abstract: The cortico-striatal-thalamo-cortical (CSTC) pathway is a brain circuit that controls movement execution, habit formation and reward. The striatum shapes the activity of the CSTC pathway through the coordinated activation of two classes of medium spiny neurons (MSNs) that express D1 or D2 dopamine receptors. It is unknown exactly how the balanced

excitation/inhibition in the entire CSTC pathway or in D1- and D2-MSNs shapes the network dynamics of the entire circuit. Here we answer this question using non-linear modeling of neuronal activity and bifurcation theory. Our findings indicate that a global and proportionate increase in excitation/inhibition pushes the system to states of generalized hyper-activity throughout the entire CSTC pathway. Certain disproportionate changes in global excitation/inhibition trigger network oscillations. Local changes in the excitation/inhibition of MSNs generate specific oscillatory behaviors inMSNs as well as in the CSTC pathway. These findings indicate that subtle changes in the relative strength of excitation/inhibition of MSNs powerfully control the network dynamics of the CSTC pathway, in ways that are not easily predicted by its own synaptic connections.

Disclosures: A.R. Radulescu: None. A. Scimemi: None.

Poster

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Grants-in-Aid for Science Research on Innovative Areas "Mental Time" (25119004)

Title: Activation of hilar mossy cells and dentate granule cells during sharp wave ripples In vitro

Authors: *A. OUCHI, N. MATSUMOTO, Y. IKEGAYA

The Univ. of Tokyo, Tokyo, Japan

Abstract: Various hippocampal oscillations may differently control memory and learning. Hippocampal sharp waves (SWs)/ ripples are high-frequency oscillations emitted mainly during slow-wave sleep or quiet rest states and play a role in memory consolidation. While it is known that SWs are initiated in the CA3 subregion and propagate to the downstream CA1 subregion, we observed that they also propagate back to the dentate gyrus. However, neither the role of CA3-to-DG SW backpropagation nor its propagation mechanism has been fully understood. We reasoned that mossy cells mediate the backpropagation, because these neurons receive direct synaptic inputs from CA3 neurons and made synaptic connection with granule cells in the dentate gyrus. In this study, we simultaneously recorded the membrane potentials from mossy cells or granule cells and the local field potentials from the CA3 stratum pyramidale. The recorded cells were morphologically confirmed based on *post-hoc* intracellular biocytin labelling. Compared with granule cells, mossy cells more reliably depolarized immediately after the generation of SWs. Therefore, mossy cells precisely reflect the activity of SWs from the CA3 subregion. Our findings shed light on a novel mechanism underlying the SW backpropagation will provide a new perspective to memory consolidation.

Disclosures: A. Ouchi: None. N. Matsumoto: None. Y. Ikegaya: None.

Poster

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Title: The characterization of hippocampal theta-driving neurons --- a time-delayed mutual information approach

Authors: *S. LI¹, J. XU², G. CHEN², L. LIN², D. ZHOU³, D. CAI¹ ¹New York Univ., New York, NY; ²East China Normal Univ., Shanghai, China; ³Shanghai Jiao Tong Univ., Shanghai, China

Abstract: Interneurons are important for computation in the brain, in particular, in the information processing involving the generation of theta oscillations in the hippocampus. Yet the functional role of interneurons in the theta generation remains to be elucidated. Here we use time-delayed mutual information to investigate information flow related to a special class of interneurons --- theta-driving neurons in the hippocampal CA1 region of the mouse --- to characterize the interactions between theta-driving neurons and theta oscillations. For freely behaving mice, our results show that information flows from the activity of theta-driving neurons to the theta wave, and the firing activity of theta-driving neurons shares a substantial amount of information with the theta wave regardless of behavioral states. Via realistic simulations of a CA1 pyramidal neuron, we further examine to which interneuron type these theta-driving neurons belong. We apply time-delayed mutual information to analyze information flow between

the experimentally recorded theta wave and the computationally obtained membrane potential trace induced by each of five candidate interneurons --- the basket cell, the axo-axonic cell, the bistratified cell, the oriensalveus/lacunosummoleculare cell, and the cholecystokinin-expressing basket cell. Based on the consistency of information flow from the activity of the pyramidal neuron to the theta wave, our analysis demonstrates that theta-driving neurons fit the characteristics of the cholecystokinin-expressing basket neurons. Our results suggest that it is important to take into account the role of cholecystokinin-expressing basket neurons in the generation and information processing of theta oscillations.

Disclosures: S. Li: None. J. Xu: None. G. Chen: None. L. Lin: None. D. Zhou: None. D. Cai: None.

Poster

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Topic: B.10. Network Interactions

Support: NIH Grant 2R01NS060757-05A1

NIH Grant 5T32 EB004314-18

Title: Can neural activity propagation be mediated by ephaptic coupling?

Authors: *R. SHIVACHARAN, C.-C. CHIANG, D. M. DURAND Dept. of Biomed. Engin., Case Western Reserve Univ., Cleveland, OH

Abstract: It is well documented that synapses play a significant role in transmission of information between neurons. However, in the absence of synaptic transmission, neural activity has been observed to continue to propagate. Previous experiments conducted in our laboratory have shown epileptiform spikes in rodent hippocampi propagate at a consistent speed of 0.1 m/s and can take place in the absence of synaptic transmission. Computer simulations and osmolarity experiments indicate that electric fields could be responsible for this phenomenon. These data suggest that electric fields can activate neighboring neurons (ephaptic coupling), thereby generating a self-propagating wave. Here, we test the hypothesis that electric fields are directly responsible for the propagation by 1) determining if an applied field with amplitude similar to the endogenous fields can trigger a propagating wave and 2) applying an uniform electric field (1-5 mV/mm) can generate a wave that travels at similar speeds to that of 4-AP activity (~0.1 m/s). We also observed that applying a uniform field (1-5 mV/mm) can speed up or slow down
propagation of the spontaneous activity depending on the polarity. In addition, short-circuiting the field resulted in a decrease in propagating speed. These results strongly support the hypothesis that ephaptic coupling can be responsible for volume conduction of neural activity and point to a novel mechanism of neural activity propagation in the brain. Funding Source: NIH 2R01NS060757-05A1 & NIH 5T32 EB004314-18

Disclosures: R. Shivacharan: None. C. Chiang: None. D.M. Durand: None.

Poster

291. Signal Propagation

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Support: CIHR Grant MOP_102482

Title: Layer-dependent inter-areal effective connectivity among anterior cingulate and prefrontal cortex in monkeys revealed by electrical microstimulation

Authors: *V. NACHER¹, T. WOMELSDORF^{1,2}

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Abstract: The anterior cingulate cortex (ACC) and prefrontal cortex (PFC) functionally coactivate during the monitoring and shifting of attention. This co-activation is evident in spiketrain correlations, burst-to-LFP synchronization, and inter-areal phase-amplitude coupling. This diverse functional connectivity pattern is paralleled by a diverse pattern of anatomical connectivity with a large proportion long-range synapses made to inhibitory interneurons. One approach to elucidate the possible link between functional and anatomical connectivity is to characterize the efficacy and temporal patterning of anatomical connections using electrical microstimulation. Here, we set out to map this so-called "effective connectivity" across layers between the ACC (areas 32, 24c) and the PFC (areas 9/46d, 46d, 46v) in two awake monkeys using electrical microstimulation consisting of single, 2, 4 or 8 biphasic square pulses (200 µs duration each, 40-500 µA current amplitude), with an inter-pulse delay of 2 ms. These protocols reliably triggered long-range evoked responses in both directions with evoked field amplitudes increasing from 1 to 8 pulses and 140 to 500 µA. We found three main results. Firstly, ACC-to-PFC stimulation resulted in shorter-latency evoked fields than PFC-to-ACC stimulation. This asymmetry could indicate differential synaptic connectivity profiles in both directions. Secondly, the majority of evoked responses showed a multiphasic pattern with 2-4 points of inflection. This indicates resonance or ringing phenomena that could help understanding the underlying mechanisms of synaptic integration. Thirdly, amplitudes of the evoked responses varied

systematically with the laminar location of either recording or stimulation electrode. This layer specific amplitude modulation likely relates to the density of synaptic contacts made between ACC and PFC circuits. In summary, we characterized the diversity of effective connectivity patterns between those two brain areas whose interactions is believed to be critical for goal directed behavior. We believe that the effective connectivity maps we report will serve as the critical link to bridge the gap between anatomical connections and the possible functional co-activations that ride on top of them during different functional states.

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Poster

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Topic: B.10. Network Interactions

Support: KAKENHI 16K15177

Title: Peak reduction of axonal spikes in the hippocamal mossy fibers during the high frequency stimulation

Authors: *S. OHURA, H. KAMIYA

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Abstract: Axons are considered as the reliable digital cable which enable the secure propagation of neuronal information from the cell body to the nerve terminal. However, axons in the central nervous system (CNS) have the "en passant" structures with multiple varicosities, which can be the risk factors of conduction block of axonal spikes. In addition, axonal voltage-gated sodium channels play critical roles in spike propagation, and thus accumulating inactivation of sodium channels might result in the frequency-dependent propagation failure. In this study, we examined the reliability of spike propagation and activity-dependent waveform changes in the hippocampal mossy fibers at different stimulation frequency. We performed the loose-patch clamp recordings from single mossy fiber boutons, which are the exceptionally large presynaptic terminals. In the mouse hippocampal slice, large mossy fiber boutons were visually identified under the IR-DIC optics. Extracellular stimulation at the granule cell layer of dentate gyrus evoked axonal spikes in all-or-none fashion. In response to supra-threshold stimulation at 0.1 Hz, axonal spikes were evoked in response to the most of stimuli and propagated without failure during repetitive stimuli. In high frequency stimulation experiments, bi-phasic stimuli were used to minimize the stimulation failure. Even at 50 or 100 Hz stimuli for 1 s, spike failure was also rarely observed. In addition, the peak amplitude of the latter spikes during the high frequency stimulation was significantly decreased compared to the first response, in contrast to the digital signal processing

in the axon. Also, the width of the last axonal spikes during 1 s stimuli at 100 Hz was significantly increased compared to the first spike, which reflected the activity-dependent broadening of axonal spikes that previous study demonstrated. Mossy fibers reported to be expressed the higher density of sodium channels compared to the somatodendritic compartments, which might secure the high-fidelity propagation of axonal spikes, even in the structures causing impedance mismatch of thin axon shafts and large axon terminals. Furthermore, the small reduction of axonal spikes might result from the accumulation of sodium channel inactivation, which implied that the abundance of sodium channels was not sufficient to propagate the full-size action potentials. Both peak reduction and broadening of action potentials in response to the repetitive-stimuli might reflect the ability of activity-dependent tuning of axonal spikes in the hippocampal mossy fibers.

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Poster

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Support: NIH IRTA postdoc fellowship

Title: Scale-free correlations in neuronal activity of behaving animals

Authors: *T. L. RIBEIRO¹, S. YU², D. WINKOWSKI³, P. O. KANOLD³, D. CHIALVO⁴, D. PLENZ¹

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Abstract: It has recently been shown that resting state networks, computed from fluctuations of the blood oxygenated level dependent signal, exhibit divergent correlation length (CL): the average distance where the correlation of the fluctuations around the mean crosses zero increases with the system size (Fraiman & Chialvo, 2012; Haimovici et al, 2013). The existence of a divergent CL implies critical phenomena, in which the dynamics at all spatial scales are explained, after rescaling, by the same law. Examples range from spins of a magnet (Stanley, 1987) to bird flocks (Cavagna et al, 2010). In the brain, evidence of critical dynamics, which has been shown to provide numerous advantages for information processing, was found mostly in the context of neuronal avalanches, spatiotemporal clusters of scale-free activity that propagate in cortical layers whose size and duration are well-described by power laws (Beggs & Plenz, 2003).

On the other hand, CL provides an important alternative to neuronal avalanches to assess the dynamical state of neuronal networks, being especially useful in situations where power-law distributed activity cascades may be hard to obtain, such as in recordings with low temporal resolution, recordings affected by subsampling and/or in the case of non-stationary activity increases due to sensory stimulation or motor command execution. Here we investigate this issue in two experimental conditions: (a) multi-electrode array recordings (10x10 array, ~400 µm between electrodes) in premotor and prefrontal cortex of behaving nonhuman primates and (b) 2photon imaging (~500 µm x 500 µm field of view) in primary visual and auditory cortex of awake mice during sensory stimulation. Our experimental findings are complemented with computer simulations to explore the origins of the divergence in CL. We identify for the first time in behaving nonhuman primates and in the awake mouse the presence of scale-free CLs during stimuli or motor command processing epochs. Numerical simulations confirm a linear relation between network size and the CL that is indeed unique to critical dynamics. In contrast, deviations from critical dynamics lead to deviations from this scaling relation. Our results demonstrate that scale-free sensory-evoked activity in awake animals is in line with predictions from a neural network exhibiting critical dynamics. We therefore conclude that the brain could take advantage of optimization principles proposed for information processing in critical systems such as optimized stimulus sensitivity, information transmission, computational capability and mnemonic repertoires size.

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Poster

291. Signal Propagation

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Topic: B.10. Network Interactions

Title: Analytical studies of activity propagation in neural networks

Authors: *R. J. ERAZO¹, C. T. THURMAN², R. M. OSAN^{1,3} ¹Neurosci. Inst., ²Physics and Astronomy, ³Mathematics and Statistics, Georgia State Univ., Atlanta, GA

Abstract: We examine mathematical and computational models of traveling waves that describe the evolution of activity propagation in networks of integrate-and-fire neurons with excitatory connections. The network dynamics are heavily influenced by the coupling function, which assumes that strength of synaptic connections depend only in the distance between neurons. The finite support function assumes that the amount of excitation produced by the spike of one neuron is equal to all neuron neighbors within a discrete synaptic footprint. We determine how wave velocity depends on the global excitability as well as on other neuron and network parameters. We take an analytical approach to compute a delayed differential equation that accurately describes the transient activity in the neuronal network, which can ultimately give rise to propagation failure or constant-speed wave solutions. We conclude that our analytical predictions are in perfect agreement with the numerical simulations. We determine that after the wave initialization, done by inducing spiking at a set region, wave speed shows transient large amplitude oscillations before converging to a constant speed solution or quiescence, i.e. activity propagation and propagation failure, respectively. We are currently examining the mechanism that supports stability and the basins of attraction to small perturbations.

Disclosures: R.J. Erazo: None. C.T. Thurman: None. R.M. Osan: None.

Poster

291. Signal Propagation

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Topic: B.10. Network Interactions

Support: HHMI

Title: Investigating the mechanisms of claustrum-cortical connectivity

Authors: *J. C. JACKSON¹, M. M. KARNANI², D. BURDAKOV², A. K. LEE³ ¹Janelia Res. Campus, Ashburn, VA; ²The Francis Crick Inst., London, United Kingdom; ³HHMI Janelia Res. Campus, Ashburn, VA

Abstract: The claustrum is a small subcortical brain region known to be highly connected with all areas of the neocortex. Claustrum inputs and outputs are topographically organized, whereby spatially segregated claustrum cells receive inputs and send outputs to different cortical regions. Despite the wealth of anatomical knowledge regarding the interactions between the claustrum and cortex, there is very little information regarding the mechanisms by which claustrum neurons influence cortical activity and the strength of inputs to different cell types in the cortex. One major reason for this shortcoming arises from the fact that the claustrum is difficult to target anatomically or genetically. To target claustrum -> cortical circuits, we used newly developed retrograde AAVs (Tervo et al., Neuron 92: 372-82, 2016) to insert ChR2 specifically into claustrum neurons projecting to different regions of the cortex. With this technique we optically activated claustrum cells projecting to specific cortical regions and assessed the influence on different cell types and network activity patterns. These studies will provide a foundation for future studies seeking to understand the mechanism and function of claustrum-cortical signaling.

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Poster

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Title: Network dynamics of GABAergic neurons

Authors: *D. POZZI¹, Q. SONG^{1,2}, V. TORRE^{1,2}

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Abstract: We analyzed properties of *in vitro* dissociated cortical cultures and organotypic cortical slices in which GABAergic neurons were transgenically labeled with a green fluorescent protein (GFP). We stained these neuronal cultures with the membrane permeable calcium dye Fura-red, so that we identified GABAergic neurons by green light illumination and simultaneously followed calcium transients ($\Delta F/F_0$) in GABAergic and non GABAergic neurons using a red light. Fluorescence images (512x512 pixels) were acquired with an Electron Multiplier CCD camera at acquisition rates ranging from 5 to 50 Hz. We calculated the cross-correlation index (ρ) of the occurrence of the peaks of calcium transients in a time window of 10 seconds, and we found that GABAergic neurons. This difference was statistically significant (p < 0.001 One-way ANOVA, Tukey post - hoc test). Specifically, calcium transients larger than 30% ($\Delta F/F_0$) had a higher value of ρ , whereas smaller calcium transient were less correlated (0.619 ± 0.007 vs 0.298 ± 0.011 for GABAergic neurons; 0.487 ± 0.008 vs 0.233 ± 0.011 for non GABAergic neurons).

After blockage of GABA-A receptors with gabazine, the synchronization of calcium transients increased in both populations, maintaining higher values for GABAergic neurons (0.865 ± 0.004 vs 0.828 ± 0.007). The mean amplitude of $\Delta F/F_0$ was similar in GABAergic and non GABAergic neurons, whereas the time interval between bursts was significantly lower in the GABAergic population (26.07 ± 2.13 seconds vs 34.62 ± 2.62 seconds; p < 0.05). The dynamics of large synchronous calcium transients was analyzed when fluorescence images were acquired at 50 Hz, in order to determine the relative timing of calcium transients in GABAergic and non GABAergic neurons, but no consistent delay was detected at the available temporal resolution of 20 msec.

Our results suggest that GABAergic neurons have more synchronized and frequent spontaneous

activity compared to other neuronal types, and preliminary experiments in organotypic cell cultures seem consistent with these conclusions. Additional properties of the GABAergic neuronal network will be investigated.

Disclosures: D. Pozzi: None. Q. Song: None. V. Torre: None.

Poster

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Title: Standard LFP reconstruction does not explain neuronal avalanches with power law scaling

Authors: *D. PLENZ, T. LINS-RIBEIRO, Y. KARIMIPANAH Sect Critical Brain Dynamics, Natl. Inst. of Mental Health, NIH, Bethesda, MD

Abstract: The local field potential (LFP) infers population dynamics from the local population of neurons. Based on LFP measures in vitro, in rodents and non-human primates using multielectrode arrays, it has been shown that ongoing neuronal activity in superficial cortical layer 2/3 organizes as neuronal avalanches characterized by power law scaling. Recently, it was suggested that even uncorrelated neuronal activity reveals LFP measures with power law scaling (Touboul & Destexhe, 2017). In this latter approach the LFP was simulated from local current sources in a 2-dimensional network using the monopolar source model. Here, we examined this approach both numerically and analytically, and show that such scale-invariance is solely contingent on the assumption of a certain spatial dependency, namely the monopole current source. Furthermore, we show that such an assumption would have serious unrealistic consequences and that it does not compare to real LFP measures. In our simulations, layer 2/3 neurons were randomly distributed in 3-dimensions (3D) and their synaptic currents were modeled by independent Poisson processes convolved with an alpha-function. An embedded electrode matrix allowed the calculation of the LFP as a spatially weighted sum of all synaptic currents. Spatial distance r dependencies of potentials including 1/r^alpha, with alpha being a constant were explored. We found that in 3D, alpha=1 constitutes a phase transition between two phases of decorrelated and extremely correlated regimes: for alpha<1, both the LFP amplitude and fluctuations are mostly determined by distant neurons, whereas alpha>1 (which includes dipole reconstructions) results in de-correlated activity with a finite spatial reach for LFP's. Furthermore, we show that contrary to what was reported by Touboul & Destexhe (2017), for a large system it would be impossible to have power law distributed LFP avalanches for alpha=1, as each electrode would be almost identical. On the other hand, for alpha<1 or finite size systems (with any alpha), we show that it is only possible to have spurious power laws for a fine-tuned set of the parameters (depending on the density and the size of the array). Finally, when compared to real LFP data from spontaneous cortical activity in nonhuman primates, power law distributed avalanches compare with significantly lower pair-wise correlations than those suggested from a monopolar source approach. Our results demonstrate the implausibility of deriving neuronal avalanches measured in the LFP from the simple integration of uncorrelated sources.

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Poster

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ERC INTERIMPACT project

Hungarian Academy of Sciences

Title: High-precision fast-spiking basket cell discharge in the complex events of human neocortex

Authors: *V. SZEGEDI^{1,3}, G. MOLNAR⁴, M. PAIZS³, E. CSAKVARI³, P. BARZO², G. TAMAS⁴, K. LAMSA³

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Abstract: In the human neocortex, solitary spikes in a subset of layer 2-3 pyramidal cells (PCs) trigger brief episodes of network activity known as complex events through strong excitatory synapses that specifically innervate GABAergic interneurons. Yet, how these layer 2-3 "master PCs" configure the local network activity is not well understood. We report that single spikes in the PCs elicit firing of fast-spiking basket cells (FSBCs) with a short delay (average 2.7 ms) and high temporal precision with small jitter. The FSBC firing is triggered by >13 mV monosynaptic EPSP from single PC. The discharge of FSBCs is observed in the first wave of the complex event interneuron activity triggered by the master PCs. The FSBCs generate fast-kinetic inhibitory postsynaptic current (IPSCs) in layer 2-3 PCs, and similar IPSCs occur phase-locked to the master PC spike in the beginning of the complex events with high probability and 4.3 ms average delay with small jitter. In comparison, IPSCs with slow kinetics from non-fast spiking

interneurons appear more incosistently in the complex events with variable delay and low probability. The high-precision discharge of FSBCs characterizes the early phase of the complex event interneuron activity in the human neocortex.

Disclosures: V. Szegedi: None. G. Molnar: None. M. Paizs: None. E. Csakvari: None. P. Barzo: None. G. Tamas: None. K. Lamsa: None.

Poster

292. Epilepsy: Synaptic Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 292.01/H7

Topic: B.05. Transporters

Support: DFG Grant LO 274/15-1

Title: Characterization of the inhibitory effects of bumetanide and several other loop diuretics on the sodium-potassium-chloride-cotransporter splice variants hNKCC1A and hNKCC1B

Authors: *P. HAMPEL^{1,2}, K. RÖMERMANN¹, N. MACAULAY³, W. LÖSCHER^{1,2} ¹Dept. of Pharmacology, Toxicology and Pharm., Univ. of Vet. Med. Hannover, Hannover, Germany; ²Ctr. for Systems Neurosci. Hannover, Hannover, Germany; ³Dept. of Neurosci. and Pharmacol., Univ. of Copenhagen, Copenhagen, Denmark

Abstract: Bumetanide is a loop diuretic that acts via inhibition of NKCC2 in the kidney. The NKCC isoform NKCC1, which is also blocked by bumetanide, is of interest in the pathology of neurological diseases such as epilepsy or neonatal seizures, which leads to new potential therapeutic indications for bumetanide. Epilepsy-associated upregulation of NKCC1 leads to a GABA shift from hyperpolarizing to depolarizing. Bumetanide has been proposed to restore GABA function in neonates with difficult-to-treat seizures as an add-on therapy to a GABA-potentiating anti-seizure drug, such as phenobarbital (PB). A European multicentral clinical trial by Pressler et al. (2015) examining PB and bumetanide in neonates with PB-resistant seizures, however, was stopped early due to low efficacy and ototoxic adverse effects. Vibat et al. (2001) described two human splice-variants, hNKCC1A and hNKCC1B, of which NKCC1B is predominantly expressed in the brain. Thus, a substance that acts more selectively on hNKCC1B than hNKCC1A and penetrates better into the brain tissue than bumetanide is expected to be an effective anti-seizure treatment while having reduced peripheral effects.

Rubidium-86-transport-assays using Xenopus laevis oocytes that expressed either the human transporter hNKCC1A or hNKCC1B were performed with several known loop diuretics, hence NKCC2-inhibitors, which to our knowledge mostly have not been tested previously towards their effects on NKCC1. The examined compounds can roughly be divided into 5-sulfamoylbenzoic acid derivatives, the azosemide-like group lacking an acidic group, and a group of non-

sulfonamide derivatives. For negative control, we included the thiazide diuretic xipamide and the antidiabetic glybenclamide.

Bumetanide was used as a reference substance. IC_{50} values for bumetanide did not show significant differences for hNKCC1A and hNKCC1B and ranged from 0.5 to 1.1 μ M in-between experiments. Similarly, furosemide and torasemide inhibited hNKCC1A and hNKCC1B at about the same potencies. In contrast, neither xipamide nor glybenclamide inhibited transport in concentrations up to 200 μ M. Experiments with various other loop diuretics are under way. To our knowledge, we are first to compare the inhibitory potencies of various clinically approved loop diuretics towards the two major human splice-variants hNKCC1A and hNKCC1B. Though yet none of the tested compounds was selective for hNKCC1B, further experiments with chemically more diverse substances will be performed.

Disclosures: P. Hampel: None. K. Römermann: None. N. MacAulay: None. W. Löscher: None.

Poster

292. Epilepsy: Synaptic Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 292.02/H8

Topic: B.11. Epilepsy

Support: NYU Finding a Cure for Epilepsy (FACES)

NIH grant NS074785

Title: Spontaneous activity and increase in seizure susceptibility in the YCaMKII KO mice

Authors: *A. SALAH¹, H. MA², O. DEVINSKY³, R. W. TSIEN¹ ¹NYU Neurosci. Inst., New York Univ. Sch. Med., New York, NY; ²Neurosci. Inst., Zhejiang Univ., Hangzhou, China; ³Comprehensive Epilepsy Ctr., NYU Univ. Sch. Med., New York, NY

Abstract: <u>Objective</u>: Activity-dependent gene expression is central for sculpting neuronal connectivity and neuronal plasticity in the brain. Recently, we identified the γ CaMKII as a shuttle protein for synapto-nuclear signaling that operates by carrying Ca⁺⁺/CaM into the nucleus to activate CaMKIV, CREB phosphorylation and gene expression in cultured neurons. However, the functional importance of this signaling pathway in vivo remains unclear. Knowing that CaMKII's are involved in neurological diseases like epilepsy and autism, we used a γ CaMKII KO mouse model to investigate their spontaneous activity and seizure susceptibility under Kainic acid (KA) by performing long-term (6 h to 2 weeks) video-electroencephalography (V-EEG). <u>Methods:</u> 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 (25 mg/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 a modified Racine Scale. Seizure latency was determined along with the time spent to reach the first stage (S1) and the first tonicclonic seizure (S4). **Results:** Spontaneous activity was increased in the *γ*CaMKII KO mice compared to controls as seen in the mean number of single spikes (p<0.0001), repetitive spikes (p<0.0035), and runs of spikes (p<0.07) 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 YCaMKII KO mice compared to controls (p<0.0001). Also, yCaMKII KO mice required less time to reach the first stage (S1) (p<0.0001) or the first tonic-clonic seizure (S4) (p<0.006). Conclusions: Taken together, our results indicate γ CaMKII, a little-studied member of the CaMKII family, may play a critical role in maintaining normal neuronal activity. Multiple genetic studies have suggested that γ CaMKII is a risk gene for autism. Importantly, our data show that γ CaMKII levels in autistic patients is only ~50% of the healthy group. Considering that about 30% ASD patients show epilepsy, our data support the idea that *y*CaMKII and, by extension, the synapto-nuclear signaling it mediates, may be involved in both epilepsy and autism.

Disclosures: A. Salah: None. H. Ma: None. O. Devinsky: None. R.W. Tsien: None.

Poster

292. Epilepsy: Synaptic Mechanisms

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Topic: B.11. Epilepsy

Support: CONACYT grants 243333 and 243247 to MC and JRE, respectively

VIEP-BUAP 2017

CA in Neuroendocrinología BUAP-CA-288

JMIH receives a fellowship from CONACYT No. 594381.

Title: The γ -aminobutyric acid receptor modulates the mean duration of absence seizures on the myelin mutant taiep rat

Authors: *J. M. IBARRA-HERNÁNDEZ¹, C. CORTES², J. EGUIBAR³

¹Lab. de Neurofisiología de la Conducta y Control Motor, Inst. De Fisiología. Benemérita Univ. Au, Puebla, Mexico; ²Physiol., Benemerita Univ. Autonoma de Puebla, Puebla, Mexico; ³Benemerita Univ. Autonoma de Puebla, Puebla, Pue., Mexico **Abstract:** The absence seizures are one type of epilepsy which is characterized by a sudden loss of consciousness and characteristic spike-wave discharges in the electroencephalogram. Absence seizures are due to a decrease in the activity of the γ -aminobutyric acid receptor type A (GABAa) in the somatosensory cortex and an increase of the inhibition mediated by GABAa in the thalamo-cortical circuit. The aim of this study was to determine the effects of two GABAergic agonists gabapentin and gaboxadol in the absence seizures in myelin mutant taiep rats.

We used 9 months old taiep rats which were implanted and fixed to a stereotactic Kopft frame there we implanted electrodes in the hippocampus, cerebral cortex, neck muscles and in the right orbit of the eye to recorded there. Gabapentin (50, 100 and 400 mg/Kg) and gaboxadol (5, 10 and 20 mg/Kg) were given intragastrically at 0800 h. We made four 24-hour video-EEG recordings, the first one is control, and three additional recordings using an increasing doses scheme every 72 hours. Off-line we analyzed the frequency and mean duration of each absence seizure and the latency of the first crisis. We followed the NIH rules for the care of experimental animals and the protocol was approved by BUAP-IACUC with the number COSM-SAL-17-I.

The mean duration of absence seizures decreased by 2 hours after gabapentin administration from to 2.72 ± 0.13 s with 100 mg/Kg, 2.94 ± 0.16 s with 200 mg/Kg to a maximum reduction to 2.84 ± 0.12 s with 400 mg/Kg, being significant (*P*<0.05). On the other hand, gaboxadol produced a maximum effect during at 7 hours after administration with mean duration from 4.10 ± 0.12 s in control conditions to 6.33 ± 0.26 s with 10 mg/Kg, 4.90 ± 0.27 s with 20 mg/Kg being significant between all doses respect to control (*P*<0.05). Importantly, frequency and the latency for the first absence seizure did not change under both treatments.

The decrease in duration of absence seizures due to gabapentin and the increase induce by gaboxadol strongly suggest that different types of GABAa receptors participate in the modulation of thalamo-cortical circuit because gabapentin act preferentially in extrasynaptic sites and gaboxadol in the postsynaptic ones.

Disclosures: J.M. Ibarra-Hernández: None. C. Cortes: None. J. Eguibar: None.

Poster

292. Epilepsy: Synaptic Mechanisms

Location: Halls A-C

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UCSF Neuroscience Graduate Program

UCSF Weill Institute for Neurosciences

Achievement Reward for College Scientists Scholarship NIH-NINDS R01 NS096369-01 K99-R00 NS078118 Gladstone Institutes

Title: Deconstruction of thalamic circuits in a mouse model of post-traumatic epilepsy

Authors: *S. HOLDEN¹, J. PAZ²

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Abstract: Post-traumatic epilepsy (PTE) accounts for 20% of symptomatic epilepsy cases and 5% of all epilepsy cases. The mechanisms underlying how traumatic brain injury (TBI) leads to PTE remain unknown, which has limited the development of interventions for preventing or curing epilepsy after injury. The cortex is typically the most affected brain area at the onset of a TBI, but the thalamus is also an important site of dysfunction after injury because of its reciprocal connections with the cortex. One of the prominent characteristics of the thalamus is its ability to produce reverberating bursting activity, which is important for sensory processing, attention, and the maintenance of sleep. Abnormal thalamic bursting is associated with cognitive dysfunction after TBI and is also pathological in some types of epilepsies. However, it remains unknown whether cortical TBI can alter thalamic function, and whether these alterations may be involved in the development of PTE. Furthermore, there is currently no cure for PTE. Here we use electrophysiological approaches to identify how cortical TBI may lead to hyperexcitability and aberrant synaptic plasticity in the thalamus, and to test whether the thalamus could be a potential therapeutic target for preventing or curing PTE. We hypothesized that TBI causes a shift in the brain's excitation/inhibition balance by altering the excitability of thalamic neurons and rewiring connections in the intra-thalamic circuit. We used the controlled cortical impact PTE model in mice and performed electrophysiological recordings in brain slices and in vivo to determine if TBI in the somatosensory cortex leads to altered function of thalamic cells. Using whole-cell patch-clamp recordings in thalamic slices, we showed that cortical TBI reduces the intrinsic excitability of inhibitory neurons and enhances the intrinsic excitability of excitatory relay thalamocortical neurons, but that glutamatergic and phasic GABAergic synaptic function are unaltered in the thalamus. Using EEG/video and thalamic depth recordings during free behavior, we showed that post-traumatic seizures are characterized by robust high-frequency bursts in the somatosensory thalamus. Our study shows that TBI alters the inhibitory/excitatory balance in the thalamus, mainly through changes in intrinsic membrane properties of thalamic cells, and that the thalamus is involved in PTE seizure expression. These results reveal how neural circuits adapt after TBI and may provide new therapeutic strategies for enhancing functional recovery while preventing seizures and/or epileptogenesis after injury.

Disclosures: S. Holden: None. J. Paz: None.

Poster

292. Epilepsy: Synaptic Mechanisms

Location: Halls A-C

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Program#/Poster#: 292.05/H11

Topic: B.11. Epilepsy

Support: R21 NS096483-01A1

Title: Impaired homeostatic potentiation of inhibitory GABAergic currents induced by slowwave oscillation in an idiopathic generalized epilepsy model with GABAR A322D mutation

Authors: *C. ZHOU¹, L. DING¹, M. J. GALLAGHER², R. L. MACDONALD³ ¹Neurol., Vanderbilt Univ. Med. Ctr., Nashville, TN; ²Neurol., Vanderbilt Univ. Sch. of Med., Nashville, TN; ³Professor & Chair, Dept Neurol, Vanderbilt Univ., Nashville, TN

Abstract: Seizures affect almost 3 million peoples in US and cause cognitive and other clinical comorbidities and in two thirds of patients, the causes are not known (possible genetic origins, idiopathic generalized epilepsy). Particularly epileptic activity onset is mostly present during sleep-wake transition or quiet wake period, suggesting that slow-wave oscillations during sleep period may have important roles on seizure onset/initiation. Here we used an idiopathic generalized epilepsy mouse model with GABAR A322D mutation to examine the homeostatic potentiation of both spontaneous excitatory and inhibitory synaptic currents in layer V pyramidal neurons of somatosensory cortex, with whole-cell recordings in ex vivo brain slice preparation. Spontaneous(s) EPSCs were recorded at Cl⁻ reversal potentials, and spontaneous(s) IPSCs recorded at -60mV with AMPARs being blocked. After 10 minutes slow-wave oscillation induction, sEPSCs or sIPSCs at 25~30 min were measured and averaged for 5 min recordings, in comparison to sEPSCs or sIPSCs before slow-wave oscillation induction. We found that 0.5 Hz cosine slow-wave oscillation (10 min) could potentiate sEPSCs (from -9.9 \pm 0.81 to -14.4 \pm 0.76 pA, n=7, percentage increase 47.8 \pm 6.46%, pair t-test p=0.001) and sIPSCs (from -19.9 \pm 2.29 to -32.9 ± 3.46 pA, n=9; percentage increase 70.6 $\pm 15.96\%$, paired t-test p=0.002) in WT littermates, suggesting that the dynamic balance of potentiated synaptic E-I in pyramidal neurons induced by slow-wave oscillation is maintained. In heterozygous mice with A322D mutation, slow-wave oscillation still potentiated sEPSCs (from -19.58 ± 5.81 to -23.16 ± 4.05 pA, n=4, percentage increase $32.36 \pm 16.50\%$, not significant different from WT increase, t-test p=0.433). However, slow-wave oscillation could not potentiate sIPSCs (from -17.09 \pm 2.54 to -18.86 \pm 3.24 pA, n=6, percentage increase 9.40 \pm 6.70%, significant from WT sIPSCs increase, t-test 0.005), suggesting that the dynamic balance of potentiated synaptic E-I in pyramidal neurons might be impaired in het mice. In conclusion, slow-wave oscillation during sleep or quiet wake period could create the dynamic imbalance between potentiated sEPSCs and sIPSCs in het mice, which would exacerbate the synaptic E-I imbalance in layer V cortical pyramidal neurons and

very likely initiate seizures in this idiopathic generalized seizures model during sleep-wake transition or quiet wake period.

Disclosures: C. Zhou: None. L. Ding: None. M.J. Gallagher: None. R.L. Macdonald: None.

Poster

292. Epilepsy: Synaptic Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 292.06/H12

Topic: B.11. Epilepsy

Support: College of Graduate Studies support

Title: Enhydrazinones have anticonvulsant activity in rat In vitro seizure models

Authors: *S. B. KOMBIAN¹, F. KHALIFOUH², I. O. EDAFIOGHO³, C. I. EZEAMUZIE² ²Fac. of Med., ¹Kuwait Univ., Safat, Kuwait; ³Pharmaceut. Sci., Univ. of St. Joseph, Hartford, CT

Abstract: Enaminones are a novel class of compounds with reported anticonvulsant activity. Enhydrazinones are similar to enaminones but have a hydrazino moiety (NHNH) joined to a keto group through a carbon-carbon double bond. We tested if this chemical modification also yielded compounds with synaptic depressant and anticonvulsant activity. 350 µM thick coronal slices of the forebrain containing the hippocampus were generated from male Sprague-Dawley rats. Evoked, population spikes (PS) were recorded in the cell body layer of area CA1 of the hippocampus using bipolar electrodes to activate Schaeffer collateral/commissural fibers. In vitro seizures were generated either chemically or electrically. The effects of 3 enhydrazinones were tested on the PS and seizure models. All the tested enhydrazinones, EMP6, EMP8 and EMP10 caused dose-dependent reduction in PS amplitude with estimated EC₅₀ values of 1.7, 0.83 and 6.62 μ M respectively. 1 μ M EMP8 consistently depressed the PS by -23.3 \pm 2.3% (n=6). This effect was blocked by 50 μ M bicuculline (-8.2 \pm 2.2%, n=4) and by 1 μ M CGP54626 (-2.4 \pm 9.3%, n=5). When *in vitro* seizure-like activity was induced by removing Mg^{2+} from the perfusion solution, (zero Mg²⁺ model) the slice developed multiple spikes(mPS) (1 vs 3.3 ± 0.3). Subsequent treatment with 1 μ M EMP8, reduced the spike number to 2.0 \pm 0.3 (36.3 \pm 2.7% reduction, n=10). A similar result was observed when picrotoxin was used to generate mPS (3.2 \pm 0.6 in picrotoxin alone vs 2.2 \pm 0.6 in EMP8, n=7). The effect of EMP8 on zero-Mg²⁺-induced mPS was blocked by 1 μ M CGP54626 (3.3 ± .4 in CGP vs 2.7± 0.4 in CGP+EMP8; n=4). EMP8 (1 μ M) also inhibited spontaneous bursting (SB) induced by zero-Mg²⁺ (8.3 ± 2.5 vs 0.3 ± 0.4, n=3), nearly 100% abolition. This effect EMP8 on SB frequency was blocked by CGP54626 (3.4 \pm 1.5 vs 3.0 \pm 0.5; n=4). Finally, SB induced by high frequency afferent stimulation (stimulus train induced bursts-STIBs) were also suppressed by EMP8 (9.0 ± 0.9 /min vs 2.8 ± 0.9 /min, n=4) yielding an average of $67.3 \pm 9.9\%$ depression. Taken together, our data indicate that enhydrazinones, like enaminones, suppress PS and chemically- and electrically-induced seizure-like activity in the rat hippocampus via a GABAergic mechanism.

Disclosures: S.B. Kombian: None. F. Khalifouh: None. I.O. Edafiogho: None. C.I. Ezeamuzie: None.

Poster

292. Epilepsy: Synaptic Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 292.07/I1

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: MCST R&I – 2013-14

Title: Opposite control by 5-HT_{2C} receptors on generalized and focal seizures

Authors: *G. DI GIOVANNI^{1,2}, G. DEIDDA^{1,2}, R. COLANGELI¹, A. CAVACCINI², M. VENZI², G. ORBAN^{1,2}, M. PIERUCCI¹, V. CRUNELLI^{1,2} ¹Physiol. and Biochem., Univ. of Malta, Msida, Malta; ²Cardiff Univ., Cardiff, United Kingdom

Abstract: The 5-HT_{2C} receptor (5-HT_{2C}R) is thought to be involved in neuronal excitability and seizure generation. 5-HT_{2C}R knockout mice display spontaneous generalized convulsive seizures which cause a high mortality rate and a reduced threshold for various convulsant stimuli. Conversely, 5-HT_{2C}R activation increased the threshold of generalized convulsive seizures induced by pentylenetetrazole and electroshock in mice. Here we show the 5-HT_{2C}R also negatively controls non-convulsive generalized seizures, in the polygenic animal model of absence epilepsy, the Genetic Absence Epilepsy Rat from Strasbourg. RO60-0175, lorcaserin and CP809101 were capable of blocking absence seizures. Interestingly, as expected, SB242084 blocked the effect of lorcaserin and CP809101, but also showed some anti-absence effects. One possible mechanism by which 5-HT_{2C}R activation exerts antiepileptic effects is via the normalization of the aberrant GABAA receptor tonic inhibition in the ventrobasal thalamus seen in different animal models of absence epilepsy. The 5-HT_{2C}R system seems devoid of any modulatory role in partial seizures, or paradoxically, have a pro-epileptic role in this type of epilepsy. Indeed, we observed that mCPP and lorcaserin, but not RO60-0175, were able to halt hippocampal afterdischarges in a rat model of temporal lobe epilepsy, an effect potentiated and insensitive to SB242084 pretreatment. We confirmed this evidence in the pilocarpine-model of status epilepticus in rats, where the RO60-0175 was devoid of any anti-seizures effect, while SB242084 increased the antiepileptic activity of cannabinoids agonists. In summary, 5-HT_{2C}R agonists may have new therapeutic utility in generalized epilepsy. Moreover, activation of the 5HT_{2C}R may also be useful for treating comorbid neuropsychiatric disorders commonly seen in people with epilepsy.

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Poster

292. Epilepsy: Synaptic Mechanisms

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Topic: B.11. Epilepsy

Support: Royal Society URF UF140596

Wellcome Trust Grant 105203/Z/14/Z

Title: An investigation into the cell-type specific effects of a dominant negative mutation in Kv3.1 underlying cases of progressive myoclonic epilepsy

Authors: *J. C. CARPENTER, G. LIGNANI, S. SCHORGE Clin. and Exptl. Epilepsy, UCL Inst. of Neurol., London, United Kingdom

Abstract: Unverricht-Lundborg disease (ULD) is the most common form of Progressive Myoclonic Epilepsy (PME); a highly heterogeneous group of rare neurological disorders characterised by action myoclonus, tonic-clonic seizures and ataxia, with largely preserved cognition. The genetic causes of ULD are diverse, occurring in genes of apparently unrelated function with no direct effects on neuronal excitability. Recently, however, a de novo, dominant negative mutation in KCNC1 has been identified as a novel cause of ULD-like PME. KCNC1 encodes Kv3.1, a member of the Kv3 family of voltage-gated potassium channels, which contribute to the rapid repolarisation of action potentials and high frequency firing. Kv3.1 channels are highly expressed in fast-spiking, parvalbumin-positive interneurons, leading some to suggest that KCNC1-based PME represents a selective dysfunction of these cell types. Here we combine optogenetic and classical electrophysiological approaches with the Cre-lox genetic system to determine how the c.959G>A mutation in KCNC1 alters the activity of fast spiking parvalbumin interneurons. Our method allows for the presynaptic effects of this mutation to be isolated and restricted to specific excitatory or inhibitory cell types and we will focus initially, on how overexpression of mutant Kv3.1 channels alters the intrinsic properties of different types of neuron.

Disclosures: J.C. Carpenter: None. G. Lignani: None. S. Schorge: None.

Poster

292. Epilepsy: Synaptic Mechanisms

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Program#/Poster#: 292.09/I3

Topic: B.11. Epilepsy

Support: NIH Grant DC009839

NIH Grant DC015525

Edward Mallinckrodt Jr. Foundation

Title: Optokindling: Catastrophic reorganization of cortical circuits by repeated optogenetic stimulation

Authors: *K. M. FRANKS¹, P. LEE², B. RYU² ¹Neurobio., ²Duke Univ., Durham, NC

Abstract: Different odors activate specific, distributed and overlapping ensembles of ~10% of principal cells in piriform cortex (PCx). These cells are also interconnected via long-range recurrent collateral connections. The formation of odor memories is thought to result by selective strengthening of recurrent synaptic connections between repeatedly co-active neurons. We sought to examine this process by directly activating random subsets of PCx neurons. To do this, we first used viral vectors to express channelrhodopsin-2 (ChR2) in random subsets of ~10% of PCx principal cells and then repeatedly co-activated these ensembles directly. We stimulated these cells several times per day with brief light trains delivered via an optical fiber implanted above PCx (20 Hz trains for 5 s. 6x/day). Optical stimulation initially had little effect, with animals pausing briefly during the light train. However, within 4 days, the same stimulation reliably triggered a cascade of activity that culminated in massive, stage 6 running-jumping seizures. Continuous local field potential (LFPs) recordings revealed that cortical activity becomes increasingly entrained to the 20 Hz light trains never produced seizures in kindled mice.

To reveal the neural circuit consequences of this process, which we call "optokindling", we isolated acute brain slices from injected mice and used whole-cell patch-clamp recordings to examine changes in synaptic connectivity. We found a large change in the balance of recurrent excitation/feedback inhibition in kindled mice. However, surprisingly, this was not due to robust increases in recurrent excitation, but rather, was due to a large decrease in feedback inhibition. These changes were synapse-specific, as both afferent excitatory inputs from olfactory bulb and the feedforward inhibitory inputs they recruit were unaffected. Consistent with this result, we found a dramatic decrease in GABA expression in PCx inhibitory interneurons in kindled

mice.Next, we examined the role of endogenous and exogenous cannabinoids in seizure expression. Low systemic doses of the CB1 agonist, Win55,255, completely suppressed light-evoked seizures in kindled mice. By contrast, low doses of the CB1 antagonist, AM251, exacerbated seizures that often killed the mice (4/7 mice, vs. 2/58 control mice). In slice experiments, we reveal a CB1-dependent DSE PCx that could support this on-demand neuroprotective effect.

Optokindling can therefore be used to probe the consequences of pathological activity in a recurrent cortical circuit.

Disclosures: K.M. Franks: None. P. Lee: None. B. Ryu: None.

Poster

292. Epilepsy: Synaptic Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 292.10/I4

Topic: B.11. Epilepsy

Title: Measurement of effects and administration of carbenoxolone in real time on high frequency oscillations involved in hippocampal epileptogenesis

Authors: *C. VENTURA, J. R. BELTRAN-RAMIREZ, M. A. CERPA-GALLEGOS, E. ARCINIEGA-VÁZQUEZ, S. A. CASILLAS-ROMERO, E. A. PADILLA-ZEPEDA, J. A. JÍMENEZ-OLVERA, G. ZARATE-RODRÍGUEZ, J. A. CHÁVEZ-GARCÍA Ctr. De Enseñanza Técnica Industrial, Guadalajara, Jalisco, Mexico

Abstract: Epilepsy is a neurological disorder that affects the world population and presents a prevalence of 2 to 5%. Animal models are a key tool for the study of the basic mechanisms of crisis generation and the Pilocarpine-induced Temporal Lobe Epilepsy (ELT) model simulates the main features of this pathology. In this model the presence of high frequency activity (250 to 600Hz) denominated Fast Ripples (FRs) has been observed, as well as in ELT patients. FRs reflect the activity of a group of pathologically interconnected neurons that play an important role in epileptogenesis of the hippocampus. Likewise, there is experimental evidence of the participation of the communicating unions (UC) in epilepsy. Until now they have related to the modulation of gap junctions but has not worked with measuring equipment in real time without the intervention of human labor, as well as the administration of drugs without human intervention so the registration and administration can be performed without interruption, which allows us to analyze the data obtained with an algorithm which does not throw errors by the manipulation periods and gives us periods of time without real analysis or with errors of detection of activity. In this work we will analyze the effect of carbenoxolone on the hippocampus of spontaneous recurrent seizures induced by pilocarpine in order to determine the possible modulation of FRs by electrical synapses by a system without errors and in real time.

Until now we have a 80% diminution of epileptiform activity in the hippocampus in only two wistar rats.

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Poster

292. Epilepsy: Synaptic Mechanisms

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Support: RO1 NS 040337

RO1 NS 044370

Title: GluA1 subunit plasticity is necessary to sustain prolonged seizures

Authors: *S. JOSHI, E. LEWCZUK, J. WILLIAMSON, M. PENMETSA, J. KAPUR Dept of Neurol., Univ. of Virginia, Charlottesville, VA

Abstract: Prolonged seizures enhance AMPAR-mediated neurotransmission and increase surface membrane trafficking of GluA1 subunits. Whether this GluA1 subunit plasticity contributes to sustaining prolonged seizures is not known.

Adult mice lacking GluA1 subunit of AMPA receptors (GluA1^{-/-}, KO) or GluA1 subunitexpressing littermates (GluA1^{+/+}, WT) were used. Electrical pulse trains (50 Hz, 0.1sec, 50-200 uA, 10 sec duration were delivered to the left hippocampus to determine afterdischarge threshold (ADT). Animals received pulse trains (2X ADT) every 15 sec (5 sec off period) for 30 or 60 min. Animals were monitored by continuous video-EEG for 24 hr. Seizure spread was determined in terms of expression of Fos protein using standard immunohistochemical techniques. ADT in the KO mice was similar to WT mice ($130 \pm 12 vA$). The electrically-evoked seizure lasted for 10-15 sec in both the strains of mice. Hippocampal stimulation for 30 min caused self-sustaining seizures in 64% of WT mice (n=12), but not in the KO mice (n=7, p<0.05, Chi-square test). Stimulation for 60 min triggered self-sustaining seizures in 77% of WT mice, n=13) as compared to 50% of KO mice (n=11, p<0.05). Although 50% of the KO animals experienced prolonged seizures, the total seizure duration in these animals was shorter than that in the WT mice (91 ± 14 min vs 342 ± 64 min, p<0.05).

Seizure spread to extrahippocampal areas could sustain prolonged seizures. In the WT animals intense Fos immunoreactivity (IR) was present in the DGCs, CA3 and CA1 pyramidal neurons, neurons in the subiculum, entorhinal cortex, lateral septum, cingulate and motor cortex, and

olfactory bulbs and olfactory cortex. In contrast, Fos IR was primarily restricted to the hippocampi in the KO mice. The number of Fos-positive neurons was also reduced in CA1 and subiculum.

WT animals in self-sustaining seizures evoked by continuous hippocampal stimulation for 60 min were treated with calcium-permeable AMPAR blocker IEM 1460 or saline. The seizures lasted for 206 ± 15 min (n=17) in saline-treated animals. In contrast, seizures terminated after 106 ± 21 min (n=5) in the mice treated with IEM 1460 30 min after the end of stimulation. Treatment of IEM 1460 at 15 min following stimulation led to a further shortening of seizure duration (51 ± 21 min, n=9, p<0.05).

In KO mice seizure were less likely to become self-sustaining (status epilepticus). When selfsustaining seizure were initiated in KO mice they were of shorter duration with limited spread. These studies combined with drug treatment study suggested a critical role for GluA1 plasticity in sustaining prolonged seizures.

Disclosures: S. Joshi: None. E. Lewczuk: None. J. Williamson: None. M. Penmetsa: None. J. Kapur: None.

Poster

292. Epilepsy: Synaptic Mechanisms

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Topic: B.11. Epilepsy

Support: RO1 NS044370

RO1 NS040337

Title: Potentiation of AMPA receptor-mediated synaptic transmission in hippocampal CA1 pyramidal activated by a single seizure

Authors: *H. SUN, J. WILLIAMSON, J. KAPUR Neurol., Univ. of Virginia, Charlottesville, VA

Abstract: <u>Rationale:</u> Seizures consist of sustained repetitive firing of a group of neurons. Seizures can enhance susceptibility to further seizures but the mechanisms remain uncertain. We tested whether single seizure potentiates AMPA receptor (AMPAR) mediated synaptic transmission on seizure-activated CA1 pyramidal neurons.

<u>Methods</u>: Transgenic mice that express EGFP under the control of tetracycline repressor and early immediate gene, cfos (Mayford) were maintained on high doxycycline diet until 48 hours prior to a single seizure, induced by pentyleneterazol (40 mg/kg, i.p.). Hippocampal slices appropriate for patch clamp electrophysiology were prepared 30 minutes after the seizure.

Whole-cell patch clamp recordings of AMPAR-mediated EPSCs, passive and active membrane properties were made from GFP^{+ve} and neighboring GFP^{-ve} cells in the same slice. Results: A small number of GFP^{+ve}CA1 pyramidal neurons dispersed among GFP^{-ve} neurons were visualized from hippocampal slices under fluorescent/DIC microscope. Passive membrane properties (resting membrane potential, membrane time constant and membrane resistance) of GFP^{+ve} and neighboring GFP^{-ve} were similar (16 pairs, from 16 animals). Although action potential threshold, amplitude, and width were similar, 11/20 GFP ^{+ve} cells fired >1 action potential in response to depolarizing current steps, only 3/20 GFP ^{-ve} (p < 0.05 c² test)did so. Miniature EPSCS were recorded after blocking GABA-A receptors (picrotoxin) and action potentials (TTX) under voltage clamp. The amplitude of mEPSCs from GFP ^{+ve} neurons (19.37 mV + 1.88, mean + 2 SD) was larger than that recorded from neighboring GFP –ve neurons (16.85 mV + 2.12) from the same slice and animal (P = 0.0002, paired t-test for 15 pairs). To investigate whether AMPAR properties were altered during seizure-induced enhancement of transmission, we investigated rectification of evoked EPSCs. There was inward rectification of eEPSCs recorded from GFP^{+ve} neurons, which was not observed in GFP^{-ve} neurons. Morphological study of the neurons performed by 3D reconstruction of biocytin filled neurons is in progress.

<u>Significance</u>: These studies suggest that a single seizure potentiates AMPAR-mediated transmission in a small number of CA1 neurons, identifiable by the activation of cfos gene. Seizures cause calcium entry into neurons, which entry could also trigger potentiation of glutamatergic transmission, through insertion of GluA1 subunit into glutamatergic synapse and activation of cfos gene.

Disclosures: H. Sun: None. J. Williamson: None. J. Kapur: None.

Poster

292. Epilepsy: Synaptic Mechanisms

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Support: Epilepsy Research UK

Medical Research Council

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Title: Functional analysis of GABAergic gene variants in genetic generalised epilepsy

Authors: *S. CHUNG¹, K. V. EVERETT², E. DUDLEY¹, C. A. HUNT¹, R. H. THOMAS³, J. S. DAVIES¹, A. HIGGINS¹, W. O. PICKRELL¹, J. G. L. MULLINS¹, A. V. DERRICK¹, S. T. BELLOWS⁴, P. S. BERGIN⁵, R. J. HARVEY⁶, I. E. SCHEFFER⁴, S. F. BERKOVIC⁴, M. I. REES¹

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Abstract: The link between inhibitory GABAergic neurotransmission and genetic generalized epilepsy (GGE) is a well-supported paradigm generated from molecular genetics of GABA receptor subunits, transgenic model systems, pharmacology, neuropathology, and electrophysiology. The focus on postsynaptic GABA channelopathies has historically attracted much attention, however, the research focus is evolving towards a proteomic synaptopathy approach. Consequently, we investigated the genetic variation within GABAergic transporters (GAT1 & GAT3) and biosynthetic proteins (GAD65 & GAD67) in 708 GGE cases from Australia, UK and New Zealand using a combination of light-scanner heteroduplex screening and direct Sanger sequencing. Eighteen novel or rare GABAergic variants were discovered in 34 GGE cases with a strong preference for absence seizure syndromes. All 18 GABAergic missense variants had deleterious outcomes in mutation-prediction software and protein-structure molecular modelling. Wild-type and mutation expression-constructs were prepared for all 18 GABAergic gene-variants in addition to a further 7 published de novo GAT1 mutations linked to myotonic-atonic seizures but without functional validation. GAT1 and GAT3 variants were analysed using various in vitro functional assays including a bespoke GABA-activity assay based on isotopically-labelled GABA with Mass Spectrometry, essential splice-site assays and cellsurface trafficking status. Reduced GABA activity was observed for the majority of GAT1 and GAT3 gene-variants. GAD65 and GAD67 mutation constructs were tested in a highly-sensitive florescence-based GAD enzymatic assay using a resazurin-linked GABase microplate format. This revealed altered GAD activity and reduced binding properties to co-factor, pyridoxal 5'phosphate (PLP). This study confirms the association between GABA transport / biosynthesis and absence seizures in GGE syndromes, which is supported by extensive in vitro evidence with important patient-specific outcomes.

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Poster

292. Epilepsy: Synaptic Mechanisms

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NIH Grant P30-NS061800

Title: Short-term depression of sprouted mossy fiber synapses from adult-born granule cells

Authors: *W. HENDRICKS¹, Y. CHEN¹, G. L. WESTBROOK¹, E. SCHNELL² ¹Vollum Inst., Portland, OR; ²Portland VA Med. Ctr., Portland, OR

Abstract: Epileptic seizures potently modulate hippocampal adult neurogenesis, and adult-born dentate granule cells contribute to the pathologic retrograde sprouting of mossy fiber axons, both hallmarks of temporal lobe epilepsy. The characteristics of these sprouted synapses, however, have been largely unexplored, and the specific contribution of adult-born granule cells to functional mossy fiber sprouting is entirely unknown. To overcome previous technical limitations, we use DcxCreER^{T2} transgenic mice to permanently pulse-label and optogenetically activate neurons born either before or after pilocarpine-induced status epilepticus (SE). We demonstrate that adult-born granule cells born prior to SE form functional recurrent monosynaptic excitatory connections with other granule cells by two months following SE. Surprisingly, although healthy mossy fiber synapses in CA3 are well characterized "detonator" synapses that potently drive post-synaptic cell firing through their profound frequency-dependent facilitation, sprouted mossy fiber synapses from adult-born cells exhibited profound frequencydependent depression, despite possessing some of the morphological hallmarks of mossy fiber terminals. Neonatally-born, mature granule cells also contributed to functional mossy fiber sprouting, but exhibited less synaptic depression. Interestingly, granule cells born shortly after SE did not form functional excitatory synapses, despite robust sprouting. Considering that adultborn granule cells are the most likely to sprout recurrent mossy fiber axons, our results suggest that although they form recurrent excitatory circuits with some of the morphological characteristics of typical mossy fiber terminals, the functional characteristics of these sprouted

synapses would limit the contribution of adult-born granule cells to hyperexcitability in the epileptic hippocampus.

Disclosures: W. Hendricks: None. Y. Chen: None. G.L. Westbrook: None. E. Schnell: None.

Poster

292. Epilepsy: Synaptic Mechanisms

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Topic: B.11. Epilepsy

Support: CIHR Grant MOP119421

Title: The role of Neuroligin 2 and inhibitory transmission in the function of thalamic circuitry during epilepsy

Authors: *F. CAO, J. LIU, Z. JIA

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Abstract: Epilepsy is one of the most common neurological disorders. At the cellular level, epileptic seizures are caused by abnormal, excessive or synchronous neuronal electrical activity in the brain. However, the exact mechanisms of epilepsy remain largely unknown. Neuroligin 2 is a postsynaptic cell adhesion protein, which is exclusively located at inhibitory synapses and serves a role in regulating the balance between brain excitation and inhibition. Importantly, the imbalance between excitation and inhibition tends to cause the disruption of neuronal activities, which may lead to epileptic seizures. By using electroencephalogram and electrophysiological recordings, we found that mice lacking Neuroligin 2 display abnormal seizure-like brain activities and an impaired inhibitory neuronal function in an epilepsy-related thalamic circuitry. Importantly, the abnormal brain activity can be rescued by the administration of a drug directed at enhancing the inhibitory synaptic transmission. These results suggest that Neuroligin 2 regulates normal brain function through modulating inhibition. Our findings provide crucial insight into the mechanisms underlying epilepsy generation and to facilitate the understanding and treatment of related brain disorders.

Disclosures: F. Cao: None. J. Liu: None. Z. Jia: None.

Poster

292. Epilepsy: Synaptic Mechanisms

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Support: NYS Office of Mental Health

Pyramid Biosciences

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Title: Effects of BDNF and trk receptors on models of seizure activity in hippocampal slices

Authors: J. J. BOTTERILL, *H. E. SCHARFMAN

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Abstract: It has been shown that brain-derived neurotrophic factor (BDNF) acting at trkB receptors may promote seizure activity in hippocampus under some conditions. Interestingly, few studies have examined the effects of BDNF and trk in models of seizures in hippocampal slices of control animals, where many investigators have examined effects of antiseizure drugs in the past. Therefore we used hippocampal slices from naïve rats and mice to test the effects of trk antagonism on seizure-like activity produced in three ways that have been commonly used in the past: reduced $[Mg^{2+}]_0$, elevated $[K^+]_0$, or disinhibition. Hippocampal slices from adult male Sprague-Dawley rats or C57BL6 mice were prepared with standard procedures. In brief, 400 µm-thick horizontal slices were cut in artificial cerebrospinal fluid (ACSF) containing sucrose substituted for NaCl and immediately placed in an interface-style recording chamber at 32°C. After 30 min a NaCl-based ACSF was used containing (in mM: 126 NaCl, 3.5 KCl, 2 mM MgSO₄, 1.25 NaH₂PO₄, 2.4 CaCl₂, 26 NaHCO₃, 10 d-glucose; pH 7.3-7.4). Recording electrodes containing this ACSF were positioned in area CA3. Three methods were used to induce seizurelike activity: 1) [Mg²⁺]₀ was decreased (to nominally 0 mM), 2) [K⁺]₀ was elevated (to 7.5 mM), or 3) GABAA receptors were blocked with 10 µM bicuculline methiodide. The types of epileptiform activity that were measured were 1) epileptiform bursts (each burst composed of a cluster of population spikes, >50 ms duration, recurring at 0.1-0.25 Hz) and 2) spontaneous episodes of spreading depolarization (SD). SD was characterized by a >10 mV DC negative shift that lasted >5 min. The trk antagonist K252a (500 nM) reduced SD in all three seizure models but there was no detectable effect on epileptiform bursts (n=2 animals per seizure model where 2-3 slices were sampled per animal). Vehicle (DMSO) had no detectable effect. If SD is considered analogous to a seizure, and epileptiform bursts in area CA3 are considered analogous to interictal spikes, the data would suggest that trk is involved in seizures but possibly not interictal spikes. Collectively, our findings suggest that trk antagonism could be an effective way

to inhibit seizures that involve area CA3. The data may also be relevant to migraine, because SD is often considered to contribute mechanistically.

Disclosures: J.J. Botterill: None. H.E. Scharfman: None.

Poster

292. Epilepsy: Synaptic Mechanisms

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Program#/Poster#: 292.17/J1

Topic: B.11. Epilepsy

Title: Physiological mapping of hippocampal networks after status epilepticus

Authors: *A. L. SOMMER, B. S. COVENTRY, N. D. SCHARTZ, A. L. BREWSTER, E. L. BARTLETT Purdue Univ. West Lafavette, IN

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Abstract: Epilepsy, characterized by unprovoked recurrent seizures, is linked to behavioral changes such as loss of memory, difficulty learning, and decreased cognition in the postictal period. Status epilepticus (SE), or an uninterrupted seizure of extended length has been seen to not only increase the risk for the development of epilepsy later in life, but also has been linked to changes in structural morphology in the neuronal network. After the occurrence of SE, dendrites undergo a morphological change which reduces the number of dendritic spines as well as the dendritic branching (Swann et al, 2000). In addition to changes in dendritic morphology, previous studies have shown that there is a significant change in the morphology and accumulation of microglia in the hippocampus after an episode of SE (Schartz et al, 2016). Although the post-SE anatomical changes in the hippocampus have been demonstrated in CA1 neurons up to 35 days after the event, the physiological changes that are predicted to accompany these changes have not been measured. In this study, we investigate the changes in hippocampal CA1 network responses using local field potential (LFP) recordings in slice to measure the synaptic efficacy at different time points following an induced episode of SE as well as induction of long term potentiation (LTP) to measure the synaptic plasticity retained. Preliminary results suggest that synaptic efficacy is diminished at and beyond 14 days post induced SE. Furthermore, we hypothesize that the plasticity of the remaining synapses is not affected at these time points. Understanding these properties in relation to microglial-mediated changes in synaptic density after a prolonged seizure will help with the understanding of the behavioral side effects that are commonly associated with epilepsy and provide information for modulation of neuronal mechanisms to reduce or prevent the accumulation of these effects.

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Poster

292. Epilepsy: Synaptic Mechanisms

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Topic: B.11. Epilepsy

Support: NIH R01 NS082046

NIH R01 NS038572

Title: Contrasting properties of active and inactive hippocampal dentate granule cells

Authors: *S. A. PARK¹, F.-C. HSU¹, H. TAKANO¹, I. PETROF¹, D. A. COULTER^{1,2,3} ¹Neurol., ²Pediatrics, Children's Hosp. of Philadelphia, Philadelphia, PA; ³Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: The hippocampus is a critical mediator of spatial learning and navigation. The dentate gyrus contributes to this function through its sparse activation properties, which aids in distinguishing between similar cortical inputs (pattern separation). Using 2-photon imaging in awake, behaving animals navigating a virtual environment, we found that a smaller number of dentate granule cells (DGCs) activate at lower frequencies than neighboring CA1 pyramidal neurons. We explored the intrinsic and local circuit mechanisms mediating this characteristic sparse DGC activation. We used fos-TRAP (targeted recombination in active populations) transgenic mice, crossed to a tdTomato reporter mouse to label active DGCs in vivo following exposure of mice to an enriched environment and 4-hydroxytamoxifen injection, and then recorded from hippocampal slices prepared from these same mice. In fosTRAP mice, in active neurons, the fos promoter drives expression of CreERT, which, in the presence of tamoxifen, drives tdTomato reporter expression. As expected, fosTRAP labeled DGCs were sparse: on average, 7.2 cells were labeled with tdTomato per dentate blade in a given slice. To understand the intrinsic and synaptic properties that differentiate active (tdTomato+) from inactive (unlabeled) DGCs, we used whole-cell patch-clamp to record intrinsic properties as well as synaptic potentials elicited by perforant path stimulation. Active DGCs had a larger IPSC amplitude, shorter IPSC time-to-peak, and a longer EPSC time-to-peak compared to neighboring inactive DGCs. These data, where larger, faster IPSCs associate with active DGCs, are counterintuitive. We further tested whether intrinsic neuronal properties might contribute to differential activation, and found that active DGCs had a lower input resistance, and a higher action potential threshold, both also inconsistent with elevated excitability. These results suggest that DGCs activate in vivo primarily due to a difference in the afferent input, and not to local circuit processing. Continued investigation of the synaptic connections and microcircuit properties may further clarify how active and inactive DGCs process their synaptic inputs to generate their characteristic behavior.

Disclosures: S.A. Park: None. F. Hsu: None. H. Takano: None. I. Petrof: None. D.A. Coulter: None.

Poster

292. Epilepsy: Synaptic Mechanisms

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Wellcome Trust / EPSRC

Newcastle Univeersity PhD Studentship

Title: Optogenetic dissection of developing synaptic activity during evolving epileptiform activity

Authors: *R. T. GRAHAM¹, E. JOHNSON¹, N. CODADU², R. R. PARRISH⁴, A. J. TREVELYAN³

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Abstract: Bathing brain slices in 0 Mg^{2+} artificial cerebro-spinal fluid (ACSF) induces an evolving pattern of epileptiform activity, including short interictal bursts of activity, and sustained discharges that replicate many features of tonic-clonic seizures in vivo. The most important, initial effect of removing Mg²⁺ ions is considered to be the removal of the voltage-dependent blockade of NMDA receptors, thereby greatly enhancing the excitatory synaptic effects mediated by glutamate. This though occurs rapidly, and the evolving epileptiform activity continues over a time course of many minutes and even hours. The epileptiform discharges therefore, cannot be ascribed simply to the NMDA effect, but rather, will involve dynamic reorganisation of the network. Changes in synaptic strength are likely to contribute to this process. We asked therefore whether the key network transitions seen in these preparations were associated with changes in synaptic function. We used an optogenetic approach to selectively stimulate three different subclasses of neurons (pyramidal cells, and two classes of interneurons: parvalbumin-positive (PV+) and somatostatin-positive (SOM+) that selectively expressed Channelrhodopsin, while monitoring the postsynaptic effect.

Brief optogenetic activation was achieved by 50ms flashes, delivered at 0.1Hz, close to the recording location through an optic fibre connected to an LED. The postsynaptic effects of

pyramidal activation was monitored using extracellular electrodes located in layers 1 and 5. Interneuronal activation was monitored by whole cell patch clamp recordings of pyramidal cells. We found clear changes in all three synaptic pathways associated with the evolving activity, and acutely with local seizure activity. Interestingly, regular "open-loop" optogenetic stimulation of the pyramidal cells appears to alter the time course of epileptic evolution in this simple model, consistent with other recent studies of open loop electrical stimulation in animal epilepsy models.

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Poster

292. Epilepsy: Synaptic Mechanisms

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Topic: B.05. Transporters

Title: Constitutive phosphomimetic inhibition of KCC2 at Thr906/Thr1007 causes GABAdependent network excitability, seizure, and early postnatal death

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Abstract: A post-natal decrease in intraneuronal Cl⁻ concentration [Cl⁻]_i dependent on the Cl⁻ extruding KCC2 cotransporter establishes fast synaptic GABA inhibition. Inhibitory phosphorylation of KCC2 at two threonines (Thr⁹⁰⁶ and Thr¹⁰⁰⁷), a powerful switch of KCC2 activity, decreases in parallel with an increase in KCC2 activity and the lowering of neuronal [Clli, but the significance of this *in vivo* is unknown. Here, using unbiased LC-MS/MS phosphoproteomics, we demonstrate that KCC2 exhibits precise developmental regulation at only several phosphorylation sites, and most significantly at Thr⁹⁰⁶. We engineered mice to express two KCC2 alleles with the missense mutations Glu^{906} and Glu^{1007} "($KCC2^{e/e}$)" to mimic constitutive phosphorylation at these sites. $KCC2^{e/e}$ mice showed body weight reduction but exhibited normal gross and histological organogenesis and the characteristic developmental up-regulation of KCC2 protein. However, all KCC2^{e/e} mice exhibited status epilepticus provoked by mild physiological stimulation such as brushing and tail pinch, and died within the first post-natal day. *KCC2^{e/e}* mice showed spontaneous seizure and the frequency of seizure gradually increased before death. Patch-clamp measurements of E18.5 ventral spinal cord neurons in WT and KCC2^{e/e} mice demonstrated that the resting Cl⁻ level was not changed in KCC2^{e/e} mice but Cl⁻ extrusion capacity was significantly impaired after Cl⁻loading. In contrast to WT mice, KCC2^{e/e}

mice a lack of spontaneous respiratory discharge recordings from cervical ventral roots (C4) and altered locomotor rhythm recordings from lumbar ventral roots (L2). *KCC2^{e/e}* mice exhibited significant abnormalities in neuronal distribution in the septum, hypothalamus, hippocampus, and cortex, but normal dendritic spine formation. These data demonstrate precisely regulated KCC2 Thr906/Thr1007 phosphorylation during brain development is essential for activity-dependent Cl⁻ extrusion, neuronal development, and post-natal survival.

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Poster

292. Epilepsy: Synaptic Mechanisms

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Support: NS090041

Title: Role of somatostatin and parvalbumin interneurons in 4-aminopyridine-induced epileptiform discharges in mouse cortex

Authors: *W. E. LADO, J. J. HABLITZ

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Abstract: 4-Aminopyridine (4-AP) induces epileptiform discharges in a variety of brain regions. These events are associated with enhanced inhibitory as well as excitatory synaptic transmission. The relative contribution of specific subclasses of GABAergic interneurons to epileptiform activity in 4-AP model has not been well characterized. We have used genetically-encoded archaerhodopsin to investigate the role of somatostatin (SST) and parvalbumin (PV) interneurons in the regulation of evoked epileptiform discharges. Whole cell patch clamp recordings were obtained from L5 pyramidal cells in somatosensory cortex of 30 to 70 day old mice. In the presence of 100 µM 4-AP, local stimulation was used to evoke epileptiform activity. Archaerhodopsin activation in SST interneurons during (Light Off: 6385.98 ± 562.1 mV*ms vs Light On: $2873.44 \pm 165.2 \text{ mV*ms}$; p < 0.05, paired t-test) and after local stimulation (Light Off: 6601.67 ± 642.82 vs Light On: 4339.20 ± 431.6 mV*ms; p < 0.05, paired t-test) reduced the area under the curve (AUC) of epileptiform discharges. Similarly, archaerhodopsin activation in PV interneurons was effective in reducing the evoked responses when electrical stimulation occurred during (Light Off: 11029.35 ± 732.7 mV*ms vs Light On: 2234.16 ± 203.8 mV*ms; p < 0.05, paired t-test) or after (Light Off: 10610.44 ± 715.1 vs Light On: 3719.62 ± 288 ; p < 0.05, paired t-test) light onset. Light activation of the PV interneurons reduced responses to a significantly greater extent than that of SST interneurons both during $(24.49 \pm 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \% \text{ vs } 55.19 \pm 2.3 \%; \text{ p} < 1.2 \%; \text{ p} < 1.$

0.05, paired t-test) and after $(44.3 \pm 1.9\% \text{ vs } 65.26 \pm 0.8\%; \text{ p} < 0.05, \text{ paired t-test})$ electrical stimulation. This suggests that PV neurons may be more effective in reducing excitability in the 4-AP model. Since both types of cells were effective in reducing epileptiform activity, it raises the possibility that non-selective activation of interneurons could be more efficacious.

Disclosures: W.E. Lado: None. J.J. Hablitz: None.

Poster

293. Epilepsy: Animal Models: Consciousness, Novel Models, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 293.01/J6

Topic: B.11. Epilepsy

Support: Branch Out Neurological Foundation

Eyes High International Doctoral Scholarship

Title: Co-morbid epilepsy in a mouse model of autism

Authors: *M. L. LEWIS¹, J. M. RHO², Q. J. PITTMAN¹

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Abstract: Autism Spectrum Disorder (ASD) and epilepsy co-occur at a high rate of 30%, suggesting that they may be linked by a common mechanism. To investigate mechanisms involved in the development of co-morbid ASD and epilepsy, a novel mouse model with a robust phenotype for both disorders was developed and validated.

BTBR T+tf/J (BTBR) mice are a well-validated model of ASD due to the ASD-like behavioural phenotype. This mouse model of ASD however, has not yet been shown to have co-morbid epilepsy. To this end, BTBR mice exposed to an immune challenge at postnatal day 7 (lipopolysaccharide, LPS; 100 μ g/kg i.p.) exhibited as adults a significant decrease in seizure thresholds (1% pentylenetetrazol; 2.5 ml/min i.v.; p<0.05), a significant increase in hippocampal electrographic seizure activity (p<0.05), and importantly, a preservation of the ASD behavioural phenotype when compared to saline treated controls.

Patients affected with co-morbid ASD and epilepsy often have a high rate of intractability with common pharmaceutical options. A limited neurobiological understanding of these disorders has precluded development of effective and personalized therapeutics. The ketogenic diet (KD) consists of a consumed ratio of 4:1 fat to combined protein and carbohydrate by weight resulting in conversion of fats into fatty acids and ketone bodies, which are used as an energy source. The KD has been shown to be an effective treatment for medically intractable epilepsy and may reduce the severity of ASD behaviours in animal models. Due to a major knowledge gap in how

the KD elicits neuroprotective and possibly disease-modifying effects, this treatment modality is not routinely used as a complementary alternative to pharmaceuticals. We asked if it would be effective in reducing the increased excitability and behavioral features in our model. We found that adult P7 LPS treated BTBR mice that were subsequently treated with the KD from P46 to P60 had a significant increase in seizure threshold compared to P7 LPS treated BTBR mice that remained on a standard chow diet (p<0.05). Lastly, BTBR mice treated with the KD had a significant reduction in a few behavioural measures of the ASD phenotype (e.g. frequency of self-grooming and communication) compared to BTBR mice on a standard diet (p<0.05). These results indicate that the KD may be effective in targeting mechanisms that are responsible for the epilepsy and ASD phenotype in this novel co-morbid mouse model. Our future directions will continue to investigate the mechanisms that lead to the development of co-morbid epilepsy in a mouse model of ASD with exposure to a postnatal immune challenge.

Disclosures: M.L. Lewis: None. J.M. Rho: None. Q.J. Pittman: None.

Poster

293. Epilepsy: Animal Models: Consciousness, Novel Models, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 293.02/J7

Topic: B.11. Epilepsy

Title: Establishment of Tsc1^{flox/flox}-GFAP-Cre (Tsc1^{GFAP}CKO) mice for evaluating potential new antiepileptic therapies for tuberous sclerosis complex

Authors: *S. C. LEISER¹, A. GHAVAMI², M. KWAN², J. BELTRAN², D. SONG¹, D. M. DEVILBISS¹, M. WONG³, N. RENSING³, S. L. ROBERDS⁴, D. BRUNNER⁴ ¹Translational EEG, PsychoGenics, Tarrytown, NY; ²PsychoGenics, Montvale, NJ; ³Dept. of Neurol., Washington Univ. Sch. of Med., St. Louis, MO; ⁴Tuberous Sclerosis Alliance, Silver Spring, MD

Abstract: In this two-part study, we evaluated incidence of seizures using EEG and changes in mRNA biomarkers using quantitative PCR (qPCR) in *Tsc1*^{GFAP}CKO mice. First, we evaluated effects of rapamycin on incidence of seizures and mortality given that previous reports indicated these mice develop spontaneous postnatal epilepsy rescued by treatment with the mTOR inhibitor. EEG from mice were continuously recorded (using Pinnacle Technology's data acquisition platform) from P35-P49. The vehicle-treated group (n=10) exhibited on week 5 and 6 an average of 3.5 ± 1.1 and 2.33 ± 1.1 seizures, respectively. In contrast, the rapamycin-treated group that started at P35 (n=8) exhibited a reduction in seizures for weeks 5 (1.75 ± 0.65) and 6 (0.38 ± 0.26) nearly reaching significance at week 6 (p=0.06, Tukey-Kramer). Moreover, the rapamycin-treated group that started at P21 (n=10) showed no seizures during week 5 and 6 (p<0.05, Tukey-Kramer). In summary, whereas *Tsc1*^{GFAP}CKO mice treated with vehicle from

P21-48 suffered robust electrographic seizures and a significant mortality over this period, mice treated with 3 mg/kg rapamycin from P21-48 showed zero seizures and zero mortality over the same period. The group receiving rapamycin 3 mg/kg from P35-48 showed reduced seizure frequency and zero mortality. Thus, we confirm the protective effects of mTOR inhibition against seizures and premature death in these mice. Secondly, in a separate cohort, we used qPCR to evaluate changes in mRNA levels of transcripts involved in axon formation, synapse function, glutamate transport, mTOR activation, cell adhesion, angiogenesis, cell regulation, inflammation and unfolded protein response activation in brain at 5 weeks of age. Expression levels between control (Cre⁻; $Tsc1^{+/flox}$) and Cre⁺; $Tsc1^{+/flox}$ mice were similar for all transcripts reported in this study. In contrast, Tsc1^{GFAP}CKO mice (Cre⁺; Tsc1^{flox/flox}) showed genotypedependent changes in some transcripts analyzed. Of particular note were decreased mGluR5 and Tyrosine Kinase c-kit and increased IBA1, CD68, ICAM1, VEGF-D, and TLR4 mRNA expression in *Tsc1*^{GFAP}CKO mice. In some cases affected transcripts in Cre⁺; *Tsc1*^{flox/flox} mice showed significant variation among individuals that cannot simply be explained by differential effectiveness of CRE-mediated knockout since, except one animal, all Cre⁺; Tsc1^{flox/flox} mice showed similarly reduced expression levels of *Tsc1* mRNA. Further, mRNA levels might covary with seizure frequency or age of seizure onset which needs to be assessed in future studies. Overall, our findings suggest a clear utility of using these mice to screen potential anti-epileptic therapeutics.

Disclosures: S.C. Leiser: None. A. Ghavami: None. M. Kwan: None. J. Beltran: None. D. Song: None. D.M. Devilbiss: None. M. Wong: None. N. Rensing: None. S.L. Roberds: None. D. Brunner: None.

Poster

293. Epilepsy: Animal Models: Consciousness, Novel Models, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 293.03/J8

Topic: B.11. Epilepsy

Title: A spontaneous deletion in Eml1 underlies the bilateral cortical malformation in the tish rat model of subcortical heterotopia

Authors: *D. K. GROSENBAUGH¹, S. JOSHI², K. S. LEE⁵, M. P. FITZGERALD⁶, M. J. ANZIVINO³, M. J. MCCONNELL⁷, H. P. GOODKIN⁴ ¹Dept. of Neurol., ²Dept of Neurol., ³Univ. of Virginia, Charlottesville, VA; ⁴Univ. of Virginia, Charlottesvle, VA; ⁵Univ. Virginia Hlth. Systems, Charlottesvle, VA; ⁶Children's Hosp. of Philadelphia, Philadelphia, PA; ⁷Biochem. and Mol. Genet., Univ. of Virginia Sch. of Med., Charlottesville, VA

Abstract: Malformations of cortical development (MCD) are common in children with pharmacoresistance epilepsy. Although surgery can be successful for some, not all forms of MCD are operable. The tish (telencephalic internal structural heterotopia) rat, which arose spontaneously in a colony of Sprague-Dawley (SD) rats, is a model of severe, non-operable MCD. The tish rat is characterized by a large bilateral heterotopic cortex separated from the overlying normotopic neocortex. Prior studies reported an aberrant proliferative zone composed of misplaced neuronal progenitors with altered cell cycle kinetics. These misplaced cells result in alterations in neuronal migration which lead to the heterotopic cortex and a neuronal network capable of generating spontaneous seizures. However, the genetic mutation underlying this MCD and the earliest age of identifiable electrographic (EEG) abnormalities have not been identified. In EEG recordings spanning from postnatal day (P) 8 - P45, spontaneous spike wave discharge (SWD) bursts were determined to begin at P18 - P20 in all tish rats examined, while no spontaneous discharges were observed in any of the age-matched control SD rats. The presence of paroxysmal discharges is consistent with altered network connectivity and hyperexcitability in these animals beginning at a young age. We performed 30X whole genome sequencing of tish and SD rats, and identified a tish-specific deletion ~50kb upstream of the *Eml1* locus (Rnor 6.0). Using RNAseq and RT-PCR, we confirmed that this deletion spans an unannotated exon 1 leading to a 4-fold down-regulation of Eml1 transcript abundance in embryonic day (E) 18 tish cortex. Rescue experiments (i.e., F2 cross) demonstrated that the deletion in *Eml1* segregates with both the observed heterotopic phenotype and the occurrence of spontaneous SWD bursts. These data link a mutation in *Eml1* to the development of a bilateral heterotopic cortex and a resultant hyperexcitable network of the tish rat. Mutations in *Eml1* have been identified in patients with subcortical heterotopia and epilepsy. These findings further strengthen the tish rat as a translational model of human non-operable MCD-associated epilepsy.

Disclosures: D.K. Grosenbaugh: None. S. Joshi: None. K.S. Lee: None. M.P. Fitzgerald: None. M.J. Anzivino: None. M.J. McConnell: None. H.P. Goodkin: None.

Poster

293. Epilepsy: Animal Models: Consciousness, Novel Models, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 293.04/J9

Topic: B.11. Epilepsy

Support: LLU Dept. of Psychology

Title: Seizure-like activity in Bang-sensitive *Drosophila* is increased by GABA_A receptor antagonists and decreased by ellagic acid

Authors: ***A. A. SMITH, JR**¹, J. PAULDURAI², A. JAEGER², A. TROFIMOVA², C. BARCENAS², W. HARDEMAN², L. VILLALPANDO², A. BRISENO², N. KALYNOVSKA²,

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Abstract: Introduction: Epilepsy is a worldwide public health concern associated with debilitating neurological, cognitive, and psychosocial consequences. Current anti-seizure drugs can have debilitating side effects and new treatments are needed for individuals with drugresistant seizures. Ellagic acid, a polyphenol present in several fruits and nuts, has shown beneficial effects in rodent models of epilepsy and anxiety, possibly mediated through GABAergic pathways. "Bang-sensitive" Drosophila experience mechanically induced seizurelike activity (SLA), possibly providing a high-throughput alternative to costly and lengthy rodent studies. The objectives of this study were to examine the anticonvulsant effects of ellagic acid in a fruit fly model of epilepsy and to explore the mechanism of action through the addition of two GABA_A receptor antagonists: flumazenil (a benzodiazepine site antagonist) and picrotoxin (a GABA site antagonist or channel blocker). Method: Adult males from two Bang-sensitive strains (bang senseless [Bss] and easily shocked [Eas]) were fed ellagic acid, flumazenil, and/or picrotoxin in their food media. Following treatment, flies received mechanical stimuli (vibration) to induce SLA, which was recorded with a digital video camera. Results: Approximately 93% of flies from each strain exhibited vibration-induced SLA. Vibration induced shorter SLA in Eas flies than in Bss flies (~4 s vs. 6 s), and ellagic acid decreased SLA duration slightly in Eas flies (to ~3 s), but not in Bss flies. Flumazenil and picrotoxin each increased SLA duration in both strains, but more so in *Eas* flies (to ~6.5 s and 8.5 s, respectively). Conclusions: Vibration reliably induced SLA in both Bss and Eas strains of Bang-sensitive Drosophila. SLA duration in Bss flies was slightly longer than in Eas flies, not affected by ellagic acid, and slightly increased by GABA_A receptor antagonists. SLA duration in Eas flies was slightly reduced by ellagic acid and significantly increased by GABAA receptor antagonists. These data show that seizure modeling with Bang-sensitive Drosophila can serve as an effective drug-screening tool for refractory epilepsy in humans and suggest that compounds found in plants may provide prophylactic benefits with regard to seizure severity.

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Poster

293. Epilepsy: Animal Models: Consciousness, Novel Models, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 293.05/J10

Topic: B.11. Epilepsy
Title: A novel user-friendly automated tool to accurately detect seizures in rodent models of acquired and genetic epilepsy

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¹Kaoskey Pty Ltd, Sydney, Australia; ²Dept. of Med., The Royal Melbourne Hospital, The Univ. of Melbourne, Melbourne, Australia

Abstract: Purpose: Prolonged video-EEG monitoring in chronic epilepsy rodent models has become an important tool in pre-clinical drug development of new therapies, in particular for anti-epileptogenesis, disease modification and drug resistant epilepsy. We have developed an easy to use, reliable, computational tool for detection of electrographic seizures from prolonged EEG recordings in rodent models of epilepsy.

Methods: We applied a novel method based on advanced time-frequency analysis which detects the episodes of EEG with excessive activity in certain frequency bands. The method uses an advanced technique of short term spectral analysis - the Similar Basis Function algorithm. The method was applied for off-line seizure detection from chronic EEG recordings from four spontaneously seizing, chronic epilepsy rat models: post-status epilepticus model of temporal lobe epilepsy (n=39 rats, n=124 seizures); the fluid percussion injury model of post-traumatic epilepsy (n=5 rats, n=49 seizures); and two genetic models of absence epilepsy - GAERS and WAG/Rij (n=41 and 14 rats, n=8733 and 825 seizures respectively).

Results: High values of power spectrum in frequency band 17-25 Hz were found to specifically indicate seizures in all four animal models. This peculiarity comes from the frequency composition of single discharges within the seizures in these animals. Focusing on this band, our computer program detected 100% of seizures in all 99 rats. Electrode artefacts, which are usually present in long-term EEG recordings, may also significantly contribute to this frequency band, so they were also selected by the program. This selection, however, generated a very low rate of false positives. For their elimination, a quick user inspection was needed. The overall processing time for 12 day-long recordings varied from few minutes (5-10) to an hour, depending on the number of artefacts.

Conclusion: Our seizure detection tool provides high sensitivity, with acceptable specificity, for chronic EEG recordings from chronic rat epilepsy models. This has the potential to improve the efficiency and rigor of pre-clinical research and therapy development using these models.

Disclosures: A. Sargsyan: A. Employment/Salary (full or part-time):; Kaoskey Pty Ltd, Sydney. D. Melkonian: A. Employment/Salary (full or part-time):; Kaoskey Pty Ltd, Sydney. P.M. Casillas-Espinosa: A. Employment/Salary (full or part-time):; Deaprtment of Medicine, The Royal Melbourne Hospital, The University of Melbourne. T.J. O'Brien: A. Employment/Salary (full or part-time):; Deaprtment of Medicine, The Royal Melbourne Hospital, The University of Melbourne. The Royal Melbourne Hospital, The University of Melbourne.

Poster

293. Epilepsy: Animal Models: Consciousness, Novel Models, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 293.06/J11

Topic: B.11. Epilepsy

Support: NIH/NINDS R01 NS073768

Title: Variable phenotype in a Dravet syndrome model in Charles river versus Jackson C57BL/6 mice

Authors: J. L. WHITE¹, K. LEE², B. TARHAN¹, J. QI³, *S. KOH¹ ¹Pediatrics, Emory Univ., Atlanta, GA; ²Pediatrics, Daegu Catholic Univ., Daegu, Korea, Republic of; ³Northwestern Univ., Chicago, IL

Abstract: Rationale: The majority (~80%) of Dravet syndrome (DS) is associated with a neuronal sodium channel alpha 1 subunit (SCN1A) mutation causing epilepsy onset in infancy, intractable seizures, sudden unexpected death, and various neurobehavioral comorbidities. The variable phenotypes in this disease are, at least in part, a result of modifier genes. Many Dravet syndrome mouse models have demonstrated the hallmark features of DS; yet none characterized the wide array of phenotypes. We present the developmental disease characteristics in three distinct C57BL/6 mice sub-strain as breeders.

Methods: We used the F1 generations of Scn1a (*129S-Scn1a^{tm1Kea}/Mmjax*) mice bred to C57BL/6 mice from (1) The Jackson Laboratory (DS-Jax) and (2) Charles River (DS-Crl). Then we bred Jackson Laboratory to Charles River C57BL/6 mice for 3 generations to establish a hybrid sub-strain (JC) as breeders for F1 DS mice. The 3 groups of F1 DS mice, DS-Jax, DS-Crl and DS-JC underwent prolonged (30 minutes) febrile (LPS +hyperthermia) seizures at P30. At P50, select mice were implanted with EEG electrodes and recorded for 24-hours, once per week, for three weeks to detect electroclinical seizures.

Results: Hyperthermia induced seizures in all (100%) 3 groups of DS mice with similar mean threshold temperatures as follows: DS-Jax, $39.7\pm0.5^{\circ}$ C (*n*=8); DS-JC, $40.3\pm0.3^{\circ}$ C (*n*=7); DS-Crl, $39.8\pm0.1^{\circ}$ C (*n*=16). Sudden unexpected and unexplained death occurred from weaning age until P49, mortality occurred in each of the DS mice groups: 5 deaths were noted (5/21, 24%) in DS-Jax while only one death each occurred in DS-JC and DS-Crl mice (1/7, 14% and 1/18, 6%, respectively). EEG recording showed abnormal EEG and spontaneous electroclinical seizures in 100% (*n*=2/2) of DS-Jax mice, 80% (*n*=4/5) of DS-J-C mice, and 60% (*n*=3/5) of DS-Crl mice. **Conclusion:** Susceptibility to hyperthermia-induced seizures was noted in all 3 sub-strains of F1 generation *129S-Scn1a* -C57BL/6 mice. There was a dose response-like trend in both mortality and electrographic seizures. DS mice from the Jackson lab C57BL/6J background appear to be more affected, while the Charles River C57BL/6NCrl bred DS mice had the milder response to

the SCN1A gene mutation. Our observation suggests that genetic modifiers may exist, even within C57BL/6 mice that originated from the same gene pool that led to variable effects on Dravet syndrome phenotype.

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Poster

293. Epilepsy: Animal Models: Consciousness, Novel Models, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 293.07/J12

Topic: B.11. Epilepsy

Support: CURE

Title: Toll-like receptor and cytokine expression changes in a rodent model of epilepsy

Authors: *C. SADANGI¹, F. ROSENOW², B. NORWOOD^{3,2} ¹Philips University, Marburg, Marburg, Germany; ²Goethe Univ., Frankfurt, Germany; ³Philipps Univ., Marburg, Germany

Abstract: Rationale: Toll-like receptors (TLRs) are innate immune receptors that are important in early host defense against pathogens. TLRs recognize pathogen- or damage-associated molecular patterns (PAMPs/DAMPs), and are either expressed on the cell surface or in the endosomes. TLRs 1, 2, and 6 are expressed on the cell surface, whereas TLR8 is expressed intracellularly. TLRs 1 and 2 have been implicated in experimental epilepsy; however, the other TLRs have yet to be studied. IL-2, a cytokine, regulates immune response through T-cell activation and it has been shown to be upregulated in epilepsy. IL-6 is both a pro- and antiinflammatory cytokine regulating inflammatory and immune response, and it has also been shown to be expressed during seizure. The chemokine RANTES (CCL5) is involved in leading leukocytes to the site of injury and has been shown to be upregulated in epilepsy. Here we investigated the gene expression changes of TLRs, cytokines, and a chemokine during epileptogenesis in rodent models of acquired epilepsy and chronic epilepsy. Methods: Freely moving male Sprague-Dawley rats (n=4 per group) received systemic administration of kainate and Lorazepam (KaL) and were sacrificed after 4 d, 14 d, or 20 weeks, and whole hippocampi were removed post treatment. The mRNA level was analyzed for the TLRs, IL-2, IL-6, and RANTES with custom-designed primers. Relative gene expression changes were calculated using the REST software (Pfaffl method). IL-2, IL-6, and RANTES

protein expressions were analyzed using ELISA.

<u>Results</u>: During epileptogenesis, except TLR1, all the other TLRs expressions were enhanced after 4 d, and all TLRs were further enhanced after 14 d. However, all the TLRs exhibited downregulation during chronic epilepsy. The mRNA expression of IL-2 was upregulated at 4 d

but declined during 14 d epileptogenesis and chronic epilepsy. The IL-6 protein expression diminished at 4 d, but elevated at 14 d epileptogenesis, however, in the chronic epilepsy phase it remained was downregulated again. RANTES was downregulated during 4 d but was upregulated during 14 d epileptogenesis, and had a slight decrease in expression during chronic epilepsy but remained upregulated.

<u>Conclusion</u>: TLRs expressions are enhanced and prolonged during epileptogenesis. Evoked seizures also upregulate expressions of these TLRs, but transiently and not to the same extent. Cytokines and chemokine expressions change during epileptogenesis and chronic epilepsy. These data warrant the further investigation on the role of these TLRs, cytokines, and chemokine in epilepto- and ictogenesis.

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Poster

293. Epilepsy: Animal Models: Consciousness, Novel Models, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 293.08/K1

Topic: B.11. Epilepsy

Support: Florida Atlantic University, Brain Institute Pilot Grant, 2017

Title: A proposed role for the nucleus of pontis oralis of the brainstem (NPO) in sudden unexpected death in epilepsy

Authors: R. P. VERTES¹, M. GIL², R. STCLAIR², R. LEMOS², K. KOROMA², *C. ISGOR² ¹Ctr. for Complex Systems & Brain Sci., Florida Atlantic Univ., Boca Raton, FL; ²Charles E. Schmidt Biomed Ctrr, Florida Atlantic Univ. Charles E Schmidt Col. of Med., Boca Raton, FL

Abstract: A network inhibition hypothesis of seizure-related vulnerability to death postulates that seizures excite the hypothalamus and lateral septum which in turn send inhibitory projections to subcortical arousal centers, one of the most prominent being the **nucleus pontis oralis** (**NPO**) of the brainstem. A marked suppression (or total inhibition) of NPO activity would generally manifest as postictal generalized electroencephalography suppression (PGES) - or the cortical EEG "flattening" that accompanies seizures and presents a major risk for fatality. PGES is reportedly one of the few predictors of susceptibility to sudden unexpected death in epilepsy (SUDEP) in human patients. We postulate that repeated seizures will result in the progressive damage to NPO cells - with further deleterious effects on arousal/consciousness in epilepsy. We will use a novel transgenic mouse model of epilepsy (brain-derived neurotrophic factor overexpressing mice under the Cam/KIIa promoter) that exhibits progressive lengthening of PGES episodes with successive seizures (as a critical marker of severity of seizures) that does not require any invasive procedures to generate epileptic activity. Spontaneous seizures are

produced in a controlled fashion by simply to tail suspension or cage shaking stimulation and which become more severe with time. Using this model, we will test whether repeated seizures cause progressive neuronal injury/death of NPO cells, which are responsible for the increased severity of PGES. Our preliminary data show that mice become more seizure prone with age and the duration of postictal generalized EEG suppression (PGES) progressively increases with each seizure episode. In addition, epileptic mice spend more time in non-REM sleep as a function of seizure frequency in inactive phase, and the average length of REM bouts decrease accordingly implicating deficits in normal NPO sleep function. In order to establish that the increased duration of PGES over repeated seizures is associated with progressive deterioration (or cell loss) in NPO and reflects subsequent alterations in sleep architecture, we crossed a commercially available live mouse strain, Thy1-eYFP (JAX 003782), with our epileptic mice and noted significant loss of neurons in the gigantocellular field of the NPO as a function of PGES severity. These findings implicate seizure-induced cellular injury in the NPO for the emerging risk for SUDEP.

Disclosures: R.P. Vertes: None. M. Gil: None. R. StClair: None. R. Lemos: None. K. Koroma: None. C. Isgor: None.

Poster

293. Epilepsy: Animal Models: Consciousness, Novel Models, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 293.09/K2

Topic: B.11. Epilepsy

Support: NIH RISE

Title: Correlation of seizure-associated central apneic episode durations with their frequency of occurrence in a rat model

Authors: *M. G. STEWART¹, S. VILLIERE⁴, K. NAKASE¹, R. KOLLMAR², R. ORMAN³ ¹Physiol. & Pharmacol., ²Dept. of Cell Biol., ³Physiology&Pharmacology, SUNY Downstate Med. Ctr., Brooklyn, NY; ⁴Res. Initiative for Scientific Enhancement (RISE) Program, CUNY Medgar Evers Col., Brooklyn, NY

Abstract: Respiratory derangements, including irregular, tachypnic breathing and central or obstructive apnea can be consequences of seizure activity. Periods of seizure-associated central apnea suggest that seizures spread to brainstem respiratory regions to disrupt breathing. Recently, we showed that seizure-associated central apnea episodes are associated with 1) a reset of the respiratory rhythm, and 2) activation of brainstem regions serving the diving reflex to suppress respiratory behavior (Villiere et al., Neurobiol. Dis. 2017; 101:8-15). Using a rat model, we demonstrated a sequence of events ending in death that begins with

obstructive apnea caused by seizure-induced laryngospasm (Nakase et al., Epilepsy Res. 2016; 128:126-139). We sought to determine if patterns of central apnea behavior (duration and/or frequency of occurrence) correlated with the tendency for obstructive apnea to occur. There were not statistically significant differences in central apneic episode duration or number of recorded episodes in animals that eventually showed obstructive apnea compared with animals that never showed obstructive apnea. There was, however, a clear positive correlation of the average apneic episode duration with the number of apneic episodes recorded (R^2 =0.54) and their crude frequency of occurrence (events/min of recording; R^2 =0.58). Whereas the frequency or duration of central apneic episode duration with frequency suggests that these episodes may reflect an attempt by brainstem networks to protect core physiology. A deeper understanding of seizure spread into brainstem regions may permit additional biomarkers (see e.g. Stewart et. al., Epilepsia 2017 in press doi:10.1111/epi.13765) to be established for identifying risk of severe systemic consequences of epileptic seizures.

Disclosures: M.G. Stewart: None. S. Villiere: None. K. Nakase: None. R. Kollmar: None. R. Orman: None.

Poster

293. Epilepsy: Animal Models: Consciousness, Novel Models, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 293.10/K3

Topic: B.11. Epilepsy

Support: RO1 NS 040337

RO1 NS 044370

Title: A continuous hippocampal stimulation model of status epilepticus and temporal lobe epilepsy in C57bl/6 mice

Authors: *J. KAPUR, E. LEWCZUK, S. JOSHI, J. WILLIAMSON Dept Neurol., Univ. Virginai Hlth. Sci. Ctr., Charlottesvle, VA

Abstract: Transgenic mice can provide insights into molecular pathophysiological processes. Available murine models of status epilepticus (SE) and temporal lobe epilepsy (TLE) include seizures induced by pilocarpine or kainic acid. However, pilocarpine causes high mortality and extensive neuronal loss occurs following infusion of kainic acid. Electrical stimulation models of status epilepticus and TLE are well characterized in rats, but not in mice. Adult (22-25 g, 10-12 week old) male and female C57bl/6 were implanted with bipolar electrodes in the left hippocampi (3 mm AP, 3 mm ML, 3 mm DV), bilateral supra-dural cortical electrodes, and a cerebellar reference. The mice were stimulated with a 1 msec biphasic square wave pulse for 10 s to determine after discharge threshold (ADT). The initial stimulation was set at 20 vA and the intensity was increased by 20 μ A until a seizure was triggered, maximum 200 vA. The current intensity was set to twice ADT, with minimum at 100 vA, and continuous stimulation was performed by cyclic application of 10 sec stimulation followed by 5 sec off period for 60 min. SE consisted of rhythmic and evolving spike-wave discharges sustained for 30 min or more. Mice were monitored continuously for 24 hr following the end of stimulation; the monitoring to determine spontaneous seizures was started 2 weeks later. Mice that developed at least 2 spontaneous seizures were classified as having TLE. Cell death was determined 3 days following SE using fluorojade labeling. Animals were perfused with PFA and horizontal brain sections (40 μ m thick) were labeled with fluorojade-C and neuronal marker protein NeuN. Images were acquired on a confocal microscope.

The ADT was $70 \pm 36 \ \mu\text{A}$ and seizures (SE) lasted $181 \pm 90 \ \text{min}$. CHS caused SE in 82% of animals (28/34). 50% of the mice survived SE (n=14) and were monitored for onset of TLE. In the first week of monitoring none of the animals experienced spontaneous seizures, during the 2^{nd} week 1 out of 5 animals monitored had spontaneous seizures and the number of epileptic animals increased to 7 out of 14 (50%) by 7 weeks.

Extensive fluorojade labeling was observed in CA3 and CA1 pyramidal neurons, and in dentate hilar neurons.

We have developed and characterized a mouse model of SE induced by CHS. More mice survived SE as compared to the pilocarpine model. The pattern of neurodegeneration in the hippocampus was similar to that described in other models. Half of the surviving animals also developed epilepsy. Thus, CHS provides an alternate model of SE and TLE in mice.

Disclosures: J. Kapur: None. E. Lewczuk: None. S. Joshi: None. J. Williamson: None.

Poster

293. Epilepsy: Animal Models: Consciousness, Novel Models, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 293.11/K4

Topic: B.11. Epilepsy

Support: Epilepsy Foundation Posdoctoral Research & Training Fellowship

Title: Variable impairment of consciousness in rodent absence seizures: Neuronal and hemodynamic mechanisms

Authors: *C. P. MCCAFFERTY¹, B. GRUENBAUM¹, Z. YUE¹, J. SAMPOGNARO¹, J. RYU¹, A. KUNDISHORA¹, P. HERMAN², B. SANGANAHALLI², F. HYDER³, A. DEPAULIS⁵, H. BLUMENFELD⁴

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Abstract: Absence seizures, the most common type of generalized epilepsy, consist of behavioral impairment and a distinctive spike-and-wave electrographic signature. These seizures, and associated attentional deficits and developmental difficulties, have a major impact on quality of life and are frequently insufficiently redressed by pharmacological treatments. Recent evidence shows that the degree of behavioral impairment associated with seizures is variable, and correlates with hemodynamic and electrographic changes. The neural mechanisms underlying this impairment can be investigated in a genetic model of absence seizures in the rat. We studied hemodynamics, electrophysiology and behavioral components of absence seizures in undrugged Genetic Absence Epilepsy Rats from Strasbourg (GAERS), a well-characterized and valid model of absence epilepsy. Incremental habituation was employed to accustom rats to head fixation and the environment of a high-field magnet (9.4T), allowing the acquisition of BOLD fMRI dynamics, cerebral blood flow, and local electrophysiology during absence seizures. Separate animals were used to investigate the behavioral impact of seizures in a task-free, unguided licking paradigm and in sensory detection and discrimination tasks. Cortical BOLD fMRI dynamics during seizure qualitatively match those of human absence seizures, reconciling previously-noted discrepancies with anesthetized rodent spike-and-wave discharges. Specifically, somatosensory cortex BOLD signal initially increased around seizure start, but then displayed a prolonged decrease as seizures continued. These dynamics were matched by those of both cerebral blood flow and cortical multi-unit activity. The initial increase in the latter was associated with increased spike-and-wave frequency rather than intensity of neuronal firing at each cycle, and its amplitude correlated with eventual duration of seizure (p<0.01). Impairment of the task-free licking behavior was observed during seizure as compared to matched control periods (p<0.001).

These data suggest that mechanisms of absence seizures must be investigated in undrugged models, as anesthesia appears to substantively alter neuronal activity and hemodynamics as well as arousal and seizure-associated behavior. With further investigation we hope that greater understanding of the physiological mechanisms of behavioral impairment in absence epilepsy can guide improved treatment.

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Poster

293. Epilepsy: Animal Models: Consciousness, Novel Models, and Mechanisms

Location: Halls A-C

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Program#/Poster#: 293.12/K5

Topic: B.11. Epilepsy

Support: Howard Hughes Medical Institute

CURE: Citizens United for Research in Epilepsy

Title: Mechanism of decreased subcortical cholinergic arousal in focal limbic seizures: wholecell recordings *In vivo*

Authors: *J. P. ANDREWS¹, Z. YUE³, G. T. NESKE⁴, D. A. MCCORMICK⁵, H. BLUMENFELD²

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Abstract: The mechanism of how seizures impair consciousness is not fully understood, but recent evidence suggests that ictal suppression of subcortical cholinergic arousal circuits, such as the nucleus basalis (NB) and pedunculopontine tegmental nucleus (PPTg), may play a role. Functional imaging and extracellular recordings show ictal suppression of activity in the NB and PPTg in a rat model of limbic seizures, but the synaptic mechanisms of this depressed firing rate are not known. The purpose of this investigation is to elucidate whether reduced neuronal firing in the subcortical cholinergic arousal system during seizures is associated with reduced excitatory input or with increased inhibitory input to cholinergic neurons. Whole cell recording is commonly used to access cortical neurons in vivo, but has not been previously reported from deep brain structures such as PPTg. In this study, whole-cell recordings were obtained in neurons of the PPTg, accessed 5.4 - 6.2 mm deep measured from the cortical surface, in the brain of head-fixed, anesthetized rats during focal limbic seizures. Micropipettes were fabricated with a ~10-mm taper and 4-6 M Ω resistance. The first pipette in each recording session was lowered to the target region and left in place for 30-60 minutes to allow the brain to form a narrow, lowresistance canal through which subsequent pipettes may pass. Intrapipette positive pressure was maintained at 500 mbar throughout the descent, and dropped to <30 mbar at the target region. A K-gluconate-based internal solution was used for all recordings. Seizures were triggered using a 2 second stimulation of the hippocampus through a twisted bipolar electrode with current titrated to seizure threshold. Continuous recordings were made using Spike2, with signal digitized using a Micro1401 (CED). Whole cell current clamp recordings of histologically identified cholinergic PPTg neurons during seizures showed hyperpolarization of membrane potential associated with decreased action potential firing. In addition, moment-to-moment input-resistance was measured using 5-10Hz 30pA hyperpolarizing current pulses. Input resistance showed an increase during seizures concomitant with hyperpolarization. Non-cholinergic neurons from the same region generally did not show these changes during seizures. These data suggest that cholinergic PPTg neurons show decreased firing during hippocampal seizures through a mechanism of reduced excitatory input. The identification of the synaptic mechanisms of depressed subcortical arousal during seizures may ultimately lead to new treatments aimed at preventing these changes and improving ictal and postictal cognition.

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Poster

293. Epilepsy: Animal Models: Consciousness, Novel Models, and Mechanisms

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Topic: B.11. Epilepsy

Support: NIH Grant R01 NS066974

NIH Grant R01 NS096088

Title: Behavioral assessment of intralaminar thalamic neurostimulation to improve consciousness during the postictal period of seizures

Authors: ***J. XU**¹, M. M. GALARDI¹, J. Y. POK¹, C. P. MCCAFFERTY¹, L. FENG¹, A. GUMMADAVELLI², A. J. KUNDISHORA², J. L. GERRARD², M. LAUBACH⁴, N. D. SCHIFF⁵, H. BLUMENFELD^{1,3,2}

¹Neurol., ²Neurosurg., ³Neurosci., Yale Univ. Sch. of Med., New Haven, CT; ⁴American Univ., Washington, DC; ⁵Weill Cornell Med. Col., New York, NY

Abstract: The postictal period is an impaired state of consciousness characterized by electrophysiological cortical depression, decreased cortical blood flow and neurocognitive disturbance immediately following seizures. Using deep brain stimulation (DBS) to improve consciousness during the postictal period may have a major benefit on life quality for patients suffering from medically refractory epilepsy. Previous work has shown that bilateral stimulation of the thalamic central lateral nucleus (CL) during the postictal period of seizures restored normal-appearing cortical electrophysiology and markedly improved spontaneous behavioral arousal in the rodent model. Here, we focused on further investigating the effect of this stimulation on purposeful behavior.

We implemented a lever-press escape/avoidance (E/A) task to evaluate task performance during the postictal period to assess for cognitive improvements provided by the DBS. During successive E/A task training sessions, animals showed an increased avoidance percentage and decreased response times. Post-training, bilateral CL and unilateral hippocampal and lateral cortex electrodes were implanted. Following recovery, animals were retrained in the task until they achieved >90% avoidance. In trials within a session, seizures were induced by 2 second hippocampal stimulation at 60 Hz. In postictal period, following the appearance of slow-wave activity, the CL was stimulated at 100 Hz while recording electrophysiology and behavior. As a natural control for behavior during an inattentive state, we compared behavior elicited during briefs arousal induced by conditioned tones during sleep to that measured during the post-ictal period.

We found that task performance was normal during the natural sleep slow-wave period (response time: 12.77 ± 3.796 , avoidance percentage: 79.18 ± 20.83 , n=17 from 3 rats), but impaired

during the postictal period with cortical slow wave activity (response time: 63.05 ± 3.377 , avoidance percentage: 16.66 ± 9.617 , n=8 from 3

rats). Moreover, CL stimulation improved task performance (response time: 40.58 ± 5.988 , avoidance percentage: 53.34 ± 10.19 , n=14 from 3 rats) when compared with sham groups (response time: 62.69 ± 1.107 , avoidance percentage: 11.11 ± 11.11 , n=11 from 3 rats). To conclude, this is the first instance in which we identified a difference in task performance ability when comparing normal sleep and the postictal slow-wave period in rats. The DBS in bilateral CL increased the ability to perform the E/A task in the postictal period. These findings further support the potential role of targeted DBS to improve cognitive function in patients following seizures.

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Poster

293. Epilepsy: Animal Models: Consciousness, Novel Models, and Mechanisms

Location: Halls A-C

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Program#/Poster#: 293.14/K7

Topic: B.11. Epilepsy

Support: NIH R01 NS066974

R01 NS096088

Title: Uncovering limbic seizure networks using optogenetic and neuroanatomical tracing approaches *In vivo*

Authors: L.-A. SIEU¹, *L. FENG¹, C. MA¹, C. W. ZHAO¹, J. CARDIN¹, H. BLUMENFELD^{1,2,3} ¹Dept. of Neurol., ²Neurosciences, ³Neurosurg., Yale Sch. of Med., New Haven, CT

Abstract: Limbic seizures are known to impair consciousness but the neural circuit responsible for this alteration of brain function is still poorly understood. During temporal lobe seizures, intracranial EEG recordings show sleep-like slow wave activity in the cortex, accompanied by reduced cholinergic neurotransmission. Considering that the nucleus basalis (NB) provides the most cholinergic input to cortex, we propose that partial seizures originated from limbic system structures (eg. hippocampus) cause depressed cholinergic neuronal activity in NB via subcortical pathways. Our previous work demonstrated that the lateral septum (LS), a putative inhibitory region strongly connected to hippocampus, could induce cortical slow waves together with a decrease of choline release during electrostimulation in absence of seizure. Here, we examined a

possible pathway that includes the LS and NB to explain how focal seizures originating from hippocampus can result in cortical depression. We applied an optogenetic approach to selectively stimulate cholinergic neurons in the NB during focal limbic seizures induced in a lightly anesthesized rat model. We found that slow oscillations in the cortex dramatically convert to fast waves in response to cholinergic stimulation, which support the hypothesis that cholinergic output from NB plays a critical role in maintaining cortical arousal. By using a combination of retrograde and anterograde tracing to identify LS and NB interconnections, our histology results did not reveal any direct anatomical connection between those two regions. However, the paratenial thalamic nuclei (PT), located among the midline thalamic nuclei known to participate in arousal, receive strong output from LS and have direct input to NB. Multiunit activity (MUA) recordings in PT showed a decrease of neuronal firing during seizures, which support an inhibitory effect from LS output and a reduced excitatory effect on NB. These results show that limbic partial seizures can exert an impact on a polysynaptic subcortical network including LS, PT and NB to facilitate the occurrence of cortical depression. Further investigation of this network may lead to novel therapeutic interventions aimed at improving cortical function and consciousness during and following seizures.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

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Topic: B.11. Epilepsy

Support: NIH Grant NS065020

NIH Grant NS062806

Title: Rapid development of structural abnormalities following PTEN deletion from newborn dentate granule cells in a mouse model of epilepsy

Authors: S. R. ARAFA¹, *C. L. LASARGE², S. C. DANZER²

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Abstract: Postnatal deletion of the mTOR negative regulator phosphatase and tensin homolog (PTEN) from a subset of hippocampal dentate granule cells is sufficient to cause epilepsy in mice. PTEN deletion leads to somatic hypertrophy, increased dendritic branching and aberrant basal dendrite formation. Whether these abnormalities appear quickly and plateau, or become

progressively more severe over time has not been fully elucidated. To answer this question, *Gli1-CreER*^{T2} hemizygous; PTEN ^{flox/flox}; Brainbow^{+/+} were treated with tamoxifen 3 weeks after birth, and mice were sacrificed at 7, 9, 14 and 18 weeks of age. The morphology of PTEN knockout cells expressing brainbow fluorochromes was compared to age-matched wildtype granule cells. The appearance of neuronal hypertrophy was rapid - evident in cells lacking PTEN for only four weeks - and showed little progression between 7 and 18 weeks. Assessment of other changes is ongoing. Additionally, we queried whether inflammatory changes occur at any of the time points examined by immunostaining for the microglial marking Iba1 and the glial marker GFAP. Iba1 immunoreactivity was similar among time points between control and PTEN knockout mice, while GFAP staining was elevated at 14 and 18 weeks. Together, these data reveal the rapid onset of morphological changes in PTEN knockout granule cells, and the delayed onset of inflammatory changes. The findings support the conclusion that disease progression following PTEN deletion is driven by the appearance of both primary and secondary changes.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

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Topic: B.11. Epilepsy

Support: NIH Grant R01NS065020

CAPES-PDSE 1452/13-4

Title: PTEN deletion increases hippocampal granule cell excitability in male and female mice

Authors: ***R. Y. PUN**¹, V. R. SANTOS², S. R. ARAFA³, C. L. LASARGE⁴, S. ROWLEY⁴, S. KHADEMI⁴, T. BOULEY⁴, K. HOLLAND⁵, N. GARCIA-CAIRASCO⁶, S. C. DANZER⁴ ¹Dept of Anesthesia, Cincinnati Children's Hosp., Cincinnati, OH; ²Dept. of Pharmacol. and Physiol., Georgetown Univ., Washington, DC; ³Univ. of Cincinnati, Cincinnati, OH; ⁴Dept. of Anesthesia, Cincinnati Children's Hsptl Med. Ctr., Cincinnati, OH; ⁵Neurol., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; ⁶Ribeirao Preto Sch. Med., Ribeirao Preto, Brazil

Abstract: Deletion of the mTOR pathway inhibitor PTEN from postnatally-generated hippocampal dentate granule cells causes epilepsy. Here, we conducted field potential, whole cell recording and single cell morphology studies to begin to elucidate the mechanisms by which granule cell- specific PTEN-loss produces disease. Cells from both male and female mice were recorded to identify sex-specific effects. PTEN knockout granule cells showed altered intrinsic excitability, evident as a tendency to fire in bursts. PTEN knockout granule cells also exhibited

increased frequency of spontaneous excitatory synaptic currents (sEPSC's) and decreased frequency of inhibitory currents (sIPSC's), further indicative of a shift towards hyperexcitability. Morphological studies of PTEN knockout granule cells revealed larger dendritic trees, more dendritic branches and an impairment of dendrite self-avoidance. Finally, analysis of cells from male and female animals demonstrated that both female control and female knockout cells receive more sEPSC's than corresponding male cells -- but also receive more sIPSC's - resulting in statistically equivalent EPSC/IPSC ratios. Consistent with this latter observation, extracellularly evoked responses in hippocampal slices were similar between male and female knockouts; albeit both groups of knockouts were abnormal relative to controls. Together, these studies reveal a host of physiological and morphological changes among PTEN knockout cells likely to underlie epileptogenic activity.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 294.03/K10

Topic: B.11. Epilepsy

Title: Cellular heterogeneity in the anti-sezire effect of ontogenetic activation of the pedunculopontine nucleus

Authors: *V. R. SANTOS¹, R. HAMMACK³, P. A. FORCELLI²

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Abstract: Epilepsy is the second most prevalent neurological disorder. One form of epilepsy, absence epilepsy, is characterized by typical absence seizures (ASs). ASs are brief (3-30 second) nonconvulsive epileptic events that consist of sudden impairment of consciousness accompanied by a generalized synchronous, bilateral, 2.5-4 Hz spike and slow-wave dischargers (SWDs) in the electroencephalogram (EEG). Various subcortical structures play a critical role in modulating seizures, including substantia nigra (SN) and superior colliculus (SC). These structures display anti- seizure effects, however, the mechanism by which they produce changes in brain-wide excitability are poorly understood. Both structures provide input to pedunculopontine nucleus (PPN), a brainstem nucleus part of the ascending reticular activating system. Activation of the PPN can trigger potent desynchronization of the cerebral cortex. Thus, the PPN is both anatomically and functionally positioned to mediate the anticonvulsant effects of SNpr and SC. The PPN contains a variety of cell types, including cholinergic, GABAergic and glutamatergic

neurons. Here, we evaluated the anti-absence seizure effects of optogenetic activation in different neuron populations of PPN. We used ChAT::Cre and GAD::Cre Long-Evans (LE) rats transfected with AAV-hSyn-FLEX-Chronos-GFP or AAV-hSyn-FLEX-ArchT-GFP to activate or inhibit cholinergic and GABAergic neurons of PPN. To study the role of glutamatergic neurons, we injected AAV-CamKII-Chr2-mCheery or AAV-CamKII-ArchT-mCherry in wildtype LE. Absence seizures were evoked by systemic administration of y-butyrolactone - GBL (SWD- thalamocortical/absence seizures model) and electrographic seizures were recorded on a within- subject basis (i.e., with and without optogenetic activation/silencing). Optogenetic activation (5 Hz) of ChAT+ neurons suppressed cortical SWD absence seizures. However, inhibition of cholinergic neurons had no effect on number of absence seizures. Meanwhile, optogenetic activation of GABAergic neurons of PPN increased absence seizures, whereas optogenetic silencing of GABAergic neurons was without effect on seizures. Finally, activation or inhibition of glutamatergic neurons from the PPN have no effect of the number of seizures. Parallel studies are underway using DREADD-mediated modulation of PPN effect on other seizure types. These data indicate that different populations of neurons in the PPN exert diverse effects in the modulation of seizures. Using optogenetic neuronal activation in cholinergic neurons from PPN is a promising target for seizures control of epilepsy.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

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Topic: B.11. Epilepsy

Support: FAPESP 2014/17959-1

CAPES-PROEX

CNPq

Title: Wistar Audiogenic Rat (WAR) strain: contributions for epilepsy and associated comorbidities

Authors: *L. D. GODOY¹, I. B. BACCHIEGA¹, J. MARQUES GUZZI¹, J. CORTES DE OLIVEIRA¹, E. H. UMEOKA², N. GARCIA CAIRASCO¹ ¹Physiol. Department, Univ. of Sao Paulo, Ribeirao Preto, Brazil; ²Mental Hlth., Ribeirão Preto Sch. of Med. - Univ. of São Paulo, Ribeirao Preto, Brazil

Abstract: Epilepsy is characterized by an enduring predisposition to generate seizures, which is usually followed by metabolic and neurobiological alterations. Wistar Audiogenic Rat (WAR) is

a strain selected from the inbreeding of Wistar rats that exhibit seizures when exposed to acoustic stimuli. Accordingly, physiological and behavioral abnormalities make WAR an interesting strain for the study of epilepsy comorbidities. Based on this, the current study aims to evaluate in the WAR strain: 1. Maternal care under undisturbed and stressful conditions 2. depressive-like behaviors basally and after audiogenic kindling (Auk) 3. metabolic function. 1. Maternal behavior was daily monitored during lactating period for 1h in undisturbed condition (30 events/block). On lactating day 2 (LD2), dams (n=6) were submitted to pup retrieval test (PRT) and maternal defense test (MDT) was performed on LD7. There was no difference for arched back nursing posture, licking/grooming or time of dam in nest. There is no difference for number of attacks, and aggressive behaviors on maternal defense test. Although WAR latency to retrieve first pup, half of the litter and last pup in PRT also were not different, 100% of Wistar dams grouped the entire litter, whereas in WAR only 40%.2. To evaluate depressive-like behaviors, rats were submitted to 20 acoustic stimuli twice a day (AuK) (Wistar-AuK;WAR-Auk), while respective controls remained undisturbed (Wistar; WAR). Animals (n=10) were subjected also to sucrose preference (SPT) and forced swim (FST) tests. WARs did not differ from Wistar in SPT, and WAR AuK group presented hedonic behavior compared to control groups [F(3,52)=7.11;p<0.001]. In FST, using a neuroethological analysis, WARs displayed a poorly connected behavioral repertoire compared to Wistar, in both pre-test and test sessions. WAR and WAR-AuK presented significant reduction of climbing in test session when compared to Wistar [F(2,16)=5.39;p=0.0162]. 3. Wistar and WARs were placed in metabolic cages (n=8). WARs presented significant reduction in body weight (t=14.07; p<0.001), water (t=5.14;p<0.001) and food intake (t=7.57;p<0.001), compared to Wistar rats. There was no difference in urine volume between strains but WARs show significant reduction in urine osmolarity (t=2.21;p<0.05). Although the WAR strain does not present differences in maternal behaviors in basal conditions, it seems to display alterations under stress. Also, based on the current findings, we suggest that inbreeding selection for seizure susceptibility is linked to altered stress-coping behavioral strategies and metabolic function. In addition, chronic seizures may alter reward systems.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 294.05/DP03/K12 (Dynamic Poster)

Topic: B.11. Epilepsy

Support: Canadian Institutes of Health Research (CIHR)

Citizens United for Research in Epilepsy (CURE)

BC Epilepsy Society

Title: *In vivo* brain imaging of seizure-induced and targeted optogenetically-induced spreading depolarization using diffusion-weighted MRI

Authors: *S. M. CAIN¹, B. BOHNET², A. C. YUNG², Y. YANG³, J. K. KASS³, P. KOZLOWSKI², T. P. SNUTCH⁴

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Abstract: Spreading Depolarization (SD) is a neurophysiological phenomenon of large-scale depolarization of brain cells followed by a period of long-lasting neuronal inactivity. SD propagates through the brain at approximately 3-5 mm per minute causing an underlying swelling in the brain tissue invaded. This swelling alters the diffusion properties of cerebral spinal fluid at the cellular level, which we have utilized to visualize SD across the entire brain with Diffusion-Weighted Magnetic Resonance Imaging (DW-MRI). We hypothesized that SD invades brainstem tissue using fatal seizures, following district propagation pathways that are spared during non-fatal seizures.

Combining DW-MRI with EEG monitoring we examined the propagation of SD during fatal and non-fatal seizures elicited by electrical brain stimulation in a mouse model of Sudden Unexpected Death in Epilepsy (SUDEP). In addition, we utilized optogenetic stimulation to induce SD in specific brain regions to examine the propagation path from targeted subcortical foci. This was further correlated with in vitro analyses of neuronal and synaptic activity at the cellular level.

DW-MRI identified two brain regions associated with SD during seizures in the mouse SUDEP model. Targeted optogenetic stimulation in these regions revealed that they are specifically-sensitive to SD only in the SUDEP model. Further, electrophysiological analyses of intrinsic neuronal and synaptic activity in these brain regions reveals distinct gain-of-function properties related to SD susceptibility. These findings highlight that distinct brain regions are involved in the propagation of SD to the brainstem during seizures and provide support to the theory that brainstem depolarization is directly linked to SUDEP.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 294.06/L1

Topic: B.11. Epilepsy

Support: NIH Grant NS094399

DoD PR1215668

Title: Datamining to quantify and characterize dynamics of In vitro mouse seizures

Authors: D. N. CRISP¹, R. PARENT², G. G. MURPHY³, *W. C. STACEY⁴ ¹Biomed. Engin., ²Mol. and Behavioral Neurosciences Inst., ³MBNI/Physiology, ⁴Neurol., Univ. of Michigan, Ann Arbor, MI

Abstract: Our recent work demonstrated that despite epilepsy's inherent heterogeneity, seizures exhibit a set of conserved dynamical features that allow them to be characterized based upon scientific principles. The types of onset and offset patterns, known as bifurcations, establish a dynamical taxonomy of seizure dynamics. We used this taxonomy to characterize seizures in in vitro models of seizure activity, using inter-spike intervals and spike amplitude trends to categorize a limited number of specific bifurcations, coupled with several traditional quantitative signal processing measurements. However, the physiological meaning and relevance behind these bifurcation types are currently unknown. We used these measurements for two purposes: to elicit the underlying physiological properties and to quantify and compare seizures across different experiments. Mouse brain slices containing hippocampus, amygdala, and entorhinal cortex regions with a variety of pro- and anti-epileptic conditions (low magnesium, epileptic mutations, anti-epileptic drugs (AEDs), ketogenic diet, and pre-ictal therapeutic electrical stimulation) were used to generate an array of different seizure types. Each seizure was quantified with our algorithm, which allowed scientifically-based measures of seizure dynamics and direct comparison of their dynamical features.

Disclosures: D.N. Crisp: None. R. Parent: None. G.G. Murphy: None. W.C. Stacey: None.

Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 294.07/L2

Topic: B.11. Epilepsy

Support: DoD PR1215668

Title: The role of K_v beta2 in modulating *In vitro* seizure activity in mice treated with the ketogenic diet

Authors: *R. PARENT¹, G. L. FISHER¹, D. N. CRISP², H. BURNS¹, W. C. STACEY³, G. G. MURPHY⁴

¹Mol. and Behavioral Neurosciences Inst., ²Biomed. Engin., ³Neurol., ⁴MBNI/Physiology, Univ. of Michigan, Ann Arbor, MI

Abstract: The ketogenic diet (KD) has been an effective antiepileptic treatment dating back to the mid-1920s. While a number of hypotheses have been advanced, the exact cellular mechanism that underlies the antiepileptic action of the KD remains unclear. Evidence has been accumulating that ketones can directly enhance voltage-gated potassium currents gated by potassium channel complexes that contain Kvβ2 auxiliary sub-units which themselves possess an aldo-keto reductase enzymatic core domain. Therefore, we advance the novel hypothesis that ketones generated under ketogenic conditions directly interact with Kvß auxiliary sub-units to increase voltage-gated potassium currents which in turn reduces neuronal excitability. At 8 weeks of age, wild-type (WT) and $Kv\beta2^{-/-}$ (KO) mice were separated into two groups, one of which was fed standard chow, and the other which was fed a KD for a minimum of 6 weeks. Ex vivo coronal and modified horizontal slices were prepared from each group. In virto extracellular field potential recordings were made from either the dorsal hippocampus (HP) of coronal slices, or the ventral HP, entorhinal cortex (EC) and lateral amygdala (LA) simultaneously in modified horizontal slices in standard aCSF, with or without the addition of ketones. Epileptiform bursts were induced by removing Mg²⁺ and increasing K⁺ concentrations in the aCSF ($[Mg^{2+}]_0$ aCSF). The percentage of slices that exhibited bursting, latency to continuous bursting, and inter-burst interval, were measured for at least one hour after perfusion with the $[Mg^{2+}]_0$ aCSF.

In WT mice, acute application of ketones to slices from animals that were not maintained on a KD significantly reduced the induction of ictal events in the ventral HP. This effect is ventral HP specific and was not seen in the dorsal HP, KO mice or animals maintained on a KD. In the ventral HP, ketogenic conditions caused a significant reduction in the latency to ictal events regardless of genotype, however, in the dorsal HP, this reduction not observed in KO slices. In both the ventral HP and the LA, maintenance of WT animals on the KD causes a reduction in the inter-burst interval, which is not seen in slices made from KO animals. This effect is not seen in the EC or the dorsal HP. Additionally, treatment with the KD appears to alter the functional connectivity in the brain, regardless of genotype, as measured by the order in which the three areas (HP, EC, & LA) begin bursting after induction with ($[Mg^{2+}]_0$ aCSF. Although it is not clear exactly how K_vB2 is modulating the effects of the ketogenic diet, it is clear that these effects differ across brain regions, indicating that the ketogenic diet affects separate regions of the brain in different ways.

Disclosures: R. Parent: None. G.L. Fisher: None. D.N. Crisp: None. H. Burns: None. W.C. Stacey: None. G.G. Murphy: None.

Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 294.08/L3

Topic: B.11. Epilepsy

Support: Citizens United for Research in Epilepsy

Title: Genetic background influences in "fast" kindling-susceptible PPKS and "slow" kindling-resistant PPKR rats transfer to kindling of the olfactory bulb

Authors: S. DUNN, C. LEVENICK, *T. P. SUTULA Dept of Neurol., Univ. of Wisconsin, Madison, WI

Abstract: Kindling is a neurobiological phenomenon of progressive seizure-induced plasticity that permanently increases susceptibility to recurring seizures, development of epilepsy, and progressive adverse effects of repeated seizures. The molecular, cellular, structural, and functional alterations induced in neural circuits by kindling are influenced by genetic background, as demonstrated by the recent development of distinct strains of "fast" and "slow" kindling rats selected and bred for "fast" or "slow" rates of kindling in response to stimulation of the perforant path. The "fast" and "slow" strains are referred to, respectively, as perforant path kindling-susceptible (PPKS) or perforant path kindling-resistant (PPKR). Compared to outbred Sprague Dawley (SD) rats which developed secondary generalized (Class V) seizures after 16.8 ± 2.5 afterdischarges (ADs), the PPKS and PPKR strains demonstrated Class V seizures after 10.0 ± 1.2 and 27.3 ± 4.4 ADs, respectively, and also demonstrated distinct behavioral phenotypes (Langberg et al., Neurobiol Dis 85:122-129, 2015). It was of interest to determine if the genetic background of these strains selected by stimulation of the perforant path also influences seizures and neural circuit plasticity evoked by stimulation of other pathways. To address this question, rates of kindling development in seizure-naïve PPKS, PPKR, and outbred SD rats were compared in response to repeated stimulation of the olfactory bulb (OB) with 1 sec trains of 60 hz biphasic constant current pulses that evoked ADs. Outbred SD rats developed Class V seizures evoked by OB stimulation after 8.6 ± 1.4 ADs, while "fast" PPKS rats required 6.8 ± 0.7 ADs and "slow" PPKR rats required 12.1 ± 1.4 ADs (p < 0.01, ANOVA). The rate of OB kindling was faster in PPKS vs. PPKR rats (p < 0.01, post-hoc t test). The results demonstrate that susceptibility or resistance to kindling development in the PPKS and PPKR strains transfers to kindling of other pathways in addition to the PP, and imply that genetic background conferring susceptibility or resistance to seizure-induced plasticity in the PPKS and PPKR strains influences multiple pathways of the central nervous system.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 294.09/L4

Topic: B.11. Epilepsy

Title: Optimization of a human stem cell derived neuron/astrocyte co-culture system for seizure liability assessment using microelectrode arrays

Authors: *G. C. LUERMAN¹, C. FLEMING¹, D. HESS², T. PALM², A. EHLICH², H. BOHLEN²

¹Axiogenesis Inc, Plymouth Meeting, PA; ²Axiogenesis AG, Köln, Germany

Abstract: Historically, animal EEG studies have been the standard for preclinical assessment of drug induced seizures. Furthermore, in a typical ex vivo study, cortical neurons derived from rat forebrain must be extracted and cultured on microelectrode arrays (MEA) for roughly 4 weeks before mature functional network activity can be utilized for seizure assessment. With recent advances in human stem cell technologies, iPS-derived neurons can provide spontaneous electrical activity closely resembling that of murine ex vivo preparations. Here, using Axion Maestro MEAs, the electrophysiological function was compared amongst three different iPSderived neuronal subtypes in the presence and absence of astrocytes. As with ex vivo preparations, we found that astrocytes are indeed necessary to provide iPS neurons with the physiological co-culture environment required for mature network level activity. Once network level activity was achieved (typically 2-3 weeks), co-cultures were exposed to 12 different compounds having a variety of seizure-related, anti-seizure, or neurotoxic activity (e.g. GABA A, K+ channel, Na+ channel, muscarinic ACh, glycine, D2 receptor, & MAO block). Though "time to assay readiness" was different, sensitivity to these compounds were similar for the three neuronal populations. Importantly, these co-culture models all demonstrated good predictivity within the 12 drug set and allowed for significantly faster "assay ready" culture times than typical murine ex vivo preparations. In conclusion, human iPS neurons + astrocytes provide a number of advantages over current models for seizure liability and anti-epileptic drug screening efforts and should be further explored to develop a more comprehensive library to better understand their predictivity for drug induced seizures.

Disclosures: G.C. Luerman: A. Employment/Salary (full or part-time):; Axiogenesis AG. C. Fleming: A. Employment/Salary (full or part-time):; Axiogenesis AG. D. Hess: A. Employment/Salary (full or part-time):; Axiogenesis AG. T. Palm: A. Employment/Salary (full

or part-time):; Axiogenesis AG. **A. Ehlich:** A. Employment/Salary (full or part-time):; Axiogenesis AG. **H. Bohlen:** A. Employment/Salary (full or part-time):; Axiogenesis AG. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Axiogenesis AG.

Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 294.10/L5

Topic: B.11. Epilepsy

Support: Startup funding, UIUC

Title: Estrous cycle stage-dependent and sex-specific alterations of GnRH neuron firing activity in a mouse model of temporal lobe epilepsy

Authors: ***J.** LI¹, J. ROBARE², M. A. GHANE², M. E. NELSON^{1,2,3}, C. A. CHRISTIAN^{1,2,3} ¹Neurosci. Program, ²Mol. and Integrative Physiol., ³Beckman Inst., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Both women and men with temporal lobe epilepsy (TLE) are at greater risk for reproductive endocrine disorders, but the neural mechanisms underlying these co-morbidities have not been elucidated. Hypothalamic gonadotropin-releasing hormone (GnRH) neurons, the neural output for controlling reproduction, likely play key roles in driving these co-morbidities. We recently demonstrated robust estrous cycle disruption in female mice in the intrahippocampal kainic acid (KA) model of TLE. Here, we made targeted extracellular recordings of GnRH neuron firing in acute coronal brain slices at 2 mo after KA/saline injection. In females, GnRH neurons showed distinct responses to KA-induced epilepsy depending on estrous cycle stage on the day of recording. On diestrus, GnRH neuron mean firing rate was increased in the KAtreated female mice that developed prolonged (≥ 7 d) estrous cycles (saline, n=16 cells, 0.30+0.04 Hz, KA/prolonged n=20 cells, 1.03+0.25 Hz, p<0.01), but KA-treated female mice that maintained regular 4-6 d cycles did not show this change (KA/normal, n=20 cells, 0.39+0.08 Hz). On estrus, however, GnRH neurons from KA-treated female mice displayed lower firing rate than control mice, and this change was not correlated with estrous cycle period (saline, n=18 cells, 0.64+0.12 Hz; KA/prolonged, n=13 cells, 0.13+0.04 Hz, p<0.01 vs. saline; KA/normal, n=6 cells, 0.21+0.14 Hz, p<0.05 vs. saline). Cells located in the medial septum and preoptic area were most strongly affected. GnRH neurons also showed a sex-specific response to epilepsy, as cells from KA-treated males did not show a change in mean firing rate (saline, n=14 cells, 0.47+0.13 Hz, KA, n=22 cells, 0.61+0.13 Hz). To examine if GnRH neuron firing pattern was also affected by KA treatment, we performed spike pattern analysis for burst detection in the recorded cells from males and diestrous females. Interspike interval joint scatter plots and kmeans clustering methods were used for burst identification and analysis. The burst duration and the number of spikes per burst were both increased (p<0.001) in all KA-treated mice, regardless of estrous cycle period or sex. Among KA-treated females, however, the intraburst firing rate increased significantly in those with prolonged estrous cycles but decreased in those with normal estrous cycles (p<0.01). In KA-treated males, by contrast, the intraburst firing rate was not changed. Together, these findings are the first direct demonstrations of aberrant GnRH neuron function in an animal model of epilepsy, and indicate dynamic and sex-specific effects of epilepsy on the neural control of reproduction in different reproductive physiological states.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 294.11/L6

Topic: B.11. Epilepsy

Title: Non-periodic electrical stimulation to the amygdala delays the kindling-induced epileptogenesis in rats

Authors: *D. MARTÍNEZ-VARGAS, F. SANTOS-VALENCIA, S. ALMAZÁN-ALVARADO, A. RUBIO-LUVIANO, V. MAGDALENO-MADRIGAL, A. VALDÉS-CRUZ Inst. Nacional De Psiquiatría, Ciudad DE Mexico, Mexico

Abstract: Deep brain stimulation has been used as a treatment for epilepsy, however, the mechanisms targets and optimal stimulation parameters are undeterminated. A novel protocol of stimulation is the non-periodic electrical stimulation pattern (NPS) which consists of changes in the temporal pattern of pulse firing, instead of periodic interpulse intervals an unstructured nonperiodic electrical stimulation is applied, with randomized interpulse interval. The delivering NPS at four pulses per second to the amygdala (AM) reduce the severity and number of seizures induced by pentylenetetrazole and pilocarpine in rats, nevertheless, the effects on epileptogenesis are unknown. The aim of this work was to analyze the effects of NPS applied to the AM on the acquisition of kindling-induced epileptogenesis in adult rats. Twenty-one adult male rats were implanted in the right basolateral AM. The implanted electrodes were used for daily kindling stimulation (AK) and NPS. Rats were divided in 3 groups: control, preemptive NPS and NPS/AK. Control animals were stimulated once a day with AK stimulus (1s, 60 Hz, 1ms/pulse). Animals in preemptive NPS group were stimulated for 10 days with NPS (20 min, four pulses per second, 0.1ms/pulse with non-periodic pattern) before the start of the AK. Rats in NPS/AK group received NPS during the development of kindling, the NPS was applied five minutes after of AK stimulus. The EEG of the AM was recorded. Seizure severity was classified according to

the Racine scale, in addition to seizure stage, the afterdischarge duration (AD) and the generalized seizure duration (GSD) were analyzed. All animals were stimulated until exhibited three consecutive stage 5 seizures. Significant differences were found between NPS during AK animals versus control group: The number of stimulations required to reach the first stage V seizures, (p < 0.02) and the number of days on stage I (p < 0.004) and II (p < 0.02) seizures increased in NPS/AK animals compared to the control ones. The daily AD (p < 0.01), the cumulative AD of the 3 stages V seizures (p < 0.02) and the GSD (p < 0.02) decreased in the NPS/AK animals compared to the control ones. The preemptive NPS did not show differences in the acquisition of AK. Our data suggest an anti epileptogenic effect by NPS when the stimulation was applied during the development of AK. On the other hand, the NPS may be an alternative treatment against the progress and the pathophysiology of epilepsy, however, a better understanding of the role of NPS in the modulation of neural networks will help us to find the physiological mechanisms to achieve a better therapeutic effect with NPS.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 294.12/L7

Topic: B.11. Epilepsy

Support: USAMRICD Commander's Innovative Research and Discovery Award

Title: Neuropathological assessment of hippocampal damage in C57BL/6J mice following convulsive motor seizures induced by repeated low-dose kainate administration

Authors: *D. L. NGUYEN, P. H. BESKE, M. R. EISEN, M. J. STENSLIK, D. M. KNIFFIN, C. L. HONNOLD, P. M. MCNUTT USAMRICD, Aberdeen Proving Ground, MD

Abstract: *Status epilepticus* (SE) is a medical emergency resulting from continuous epileptic seizures that persist for longer than 5 minutes. If left untreated, SE can result in devastating damage to the brain. While chemoconvulsants such as pilocarpine and kainate (KA) are commonly used to induce SE in rats, efforts to transition these models to inbred mouse strains have been met with numerous challenges (high mortality, inconsistent SE behavior, resistance to pathology). Recent studies have suggested that repeated low-dose (RLD) administration of KA to adult mice may provide a reliable and survivable approach for SE induction. To evaluate this model, KA was repeatedly administered to male C57BL/6J mice (7-8 wk; 9-10 wk; 11-12 wk) until onset of Racine scale 4-5 convulsive motor seizures (CMS). The effects of seizure

induction on neuronal damage and GFAP immunoreactivity were then evaluated in medial temporal lobe brain regions and correlated to age. While CMS onset consistent with SE induction was observed in all age groups, survival and neuropathology were found to be age-dependent, as survival rates and resistance to neuronal damage increased with age. Most survivors of severe KA-induced CMS exhibited frank neuronal loss of *cornus ammonis* (CA) area 1 and 3 neurons and increased GFAP staining in the hippocampal formation. The spatial distribution of hippocampal damage was assessed across 3 bregmas (-1.4, -2.0, and -2.6), with neuropathology found to be largely consistent along the ventral-dorsal axis. Interestingly, KA-induced damage was restricted to the hippocampus, with minimal evidence of neurotoxicity observed in other brain regions. Collectively, while RLD administration of KA to C57BL/6J mice represents a reliable CMS induction method, further optimization of this model is necessary to reduce variability in survival rates and neuropathological outcomes.

Disclosures: D.L. Nguyen: None. P.H. Beske: None. M.R. Eisen: None. M.J. Stenslik: None. D.M. Kniffin: None. C.L. Honnold: None. P.M. McNutt: None.

Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

Location: Halls A-C

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Program#/Poster#: 294.13/L8

Topic: B.11. Epilepsy

Support: 1R03 NS098015-01

NSF DGE-1069104

MnDRIVE

Title: Dorsal versus ventral hippocampus: Does target location matter in the kainic acid mouse model of temporal lobe epilepsy?

Authors: *Z. ZEIDLER¹, C. LEINTZ², M. BRANDT-FONTAINE², E. I. KROOK-MAGNUSON² ¹Dept. of Neurosci., ²Univ. of Minnesota, Minneapolis, MN

Abstract: Temporal lobe epilepsy is a highly prevalent form of adult epilepsy and commonly modeled in mice using an intrahippocampal injection of kainic acid into the dorsal hippocampus. However, the hippocampus in mice is heterogeneous; targeting different aspects of the hippocampus may produce distinct phenotypes in an intrahippocampal kainic acid model of temporal lobe epilepsy. The ventral hippocampus is known to have differences in connectivity and function compared to the dorsal hippocampus, including greater connectivity with the amygdala. Furthermore, acute seizures induced via manipulation of the ventral hippocampus are

more likely than dorsal hippocampal seizures to generalize into overt behavioral seizures. Therefore, mice were subjected to a unilateral injection of kainic acid in either the dorsal (n=17) or ventral (n=17) hippocampus to induce an initial epileptogenic insult in order to monitor chronic, spontaneous overt behavioral seizures as well as behavioral and anatomical phenotypes. These mice were compared to saline controls (n=19). The dorsally and ventrally injected kainic acid groups exhibited similar deficits on a spatial memory task compared to saline injected mice (p<0.01). Ventrally injected kainic acid mice additionally displayed a hypohedonic phenotype (p<0.05). Surprisingly, both dorsally and ventrally injected kainic acid groups had similar levels of spontaneous overt behavioral seizures (3 or greater on the Racine scale). Hippocampal pathology will also be compared - examining the extent and focality of several anatomical phenotypes of temporal lobe epilepsy including cell loss, granule cell dispersion, and mossy fiber sprouting. Correlations between seizures, behavior, and pathology will also be tested. These results inform how different aspects of the hippocampus may contribute to distinct or common pathologies and comorbidities observed in temporal lobe epilepsy.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

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Program#/Poster#: 294.14/L9

Topic: B.11. Epilepsy

Support: IMSS Grant R-2012-785-062

Title: Increased expression of proinflammatory cytokines IL-1 α , IL-6 and TNF- α by a kainic acid-induced status epilepticus model in the neonatal rat brain

Authors: *M. J. T. HERRERA¹, C. R. DE LA CRUZ-NEGRETE¹, S. OROZCO-SUÁREZ¹, M. A. DELI², A. HARAZIN², F. WALTER³, L. ROCHA⁴, P. CAMPOS-BEDOLLA¹ ¹Unidad de Investigación Médica en Enfermedades Neurológicas, Inst. Mexicano Del Seguro Social, Ciudad de México, Mexico; ²Lab. of Mol. Biol., Hungarian Acad. of Sci., Szeged, Hungary; ³Dept. of Pathology and Lab. Med., Univ. of Wisconsin, Madison, WI; ⁴Dept. de Farmacología, CINVESTAV, Ciudad de México, Mexico

Abstract: Introduction. Status epilepticus (SE) is an important neurological condition that requires immediate clinical attention. Neonatal seizures constitute one of the most common neurologic disorders in the newborn with the highest incidence in the first year of life. Neonatal status epilepticus has been extensively studied and it's been reported it can lead to neuronal death by apoptosis, morphological alterations in neural networks, among others, mediated by

excitatory amino acids and inflammatory cytokines. Neuroinflammation promotes the activation of microglia, astrocytes and brain endothelial cells, causing the release of glutamate, interleukin- 1α (IL- 1α), interleukin-6 (IL-6) and Tumoral Necrosis Factor- α (TNF- α), which provoke neuronal damage. Until now, the expression of proinflammatory cytokines IL-1 α , IL-6 and TNFα by kainic acid-induced status epilepticus in the neonatal rat brain (P2) is not known. For this reason, we have proposed to evaluate it. Material and methods. It was standardized a model of SE induced by KA in newborn rats (P2). The behavioral assessment to determine SE was performed using the modified Racine scale. Control and kainic acid treated rats (1.5 mg/kg, KA) were sacrificed 30 minutes and 24 hours after the treatment. The brains were obtained to determine the histological damage and expression of IL-1 α , IL-6 and TNF- α in hippocampus and cortex by immunological techniques. We used monoclonal antibodies against the NeuN protein to evaluate the functional status of the neurons. The immunological staining was performed using previously published methods. Results. The SE model was established in newborn rats (P2) at a dose of 1.5 mg/kg AK, with 80% survival. The SE model caused morphological damage in the hippocampus and cortex regions, quantified by densitometry. We observed a decrease in the neuronal integration of AK-treated rats. On the other hand, there was a significant increase in expression of IL-1 α , IL-6 and TNF- α proinflammatory cytokines in the neonatal rat brain by kainic acid-induced SE. A greater expression of these cytokines was observed at 30 min compared to 24 h later. Conclusions. We obtained an appropriate SE model with a high survival rate and an extraordinary reproducibility. We also defined that KA produces morphological damage in the hippocampus and cortex regions, both in rats sacrificed 30 minutes later and 24 hours after the stimulus. The immunohistochemical expression of proinflammatory cytokines (IL-1 α , IL-6 and TNF- α) is higher in the group of animals sacrificed at 30 minutes compared to the 24-hour group.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

Location: Halls A-C

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Program#/Poster#: 294.15/L10

Topic: B.11. Epilepsy

Support: CNPq grant 307812/2014-6

CAPES

FAPERGS

Title: Cyclooxygenase-2 inhibitors differentially attenuate pentylenetetrazol-induced seizures and increase of pro- and anti-inflammatory cytokine levels in the cerebral cortex and hippocampus of mice

Authors: *C. F. MELLO¹, F. R. TEMP², T. DUARTE², J. R. MARAFIGA², M. M. PILLAT² ¹Fed Univ. S. Maria (UFSM), Santa Maria, Brazil; ²Univ. Federal de Santa Maria, Santa Maria, Brazil

Abstract: Seizures increase prostaglandin and cytokine levels in the brain. However, it remains to be determined whether cyclooxygenase-2 (COX-2) derived metabolites play a role in seizureinduced cytokine increase in the brain and whether anticonvulsant activity is shared by all COX-2 inhibitors. In this study we investigated whether three different COX-2 inhibitors alter pentylenetetrazol (PTZ)-induced seizures and increase of interleukin-1ß (IL-1ß), interleukin-6 (IL-6), interferon- γ (INF- γ), tumor necrosis factor- α (TNF- α) and interleukin-10 (IL-10) levels in the hippocampus and cerebral cortex of mice. Adult male albino Swiss mice received nimesulide, celecoxib or etoricoxib (0.2, 2 or 20 mg/kg in 0.1% carboxymethylcellulose (CMC) in 5% Tween 80, p.o.). Sixty minutes thereafter the animals were injected with PTZ (50 mg/kg, i.p.) and the latency to myoclonic jerks and to generalized tonic-clonic seizures were recorded. Twenty minutes after PTZ injection animals were killed and cytokine levels were measured by ELISA. Behavioral data were analyzed by Kruskal-Wallis followed by nonparametric Dunn's test. Cytokine data were analyzed by three-way ANOVA, considering the brain structure as within-subject-factor followed by Bonferroni test. PTZ increased cytokine levels in the cerebral cortex and hippocampus. While celecoxib and nimesulide attenuated PTZ -induced increase of proinflammatory cytokines in the cerebral cortex, etoricoxib did not. Nimesulide was the only COX-2 inhibitor that attenuated PTZ-induced seizures. This effect coincided with an increase of IL-10 levels in the cerebral cortex and hippocampus, constituting circumstantial evidence that IL-10 increase may be involved in the anticonvulsant effect of nimesulide.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

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Program#/Poster#: 294.16/M1

Topic: B.11. Epilepsy

Title: Mild TBI generated by a supersonic helium wave provides mechanistic insight into induced pathological states such as absence seizures

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Abstract: <u>Purpose.</u> Mild traumatic brain injury (mTBI) is a risk factor for neurodegenerative diseases, Post-Traumatic Stress Disorder (PTSD), major depression and epileptic seizures. It is generated by explosions, traffic accidents, falls or impact sport events. The resulting closed-head injuries can be identified with Diffusion Tensor Imaging (DTI) by axonal reduction of Fractional Anisotropy (FA). The clinical severity is correlated with the concussion duration. mTBI produces Chronic Traumatic Encephalopathy (CTE), biomarked by hyperphosphorylated tau protein (pTau). mTBI also serves as a tool in animal models to investigate the cell biological mechanisms of the induced pathological states.

Experimental. In most experiments, we used male C57BL/6N mice (7-8 weeks old) anesthetized with isoflurane. Their lungs and ears were protected with nylon ballistic coats and plastic plugs, respectively. The anesthetized mice were exposed for 0.2 msec to a supersonic helium wave produced by an aluminum 6 feet long and 2 inches wide shock tube. In some experiments, the animals were repetitively (twice or four times on subsequent days) exposed at 70 or 36 psi. Controls were treated similarly except for the missing helium wave. Subsequently, all mice were cardioperfused 2, 5, or 12 days after the last blast. The isolated brains were subjected to DTI and pTau histology. For absence seizure detection, three stainless steel screw electrodes were inserted into the skull over the hippocampus and the cerebellum. Two wire electrodes were additionally inserted into the neck musculature to record electromyography (EMG) activity. After recovery for one week, EEG and EMG signals were amplified in a range of 0.3 to 70 Hz. Absence seizures were identified by i.p. injection of pentylenetetrazole (PTZ). Thereafter, the mice were subjected to forced swim and sucrose preference tests to check on depression-like behavior.

<u>Results</u>. No mortality was observed. The mice required 5-7 minutes to recover from one blast. After repetitive blast (2 x 70 psi), the recovery process needed significantly less time. Four hours after one exposure to 70 psi, the blood brain barrier was found to be open. By PTZ injection, a significant discharge at 2-4 Hz in mTBI-induced mice indicated absence seizures. No evidence for depression-like behavior was observed, possibly because of the antagonism between epileptic and depressive actions. The role of the mTBI-induced increase of pTau will be discussed.

<u>Conclusion</u>. The hypothetical inverse relationship between epileptic and depressive processes will be further investigated by antiepileptic intervention.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

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NIH CTSC UL1 RR 024996

Daedalus Fund for Innovation

Title: A novel fluorescamine-based imaging approach to investigate the diffusion of 4aminopyridine in rat neocortex

Authors: *M. ZHAO^{1,2}, R. E. RADWANSKI^{1,3}, E. BAIRD-DANIEL¹, H. MA^{1,2}, N. NISHIMURA³, C. B. SCHAFFER³, T. H. SCHWARTZ^{1,2,4} ¹Dept Neurolog Surg, ²Brain and Mind Res. Inst., Weill Cornell Med. of Cornell Univ., New York, NY; ³Dept. of Biomed. Engin., Cornell Univ., Ithaca, NY; ⁴New York Presbyterian Hosp., 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. Animal models of epilepsy are very important not only for understanding the fundamental neuronal mechanism of epilepsy but also for testing the efficacy of new antiepileptic drugs or other therapeutic interventions. 4-Aminopyridine (4-AP) is a potent convulsant when applied to the neocortex. Microinjection of 4-AP in vivo can induce focal seizures, but the volume of tissue in which the 4-AP is released over time is poorly understand. Here, we combined the 4-AP with fluorescamine, a fluorescent agent that reacts with primary amines to form a highly fluorescent compound, to characterize the radial diffusion pattern of 4-AP. 0.5 µl fresh fluorescamine/4-AP solution was injected into rat neocortex (25mM 4-AP and 270mM fluorescamine). We excited fluorescamine/4AP with a 395 nm LED light passed through a 395 ± 2 nm bandpass filter. The excitation light was diverted onto the neocortex via an extended reflectance dichroic mirror. The emitted fluorescence was passed through a band-pass 474±13.5 nm emission filter and collected by an Adimec 1000M/D camera. Serial imaging of the fluorescence of the mixture of fluorescamine and 4-AP can be used to trace the diffusion of 4-AP over time. The mixture of fluorescamine/4-AP showed strong fluorescence signals compared to

the background autofluorescence of cortex. On average, injection of 4-AP leads to an early rapid increase in the area of fluorescence with a second slower increase from diffusion. The delay of 4-AP starting diffusion is $210\pm105.9 \text{ sec}$ (n=5 rats). The averaged diffusion distances at 1800, 2400, 3000, 3600, 5400, and 7200 sec were $372.77 \pm 54.30 \mu m$, $466.48 \pm 54.62 \mu m$, $526.67 \pm 48.33 \mu m$, $589.60 \pm 42.86 \mu m$, $497.94 \pm 78.35 \mu m$, and $428.17 \pm 54.15 \mu m$, respectively. The average maximum distance of diffusion of $589.60 \pm 85.72 \mu m$ was reached at 3600 seconds. Taken together, these results demonstrate that the fluorescamine-based bioassay could be used as a novel method to investigate the distribution of 4-aminopyridine or other compounds with primary amines in the brain.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

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NIH/NCATS TL1 TR000151

Beth and Nathan Tross Epilepsy Research Fund

Title: The effect of time of day on respiratory outcomes and survival following maximal electroshock seizures in mice

Authors: *B. S. PURNELL^{1,2}, M. A. HAJEK³, G. F. BUCHANAN^{1,3,2} ¹Neurol., ²Interdisciplinary Grad. Program in Neurosci., Univ. of Iowa, Iowa City, IA; ³Neurol., Yale Sch. of Med., New Haven, CT

Abstract: One in twenty-six Americans are diagnosed with epilepsy at some point during their lifetime. While the majority of these patients achieve seizure control with medications, approximately one-third of epilepsy patients are refractory to therapy with antiepileptic drugs. Patients with refractory epilepsy are at high risk for sudden unexpected death in epilepsy (SUDEP). The preponderance of evidence suggests that SUDEP occurs due to seizure-induced

respiratory and/or cardiac dysfunction. In addition, the majority of SUDEP cases occur during the night. Since humans tend to sleep during the night, this has been attributed to SUDEP occurring during sleep. Sleep state certainly affects seizures, and there is evidence that sleep state influences SUDEP; however, the observation that SUDEP tends to occur during the night might also implicate circadian influences on SUDEP that are independent of the influences of sleep state. We investigated this possibility here. Adult male wild-type mice were implanted with EEG, EMG, and EKG recording electrodes. After recovery from surgery and acclimation to the recording chamber, seizures were induced via maximal electroshock (50 mA, 200 msec, 60 Hz, biphasic sine wave) during wakefulness, non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep during the daytime and the nighttime (n = 12 per condition). EEG, EMG, EKG, and breathing plethysmography data were collected during each seizure induction. Seizure severity and duration, and the effects of seizures on breathing, cardiac function, EEG power, and survival were assessed. Seizures induced during the daytime, the inactive phase of rodents, were longer in duration, more severe, and more likely to be fatal than seizures induced during the nighttime. Seizures induced during the daytime were also associated with more profound respiratory dysregulation and post-ictal EEG suppression compared to those induced during the nighttime. There were no statistically significant differences in the effects of seizures induced during different vigilance states and times of day on cardiac function. Seizures induced during REM sleep during either the daytime or the nighttime were fatal. Taken together these data suggest that time of day influences the physiological consequences of seizures that may lead to seizure-induced death. Additional work is needed to determine the mechanisms by which time of day affects seizure outcomes. Understanding these mechanisms may inform strategies to prevent SUDEP.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

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Topic: B.11. Epilepsy

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NIH RO1 NS070261

Title: Ketogenic diet-induced extension of longevity in epileptic Kcna1-null mice is influenced by gender and age at treatment onset

Authors: K.-C. CHUN^{1,2}, S.-C. MA², *H.-J. CHOI³, J. M. RHO⁴, D. KIM² ¹Dept of Ob&Gyn, Inje university, ilsan paik hospital, Goyang-si, Korea, Republic of; ²Barrow Neurolog. Institute, St. Joseph's Hosp. & Med. Ctr., Phoenix, AZ; ³Harvard Med. Sch. (SERI/MEEI), Somerville, MA; ⁴Alberta Children's Hospital, Univ. of Calgary, Calgary, AB, Canada

Abstract: Sudden unexpected death in epilepsy (SUDEP) is a leading cause of premature mortality in patients with medically intractable epilepsy. While the pathophysiology of SUDEP remains unclear, multiple risk factors have been identified, including generalized tonic-clonic seizure (GTC) frequency, gender, early age at seizure onset and polytherapy with anti-seizure drugs. The spontaneously epileptic Kcnal-null (KO) mouse, lacking the delayed rectifier potassium channel protein Kv1.1, has been validated as a model of SUDEP, and a recent study has shown that a high-fat, anti-seizure ketogenic diet (KD) significantly extends longevity relative to KO mice fed normal rodent chow (PMID: 27346881). Here, we investigated whether the KD-induced extension of lifespan in KO animals is dependent upon gender and/or age of onset of KD administration. As mice aged, we found that KO animals had a steadily increasing daily seizure frequency (6.5 \pm 4.7 at postnatal days [PD] 33-38 vs. 10 \pm 2.2 at PD40-45, p < 0.01) and early demise by PD 46.9 \pm 0.8 (n=112). In standard diet (SD)-fed KO mice, there was no difference in lifespan between male and female animals. By contrast, following KD initiation at PD30, KD-fed mice exhibited significantly extended longevity (PD 69.8 \pm 1.7; n=36; p <0.01), and concomitantly had a decreased frequency of daily seizures (4.1 ± 0.9 ; n=36; p < 0.01). Seizure control on the KD was similar between male and female mice, but KD-fed female KO mice survived longer than their male counterparts. The average ages of mortality were PD 73.1 ± 2.5 (n=21) and PD 65.1 ± 1.4 (n=15) for females and males, respectively (p < 0.05). Comparing KO mice with KD onset at two different ages, we found that diet initiation at PD25 led to longer lifespans compared to those who were placed on the KD starting at PD35. Collectively, these data further support the notion that the KD can retard disease progression and sudden death in the Kcna1-null mouse model of early developmental epilepsy, but that these effects are dependent on sex and age at initiation of therapy. The profound alterations in cellular metabolism and mitochondrial bioenergetics elicited by the high-fat diet are likely to be influenced by other factors as well, and future studies are necessary to clarify the specific mechanisms underlying the disease-modifying effects of the KD.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

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Research Participation Program for the U.S. Army Medical Research Institute of Chemical Defense administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. DoE and U.S. Army MRMC

Title: Validating a nerve agent-induced seizure model by evaluating the anticonvulsant and neuroprotective effects of scopolamine, memantine, phenobarbital, and ganaxolone

Authors: C. JACKSON, C. ARDINGER, D. BALLOUGH, H. CRAIG, J. H. MCDONOUGH, *H. S. MCCARREN US Army Med. Res. Inst. of Chem. Def, Aberdeen Proving Ground, MD

Abstract: Nerve agents are organophosphorus (OP) compounds which have been used by terrorist organizations and militant governments in civilian attacks. Nerve agents irreversibly block acetylcholinesterase activity, resulting in an accumulation of excess acetylcholine at neural synapses. This leads to a state of prolonged seizures known as status epilepticus, which is associated with significant morbidity and mortality. Benzodiazepines, the current anticonvulsant standard of care, become less effective as the time to treatment increases. In a mass civilian nerve agent exposure, concurrent trauma and limited resources would likely cause a delay in first response time. We have developed a rat model to test the anticonvulsant and neuroprotective efficacy of novel therapeutics at delayed time points. Adult, male rats with cortical EEG electrodes are exposed to the nerve agent soman and administered test treatments along with midazolam 20 minutes after seizure onset. FluoroJade B (FJB) staining is used to assess the neuroprotective effects of the treatments. We have validated our model using four drugs with established efficacy, or lack thereof, in other seizure models. Scopolamine, an anticholinergic, stopped seizures within one hour of treatment, in 5/9 rats treated with 5.6 mg/kg, 7/11 rats treated with 10 mg/kg, and 6/10 rats treated with 30 mg/kg, while saline + midazolam was ineffective (n = 11). Scopolamine also reduced the number of FJB+ neurons compared to controls in the five brain regions considered (amygdala, thalamus, piriform cortex, hippocampus, and parietal cortex). Memantine, an NMDA antagonist, had no anticonvulsant or neuroprotective effect and led to significant mortality (4/10 survivors) when tested at 56 mg/kg. Phenobarbital, a barbiturate, stopped seizures in rats 51.98 minutes (SEM = 14.23) after treatment in 8/8 rats treated with 100 mg/kg. In 4/10 rats treated with 56 mg/kg, seizures terminated, but returned an average of 19.63 minutes (SEM = 3.06) later and lasted throughout the day. Phenobarbital dosedependently reduced the number of dying cells in the amygdala only. Ganaxolone, a neuroactive steroid, also demonstrated dose-dependent anticonvulsant efficacy with seizures terminating in 2/13 rats at 3 mg/kg, 3/9 rats at 6 mg/kg, and 5/9 rats at 10 mg/kg. Our results align with previous findings, thus validating our model as a way to successfully test the anticonvulsant and neuroprotective effects of novel drugs that may stop nerve agent-induced seizures.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

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Title: Novel insights into the mechanisms underpinning modulation of cortical seizure propagation by sensory activation

Authors: *S. S. HARRIS¹, L. BOORMAN¹, A. KENNERLEY¹, P. OVERTON¹, Y. ZHENG², T. H. SCHWARTZ³, J. BERWICK¹

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Abstract: Understanding how seizures propagate across the brain is important to elucidating seizure dynamics and improving treatment strategies in pharmacoresistant focal epilepsy. While it has long been recognised that sensory stimuli can facilitate or inhibit epileptic seizures, very little is known about the cortical mechanisms that underpin such behaviour. Since it is thought that seizure propagation is restrained by feed-forward inhibition, and sensory adaptation during repetitive stimulation alters the balance between cortical excitation and inhibition, we sought to perturb focal seizure propagation dynamics through activation of sensory regions adjacent to the seizure focus. To this end, we exploited our established multi-modal methodology to examine neural and hemodynamic responses in cortex during physiological stimulation and recurrent focal acute seizures, and our mathematical model that allows decomposition of evoked LFPs into excitatory and inhibitory components. Two multi-channel depth electrodes were implanted into visual and adjacent vibrissal cortex of the urethane-anesthetized rat, the latter loaded with 4aminopyridine (4-AP, 15mM) for induction of recurrent focal seizures. 4-AP was infused into layer 6 visual cortex after a pre-infusion baseline recording. High-resolution spatial measures of total haemoglobin concentration were obtained using optical imaging spectroscopy. Whisker stimulation (16s, 5Hz, 1.2mA) was delivered at specific time-points following 4AP infusion and responses compared to controls to determine the effect of nearby epileptic activity on sensory processing and assess the manner in which ictal propagation is modulated by nearby sensory stimulation. Laterolaminar properties of epileptiform activity and resultant neurovascular coupling processes during the horizontal spread of epileptic activity were concurrently assessed using a suite of analysis techniques. Somatosensory activation in the ictal surround during ongoing recurrent seizures was associated with: 1) an increase in synchronisation between electrodes in the seizure onset zone and neighbouring S1 cortex within the gamma-frequency range of the LFP; and 2) a profound decrease in multi-unit activity in deep cortical layers.

Notably, the reverse of the latter was observed in an isolated case where sensory responses and background cortical activity were consistent with a lighter anesthetic state. Our findings provide new insights into the complex effects of sensory stimulation on the propagation of adjacent acute focal seizures that may have important implications for our understanding of seizure dynamics.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

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Tab Williams Family Fund

Title: Optogenetically induced population discharge threshold (oPDT) as a sensitive measure of excitability

Authors: *D. C. KLORIG¹, G. ALBERTO², D. W. GODWIN³

¹Neurosci. Program, Wake Forest Hlth. Sci., Winston Salem, NC; ³Neurobiology/Anatomy, ²Wake Forest Sch. of Med., Winston Salem, NC

Abstract: Current pre-clinical methods for characterizing the efficacy of anti-epileptic drugs and/or surgical interventions rely on the ability to prevent, delay, or lessen the severity of artificially induced seizures, either as a result of electric shock or the administration of proconvulsant drugs. These measures exhibit a large degree of baseline variability, requiring large numbers of replicates to achieve the statistical power required to demonstrate efficacy. We have developed a new method to detect small shifts in network excitability (e.g. seizure susceptibility) using optogenetically induced population discharge thresholds (oPDT), in mice, as a surrogate for seizure. By combining optogenetic stimulation of the hippocampus with chronic multi-site recording in peri-hippocampal structures of awake behaving animals, we can induce and detect abnormal network wide population discharges that mirror spontaneous interictal spikes in terms of waveform shape and latency. By varying the intensity of light we can compare the magnitude of the optogenetically mediated current to the probability of population discharge. This probability curve is well fit by a Boltzmann curve, allowing calculation of a V50. Here we demonstrate that the V50 is a sensitive and reliable metric of network excitability. Manipulations known to increase excitability such as chronic EtOH withdrawal, or sub-convulsive doses (20 mg/kg) of pentylenetetrazol (PTZ) produce a leftward shift in the curve compared to baseline.
Anti-epileptic drugs, in combination with pro-convulsive manipulations, produce a rightward shift to baseline. oPDT baselines are remarkably stable over time, allowing for within-subject experimental design with multiple pharmacological manipulations, reducing the total number of animals needed. Furthermore we have fully characterized the close relationship between the oPDT and optogenetically induced seizure thresholds, thus demonstrating that the oPDT is a useful surrogate. The oPDT is the first example of a sensitive measure of subconvulsive network excitability, with broad applicability to a number of areas of investigation.

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Poster

294. Epilepsy: Animal Models - Genetic Strategies, Optogenetics, and Mechanisms

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Support: NINDS RO1NS039587

Title: Forebrain neuron-specific deletion of Sod2 results in epilepsy, mitochondrial oxidative stress, and altered bioenergetics and gene expression

Authors: R. FULTON¹, J. N. PEARSON², S. AIVAZIDIS¹, T. SHIMIZU³, *M. N. PATEL⁴ ¹Pharmaceut. Sci., Univ. of Colorado Anschutz Med. Campus, Aurora, CO; ²Univ. of Colorado, Aurora, CO; ³Advanced Aging Med., Chiba Univ., Chiba, Japan; ⁴Dept Pharmaceut. Sci., Univ. Colorado, Anschutz Med. Ctr., Aurora, CO

Abstract: Superoxide dismutase-2 (Sod2) is a critical mitochondrial antioxidant enzyme that catalyzes the detoxification of superoxide radicals in the mitochondrial matrix. Global deletion of Sod2 causes neonatal lethality. The goal of this study was to determine the consequences of mitochondrial oxidative stress in forebrain neurons during adulthood. A brain specific knock out (KO) mouse was generated by crossing an Sod2 p lox mouse strain with a neuronal basic helix-loop-helix (NEX) cre-recombinase mouse. The homozygous lox/lox offspring (Sod2-/-) were compared to their wild type littermates (Sod2+/+) for redox and metabolic parameters, behavioral deficits and cell death. Compared to Sod2+/+ mice, Sod2-/- mice displayed lower body weights at 1 month (p=0.03) and 2 months (p=0.006) of age with average age of death of approximately 2 months. Sod2-/- mice exhibited progressive age-dependent ataxia, kyphosis, and eventual hind limb paralysis. A rotarod test at 6 weeks revealed a deficit in balance, grip strength, and/or motor coordination (p=0.001). Mitochondrial aconitase, but not fumarase activity, a measure of oxidative stress, was decreased in 1 month (p<0.0007) and 2 month (p<0.0001) old Sod2-/- mice compared to Sod2+/+ mice. Spontaneous seizure activity was detected in 6 week old Sod2-/- mice. Cell death, identified by Fluoro Jade B stain, was

progressive and specific to the prefrontal cortex ($p \le 0.01$) and somatosensory/motor cortex ($p \le 0.05$) in Sod2-/- mice. Primary cortical cultures isolated from 5 day-old Sod2-/- and Sod2+/+ littermates revealed increased glycolytic rates in Sod2-/- cultures. Correspondingly, blood glucose levels were decreased in 6 week old Sod2-/- mice (p=0.0101). Finally, using a PCR array, we identified nine genes with increased expression in the prefrontal cortex of Sod2-/- mice. These results suggest that mitochondrial oxidative stress in forebrain neurons results in seizures and behavioral deficits, selective neuronal death and increased glycolysis.

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Poster

295. Biology of Microglia

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 295.01/M9

Topic: B.12. Glial Mechanisms

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The Hartwell Foundation

Title: Are microglia replaceable?

Authors: *A. J. FILIANO, J. C. CRONK, D. GOLDMAN, J. KNOPP, A. LOUVEAU, I. MARIN, R. MARSH, E. JI, I. SMIRNOV, C. C. OVERALL, J. KIPNIS Neurosci., Univ. of Virginia, Charlottesville, VA

Abstract: Ontogeny and function have been the major foci of microglia research in recent years. However, numerous questions remain unanswered regarding their role in pathology and whether monocyte-derived macrophages can assume microglia function in the adult brain. Whether or not monocyte-derived macrophages can engraft the brain and whether engrafted cells become microglia or instead retain unique functions remain major points of contention in the field. Here, we demonstrate that adult microglia, despite prevailing dogma, can be replaced spatially by peripheral monocytes without brain irradiation or blood-brain barrier disruption. However, these monocyte-derived engrafted macrophages do not differentiate to microglia, but acquire a unique transcriptomic profile. Using a bioinformatics approach, we defined signatures that detect the presence and unique function of engrafted macrophages among putative myeloid transcriptomes. Further, we developed a technique to replace microglia with hematopoietic-derived macrophages and compared their responses to the response of microglia under numerous physiological and pathological conditions. Under baseline conditions brain-engrafting macrophages cause no overt deficits in behavior, yet have aberrant response to peripheral or local challenges. These aberrant responses may be relevant for many neurological conditions and may impact neural function over the life of an individual.

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Poster

295. Biology of Microglia

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Topic: B.12. Glial Mechanisms

Support: JSPS KAKENHI 16H01329

JSPS KAKENHI 17H03988

JSPS KAKENHI 17H05738

Title: cAMP modulates microglial phagocytosis

Authors: *M. ANDOH, R. KOYAMA, Y. IKEGAYA Grad Sch. Pharma Sci, Univ. Tokyo, Tokyo, Japan

Abstract: Phagocytosis is one of the crucial functions of microglia. Recently, cAMP signaling in microglia is suggested to regulate their phagocytic capacity. However, it remains unclear whether cAMP signaling enhances or attenuates microglial phagocytosis. We investigated how changes in the intracellular cAMP levels in microglia modulate their phagocytic capacity. For this purpose, we developed a coculture system of microglia with astrocytes and neurons, in which the in vivo-like ramified shape of microglia is maintained. We found that pharmacological activation of cAMP slightly facilitated microglial phagocytosis of E.coli particles. In addition, we investigated the role of cAMP in microglial phagocytosis by optogenetically stimulating photoactivated adenylyl cyclase (PAC) in cultured microglia. We also applied this system for the live imaging of synaptic pruning by microglia. To image microglia-synapse interactions in real time, hippocampal neurons were transfected with synaptophysin-mCherry and were cocultured with GFP-expressing microglia prepared from CX3CR1^{GFP/+} mice. Using this system, we observed a translocation of mCherry-labeled synaptophysin from axons of living neurons to GFP-labeled microglial processes. Thus, our experimental system is useful to examine the mechanisms that regulate the phagocytic capacity of microglia.

Disclosures: M. Andoh: None. R. Koyama: None. Y. Ikegaya: None.

Poster

295. Biology of Microglia

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Topic: B.12. Glial Mechanisms

Support: NUS Strategic Research Grant (Memory Networks in Rodent and Primate) DPRT/944/09/14 (R185-000-271-646)

Title: Epigenetic regulation of microglial phosphatidylinositol 3-kinase (PI3K) pathway involved in synaptic plasticity

Authors: *G. SAW¹, M. KARTHIK², T. DHEEN¹ ¹Anat., ²Physiol., Natl. Univ. of Singapore, Singapore, Singapore

Abstract: Microglial cells are the immune-competent macrophages present in the central nervous system (CNS). Microglia-mediated neuroinflammation has been shown in various neurological disorders such as Alzheimer's disease, Parkinson's disease, brain injury and infection. Phosphatidylinositol 3-kinase (PI3K) is known to play a significant role in synaptic plasticity in neurons. It is also expressed in microglia and its role in microglia has been mainly studied in the context of inflammation and phagocytosis. Studies have also shown that PI3K can bring about a phenotypical switch in microglia from pro-inflammatory (M1) to antiinflammatory (M2) which may prevent neurotoxicity. Downstream effectors of microglial PI3K such as protein kinase B (Akt), cAMP response element binding protein (CREB) and brainderived neurotrophic factor (BDNF) have been found to influence synaptic plasticity. We therefore hypothesise that the PI3K/Akt pathway in microglia regulates the production of BDNF which in turn alters synaptic plasticity. In this study, we investigated the regulation of microglial PI3K epigenetically through histone modification and post-translationally through sumoylation. Western blot and immunohistochemistry (ICC) analysis showed that sodium butyrate, a histone deacetylase inhibitor (HDACi) upregulated PI3K expression and the phosphorylation of Akt and CREB in microglia, suggesting that BDNF secretion from microglia may be altered via epigenetic regulation of PI3K. Knockdown of SUMO1 in stable BV2 microglia decreased the phosphorylation of Akt and CREB as well as the expression of BDNF. These results suggest that microglial PI3K is epigenetically regulated by histone modifications and post-translationally modified by sumoylation. We will next explore the role of microglial PI3K in long-term potentiation (LTP) in hippocampal slice cultures. Understanding the mechanisms by which microglial PI3K influences synapses may give us an insight into the ways by which it can modulate synaptic transmission and subsequently synaptic plasticity in learning and memory.

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Poster

295. Biology of Microglia

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Topic: B.12. Glial Mechanisms

Support: Grant-in-Aid for Scientific Research-16K09520

Grant-in-Aid for Scientific Research on Innovative Areas

Title: CRISPR/Cas9-mediated disruption of SGK1 enhances potential inflammatory activity of microglial BV-2 cells

Authors: H. ASAI, *K. INOUE, E. SAKUMA, T. UEKI Nagoya City Univ. Sch. Med., Nagoya, Japan

Abstract: Distinct from other type of cells in brain, microglia are derived from myelogenous cells and responsible for immunological and inflammatory events in central nervous system. While they play a major role in acute inflammation on a variety of disorders, recent studies have revealed that chronic inflammation caused by active microglia leads to neurodegenerative diseases such as Alzheimer's disease and psychiatric diseases such as schizophrenia. Thus, the comprehensive understanding of brain inflammation elicited by microglial activation would refine therapeutic strategies of multiple neurological disorders. We have recently shown that two subunits of serum- and glucocorticoid-inducible kinases (SGKs), SGK1 and SGK3, are expressed in multiple microglial cell lines. Application of an SGK inhibitor gsk650394 results in enhanced lipopolysaccharide (LPS)-induced expression of iNOS and TNFα and nitric oxide (NO) production. These results imply the involvement of SGKs in cellular inflammatory responses (Inoue et al, 2016 BBRC). Here, to explore further the mechanism by which SGKs participate in microglial function, we focus on the roles of SGK1 in microglial BV-2 cells. We first employed the CRISPR/Cas9 system to introduce mutations in SGK1 gene, and successfully obtained heterozygous and homozygous knockout cell lines. Disruption of SGK1 gene leads to deramification of BV-2 cells; proportion of relatively round amoeboid cells is increased while that of ramified cells having long process(es) is decreased, suggesting the likelihood of microglial activation in SGK1^{-/-} cells. In support of this, deleting SGK1 accelerates proliferation rate, another potential parameter of activation. NO is generated by an administration of LPS, and it is enhanced in SGK1^{-/-} cells. In addition, those cells are treated with ATP to induce activation and injury, and SGK1^{-/-} cells turn out to be more susceptible to ATP-induced cell death, suggesting an increase in sensitivity to apoptotic signals. Taken together, these findings

demonstrate that SGK1 contributes to fundamental cellular function in regard with microglial activation. Further experiments will be performed.

Disclosures: H. Asai: None. K. Inoue: None. E. Sakuma: None. T. Ueki: None.

Poster

295. Biology of Microglia

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 295.05/N3

Topic: B.12. Glial Mechanisms

Title: Aif1-iCre knock-in mouse line: A tool for conditional gene manipulation in microglia

Authors: *M. ABE, F. PENG, K. SAKIMURA

Cell. Neurobiol, Brain Res. Inst, Niigata Univ., Niigata, Japan

Abstract: Microglia, the resident immune cells of the brain, are known to play important roles in pathological and physiological conditions. Their main function, as immune cells, is to detect the signs of pathogenic invasion or tissue damage, and moderate the potential damage to the CNS and support tissue repair. In addition, recent findings have revealed that microglia are also involved in maintaining the neurodevelopment process and synaptic plasticity and controlling other neural functions. In order to investigate detailed functions of microglia, we have undertaken development of a conditional gene manipulation system in microglia using Cre/loxP recombination. We generated a new knock-in mouse line expressing codon-improved Cre recombinase (iCre) under the control of the Aif1 (Allograft inflammatory factor 1, also known as Iba1) promotor. This Aif1-iCre line was established by insertion of iCre gene into the translation initiation site of the Aif1 in frame using C57BL/6N ES cells via homologous recombination. Resulting Aif1-iCre mice were viable, grew normally, and were fertile. By crossing the Aif1iCre line with the tdTomato reporter mouse line, we investigated its recombination pattern in the brain. We found that tdTomato fluorescence was detected in the almost cells expressing Iba1 protein, indicating that conditional recombination was performed in a very large population of microglia. Although the recombination seemed to be highly microglia-selective, we also detected fluorescence in a minor population of excitatory neurons, which express a neuronal marker NeuN but contain no inhibitory transmitter GABA, in the forebrain and cells that comprise a part of capillary vessel throughout the brain. It seems that the recombination in these cells was due to transient expression of Cre recombinase at an early stage of development because no immunoreactivity for Iba1 was detected in the adult brain. However, no tdTomato fluorescence was not overlapped with the immunofluorescence for astrocyte marker GFAP, glial fibrillary acidic protein, or oligodendrocyte marker APC, adenamatous polyposis coli. Thus, the Aif1-iCre line on the C57BL/6N background will be a useful genetic tool to manipulate gene expression in microglia without affecting the functions of other glial cells.

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Poster

295. Biology of Microglia

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Topic: B.12. Glial Mechanisms

Support: NIEHS grant ES007062

Title: TSPO interacts with NOX2: a novel function in murine primary microglia

Authors: *M. K. LOTH^{1,2}, T. R. GUILARTE²

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Abstract: Translocator protein 18 kDa (TSPO) is a glial stress-response protein used in preclinical and clinical neuroimaging studies as a biomarker of neuroinflammation. Recent studies using TSPO knockout mice have questioned the long-held view that TSPO functions to transport cholesterol into the mitochondria for steroid synthesis. Further, the understanding of TSPO function in microglia is lacking. We have previously shown that TSPO ligands (TSPO-L) induce microglia functions consistent with an activated state, suggesting a role of TSPO in the inflammatory response to brain injury. Primary microglia exposed to TSPO-L (1-100 nM) induces ROS production which is abrogated by NADPH oxidase (NOX2) inhibitors, suggesting an association between TSPO and NOX2. NOX2 is a multi-subunit enzyme highly enriched in microglia and a major source of ROS in the CNS. To further elucidate the TSPO-NOX2 association in microglia, we used different molecular approaches to assess protein-protein interaction under unstimulated or stimulated conditions (100 ng/mL lipopolysaccharide (LPS) for 18 hours). 1) Co-immunoprecipitation (co-IP) revealed that the NOX2 subunits, gp91phox (gp91) and p22phox (p22), co-IP with TSPO supporting a protein-protein interaction. TSPO's association with gp91 and p22 decreased with LPS stimulation, whereas TSPO's association with VDAC, a mitochondrial protein, remained constant. These findings suggest that microglia activation changes the dynamics of the TSPO-NOX2 interaction. 2) Confocal imaging and colocalization analysis of TSPO/gp91/VDAC or TSPO/p22/VDAC immunofluorescence in microglia confirmed that TSPO colocalizes with both NOX subunits, as well as VDAC. Under LPS-stimulated conditions, TSPO associated with gp91 and TSPO associated with p22, exhibit decreased colocalization with VDAC. 3) Duolink Proximity Ligation Assay confirmed that TSPO interacts with p22, gp91 and VDAC in murine primary microglia. TSPO interaction with these proteins mirrored our co-IP data in that TSPO's association with NOX2 subunits decreased with LPS activation, whereas TSPO's association with VDAC did not change. Our findings suggest a novel TSPO-gp91-p22 interaction with VDAC in primary microglia that

is disrupted by microglia activation with LPS. This TSPO-NOX2-VDAC putative complex may be disrupted by LPS-induced changes in membrane lipid composition, and promote the trafficking of TSPO, gp91, and p22 away from VDAC. We propose that the TSPO-gp91-p22-VDAC interaction modulates the subcellular localization of gp91 and p22 proteins, thereby altering NOX2 activity and mitochondrial function.

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Poster

295. Biology of Microglia

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Topic: B.12. Glial Mechanisms

Support: Canadian Institutes of Health Research

NIH Grant R21NS098514

Title: Dark microglia: A follow-up study across the lifespan

Authors: *K. BISHT¹, K. PICARD¹, N. VERNOUX¹, K. SHARMA¹, Y. Y. GRINBERG², J. FAUSTINO⁴, M. J. CARSON³, Z. S. VEXLER⁵, M.-E. TREMBLAY⁶ ¹Dept. of Mol. Medicine, CHUL, Laval Univ., Quebec, QC, Canada; ³Div. of Biomed. Sci., ²Univ. of California Riverside, Riverside, CA; ⁴Univ. California, San Francisco, CA; ⁵Dept Of Neurol., UCSF, San Francisco, CA; ⁶Dept. of Mol. Med., Univ. Laval, Quebec, QC, Canada

Abstract: Previously, we reported the existence of dark microglia, a newly defined microglial phenotype found during chronic stress, aging, fractalkine signaling deficiency, and Alzheimer's disease pathology (APP-PS1 mice), but rarely present under steady state conditions (Bisht *et al.*, Glia, 2016). Ultrastructurally, the dark microglia exhibited characteristic features of oxidative damage, and also expressed TREM2 (triggering receptor expressed on myeloid cells 2), a cell surface receptor that mediates phagocytosis, when found in association with the plaques of amyloid β . These cells appeared to be phagocytically more active than normal microglia, with their highly ramified and extremely thin processes extensively encircling axon terminals, dendritic spines, and even entire synapses, suggesting their implication in the pathological remodelling of neuronal circuits. Since brain development is also characterized by massive synaptic remodelling, including synaptic pruning, that requires microglial activity, it is important to look at the contribution of these highly phagocytic cells in development mechanisms to better understand their implication in diseases. In continuation with the previous study, we have attempted to characterize further these cells across stages of the lifespan, employing immunohistochemistry followed by transmission electron microscopy analyses. Previously, we

found these cells to be associated with pathological states. Nevertheless, surprisingly, they are also abundant in the normal developing brain, as early as postnatal day 5 (P5). Detection of dark microglia in the early developing brain could be associated with the clearance of cellular debris and synaptic connections overproduced during early postnatal development, however, direct evidence for the involvement of dark microglia in early brain development is lacking. Recent studies have shown the expression of TREM2 mRNA in embryonic and postnatal brain, primarily in microglia. Inducing TREM2 expression in microglial cells results in an increased phagocytosis, whereas its knockdown causes a reduced phagocytic ability. Given the importance of TREM2 in mediating microglial survival and phagocytic response, we attempted to detect dark microglia could not be detected in brain sections prepared from TREM2 knockout animal brains in comparison to their wildtype controls. Our focus is on the identification of specific markers that could selectively label these cells, in order to characterize their implication across brain development, plasticity, and disease.

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Poster

295. Biology of Microglia

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Support: Japan Society for the Promotion of Science - KAKENHI ((C)

Japan Agency for Medical Research and Development (AMED)

Title: Donepezil suppresses intracellular Ca^{2+} mobilization through the PI3K pathway in rodent microglia

Authors: *Y. MIZOGUCHI, Y. HARAGUCHI, T. MURAKAWA-HIRACHI, Y. IMAMURA, A. MONJI

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Abstract: Microglia are resident innate immune cells which release many factors including proinflammatory cytokines or nitric oxide (NO) when they are activated in response to immunological stimuli. Pathophysiology of Alzheimer's disease (AD) is related to the inflammatory responses mediated by microglia. Intracellular Ca^{2+} signaling is important for microglial functions such as release of NO and cytokines. In addition, alteration of intracellular

Ca²⁺ signaling underlies the pathophysiology of AD, while it remains unclear how donepezil, an acetylcholinesterase inhibitor, affects intracellular Ca²⁺ mobilization in microglial cells. In this study, we observed that pretreatment with donepezil suppressed the TNFα-induced sustained intracellular Ca^{2+} elevation in rodent microglial cells. On the other hand, pretreatment with donepezil did not suppress the mRNA expression of both TNFR1 and TNFR2 in rodent microglia we used. Pretreatment with acetylcholine but not donepezil suppressed the TNFainduced intracellular Ca^{2+} elevation through the nicotinic α 7 receptors. In addition, sigma 1 receptors were not involved in the donepezil-induced suppression of the TNFα-mediated intracellular Ca²⁺ elevation. Pretreatment with donepezil suppressed the TNFα-induced intracellular Ca²⁺ elevation through the PI3K pathway in rodent microglial cells. Using DAF-2 imaging, we also found that pretreatment with donepezil suppressed the production of NO induced by TNFα treatment and the PI3K pathway could be important for the donepezil-induced suppression of NO production in rodent microglial cells. Finally, phagocytosis assay showed that pretreatment with donepezil promoted phagocytic activity of rodent microglial cells through the PI3K but not MAPK/ERK pathway. These suggest that donepezil could directly modulate the microglial function in vitro through the PI3K pathway.

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Poster

295. Biology of Microglia

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Title: Exosomes regulate microglia responses to inflammation and aging

Authors: *J. C. UDEOCHU^{1,2}, A. CAI², A. JOVICIC³, C. SANCHEZ DIAZ⁴, P. VENTURA², S. VILLEDA²

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Abstract: Microglia are important cellular sentinels in the brain, however mechanisms of communication directing homeostatic responses are poorly understood. Brain aging represents a unique model for studying such mechanisms given prominent alterations in microglia homeostasis driven by aging-associated inflammation and loss of proteostasis. Here, we focus our study on exosomes, a subtype of extracellular vesicles containing bioactive proteins, RNA molecules and lipids, based on their endosomal origin and previously reported involvement of exosomes in regulating peripheral immune cell activation. We hypothesized that aging alters microglia exosome production and that these changes serve to regulate microglia inflammatory activation. Our initial characterizations revealed increased exosome abundance in the interstitial space of aged compared to young brains. Additionally, aged microglia expressed significantly higher levels of exosome release gene, rab27a, and showed enhanced abundance of exosomeassociated proteins compared to young microglia. Utilizing in vitro approaches, we investigated the signaling capability of microglia exosomes by analyzing exosome content. Microglia exosomes contained heterogeneous mixtures of proteins and microRNAs, enriched with molecules involved in the regulation of inflammatory processes including cytoskeletal rearrangement and cytokine signaling. To directly test the signaling capability of these exosomeassociated molecules, we performed stimulation assays using exosomes from activated microglia. RNA sequencing identified differential induction of numerous genes involved in immune activation, cytokine production and phagocytosis in exosome- relative to vehicle-treated microglia. Functional in vitro assays corroborated these gene expression changes confirming exosome-mediated inflammatory propagation in microglia. To determine if exosomes regulate such responses during microglia aging, we performed unilateral stereotaxic injection of exosome inhibitor, GW4869, or vehicle into aged brains. Subsequently analysis of microglia activation revealed significantly higher expression of CD68 in response to exosome inhibition. Together, we identify a novel form of cell-cell communication in microglia with important implication in regulating homeostatic and inflammatory responses during aging.

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Poster

295. Biology of Microglia

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Program#/Poster#: 295.10/N8

Topic: B.12. Glial Mechanisms

Title: Spatial and temporal activation of microglia and astrocytes following transcranial focused ultrasound

Authors: *J. SILBURT^{1,2}, S. HEINEN², K. MARKHAM-COULTES², M. A. O'REILLY², K. HYNYNEN⁴, I. AUBERT³

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Abstract: Transcranial focused ultrasound (FUS) enables non-invasive and controlled drug delivery across the blood-brain barrier (BBB) in a temporal (6- to 12-hours) and localized manner. Previous work reported varying degrees of microglial and astrocytic activation triggered by FUS over time. For example, Jordão et al (2013) showed that microglia were activated by 4 hours, and astrocytes by 4 days, both returning to baseline levels by 14 days post-FUS. This work did not use a feedback controller, which can better control opening. Depending on the type and severity of brain challenge, microglia can display a gradient of activation, and can contribute to inflammation and pathology, or reduce pathology (e.g. clear amyloid-beta) and promote regenerative processes. Mild challenges result in earlier resolution of microglia and astrocyte activation, and are not associated with the detrimental characteristics of glial scars. Here, we investigated a time-course of glial activation following FUS coupled with a feedback controller. We hypothesized that FUS treatment would transiently activate microglia and astrocytes near permeabilized, but not ruptured, blood vessels. We developed a machine learning protocol to analyze, in an unbiased manner, the spatio-temporal activation of microglia and astrocytes as determined by changes in morphology and the intensity of established glial markers. We show that microglia activation occurs by 1 day post-FUS and is significantly reduced by 10 days post-FUS. Astrocytes show activation beginning by 1 day post-FUS and remain activated at 10 days post-FUS. Furthermore, we quantified microglia and astrocyte proliferation, and the levels of stress proteins, including heat shock proteins. Microglia showed an increase in proliferation consistent with activation, while astrocytes did not proliferate. We observed no changes in heat shock proteins 70 and 90. Finally, neuronal cell death following FUS was not observed and erythrocyte extravasation was minimal. We conclude that following FUS, microglia and astrocytes are activated for at least 10 days, with progressive reductions in microglia activation. This activation was not associated with cell death, significant red blood cell entry in the brain, or changes in common heat shock proteins.

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Poster

295. Biology of Microglia

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Topic: B.12. Glial Mechanisms

Support: NIDA IRP

Title: Local cues establish and maintain region-specific phenotypes of basal ganglia microglia

Authors: *L. M. DE BIASE¹, K. E. SCHUEBEL², Z. H. FUSFELD¹, K. JAIR², I. A. HAWES³, R. CIMBRO⁵, H. ZHANG⁶, Q.-R. LIU⁷, H. SHEN⁸, Z.-X. XI¹, D. GOLDMAN⁹, A. BONCI⁴ ¹Natl. Inst. on Drug Abuse, Baltimore, MD; ²Natl. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD; ⁴Office of the Scientific Director, ³Natl. Inst. On Drug Abuse, Baltimore, MD; ⁵Johns Hopkins Univ. Sch. of Med., Baltimore, MD; ⁶Natl. Inst. on Drug Abuse Intramural Res. Program, Baltimore, MD; ⁷Neuronal Networks Section, Integrative Neurosci. Res. Br., Intramural Res. Program,Nia/ NIH, Baltimore, MD; ⁸NIDA/IRP, Baltimore, MD; ⁹Lab. Neurogenetics, Natl. Inst. on Alcohol Abuse and Alcoholism Lab. of Neurogenetics, Rockville, MD

Abstract: Microglia play critical roles in CNS injury responses, tissue homeostasis, and can also modulate neuronal function and synaptic connectivity. In contrast to astrocytes and oligodendrocytes, which arise from multiple progenitor pools, microglia arise from yolk sac progenitors and are widely considered to be equivalent throughout the CNS. However, little is known about the basic properties of deep brain microglia, such as those residing within the basal ganglia (BG). Here, we show that microglial anatomical features, lysosome content, and membrane properties differ significantly across BG nuclei. In addition, transcriptome sequencing revealed significant differences in gene expression between midbrain and cortical microglia. Region-specific phenotypes of BG microglia emerged during the second postnatal week and were re-established following genetic or pharmacological microglial ablation and repopulation, indicating that local cues play an ongoing role in shaping microglial diversity. Together, these observations demonstrate that microglia in the healthy brain exist along a spectrum of distinct functional states and these data provide a critical foundation for defining microglial contributions to BG circuit function.

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Poster

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Topic: B.12. Glial Mechanisms

Title: Effect of microglia depletion on cerebral kynurenine pathway metabolism in control and R6/2 mice

Authors: *K. V. SATHYASAIKUMAR¹, D. W. DONLEY², M. A. R. THOMAS¹, F. M. NOTARANGELO¹, P. SEVERSON³, B. L. WEST³, J. H. FOX², R. SCHWARCZ¹ ¹Univ. of Maryland Sch. of Med., Baltimore, MD; ²Vet. Sci., Univ. of Wyoming, Laramie, WY; ³Translational Pharmacol., Plexxikon Inc, Berkeley, CA

Abstract: Kynurenine 3-monooxygenase (KMO) plays a key regulatory role in the kynurenine pathway (KP) of tryptophan degradation, which contains several neuroactive metabolites. In vitro studies suggested that KMO in the mammalian brain is predominantly localized in microglial cells (Alberati-Giani et al., 1996; Heyes et al., 1996, Guillemin et al., 2001), but verification in vivo has not been provided so far. We showed recently that KMO activity is unchanged in adult wild-type (WT) mouse brain after depletion of microglia (Sathyasaikumar et al., SfN Abstr., 37.19, 2016). As KMO activity is substantially increased in the brain of R6/2 mice, an established animal model of Huntington's disease (HD) (Sathyasaikumar et al., 2010), we now examined possible impairments in KMO and other elements of cerebral KP metabolism in R6/2 mice after pharmacological depletion of microglial cells. To this end, we treated animals with the colony stimulating factor 1 receptor inhibitor PLX5622, which depletes microglia in normal mice by 80% after 1 week of treatment (Dagher et al., 2015). WT and R6/2 mice were fed either PLX5622 (1,200 ppm in chow) or normal chow for 28 days starting at 7.5 weeks of age (N=10-12 per group). Animals were euthanized on the last day of treatment, and KMO, kynureninase and 3-hydroxyanthranilic acid dioxygenase (3-HAO) activities, as well as 3hydroxykynurenine (3-HK), kynurenic acid (KYNA) and quinolinic acid (QUIN) levels were determined in the forebrain. In addition, we analyzed brain weight, body weight and rotarod behavior in all animals. Compared to WT animals, R6/2 mice showed increased KMO (+214%) and decreased kynureninase (-42 %) activity. Neither of these changes was affected in PLX5622treated animals. In contrast, brain 3-HAO activity was essentially identical in WT and R6/2 mice, and PLX5622 treatment caused dramatic reductions in enzyme activity in both genotypes (WT: -65%; R6/2: -77%), indicating microglial localization of the majority of 3-HAO in the brain of both WT and HD mice. PLX5622 treatment had no significant effects on body weight, or on the brain levels of 3-HK, KYNA and QUIN in either genotype. Brain weight was significantly reduced in R6/2 mice, but PLX5622 treatment had no effect. The latency to fall measured by rotarod task worsened with age in R6/2 mice, but was unaffected by PLX 5622 in either genotype. Taken together, our data suggest that microglial cells do not harbor the majority of KMO and kynureninase in either physiological or pathological conditions. Possible compensatory events following PLX5622 treatment are currently under investigation.

Disclosures: K.V. Sathyasaikumar: None. D.W. Donley: None. M.A.R. Thomas: None. F.M. Notarangelo: None. P. Severson: None. B.L. West: None. J.H. Fox: None. R. Schwarcz: None. Poster

295. Biology of Microglia

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 295.13/N11

Topic: B.12. Glial Mechanisms

Support: Western Michigan University Indirect Funds

Title: The role of microglia/macrophages in the response to olfactory bulb damage in adult zebrafish

Authors: *S. R. VAR, C. A. BYRD-JACOBS

Biol. Sci., Western Michigan Univ., Kalamazoo, MI

Abstract: The inherent plasticity of the zebrafish olfactory system serves as a useful model for examining immune cell response after injury. Microglia are the resident immune cells of the central nervous system that respond to damage by migrating to the site of injury and phagocytizing neuronal debris. We have shown a microglial response to olfactory deafferentation and direct injury; however, the timing and pattern of microglial migration remains unclear. We hypothesize that the microglial response to deafferentation or direct bulb injury facilitate regeneration and that liposome-encapsulated clodronate treatment will induce specific and localized macrophage depletion after damage and inhibit regeneration efficiency. Peripheral deafferentation and direct injury to the olfactory bulb in the whole fish was performed and compared to results in the isolated brain removed of all afferent input and peripheral influence. Comparisons of whole fish treatment groups to controls showed a significant increase in activated microglia in the damaged bulb following peripheral deafferentation at 4, 12, 24, 48, and 72h. Amoeboid profiles significantly increased between 1-4h, decreased between 4-12h, increased again at 12-24h, and decreased again at 24-48h. Following direct injury to the bulb, there was a significant increase in activated microglia in the ipsilateral and contralateral bulbs at 1 and 4h after injury. At 4h after injury, there was a significant decrease in amoeboid profiles, which remained low until 72h. Comparisons of isolated brain treatment groups to controls showed significantly more activated microglia in the olfactory bulbs after 4 and 12h in culture. Isolated brains that received a direct injury showed a significant increase in activated microglia after 1, 4, and 12h in culture; however, isolated brains that received this injury showed significantly fewer responsive microglia in the damaged bulb when compared to direct injury to the bulb in the whole fish at 12h, suggesting that microglia can respond to damage without afferent input or peripheral influence, but only up to a certain time after injury. When liposomeencapsulated clodronate was injected into the telencephalic ventricle 24h before insult in whole fish, there appeared to be a significant reduction in microglia 4h after injury but only a small reduction in microglia 24h after injury, perhaps suggesting a temporal significance and selective

phagocytosis during the resolution of inflammation, particularly between the hours of 4 and 24h. Additional work is required to explore further the timing of the response and the potential role of these immune cells in recovery and regeneration after injury.

Disclosures: S.R. Var: None. C.A. Byrd-Jacobs: None.

Poster

295. Biology of Microglia

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 295.14/N12

Topic: B.12. Glial Mechanisms

Title: Improved culturing conditions for the generation of In vivo-like primary microglia

Authors: *H. CYNIS¹, S. BARENDRECHT¹, B. HIETEL¹, R. EICHENTOPF¹, S. SCHILLING¹, H. U. DEMUTH¹, D. C. WAGNER² ¹Fraunhofer IZI-MWT, Halle, Germany; ²Johannes-Gutenberg Univ., Mainz, Germany

Abstract: Microglia cells are phagocytic immune cells in the CNS. They make up 8-13% of the total cell population in the brain and derive from primitive yolk-sac macrophages. Microglia cells have many different functions in the brain, both in normal brain development and in pathological conditions such as Alzheimer's disease (AD). The exact role of microglia in AD remains unknown: while some studies show a positive contribution of microglia through clearance of amyloid-beta plaques, other studies report a negative effect of inflammatory cytokines released by microglia during pathology. Primary murine microglia are often used to model the functioning of microglial cells in vitro. However, these experiments suffer from a rapid loss of the *in vivo* microglial phenotype, due to the lack of input from other CNS cells, which is necessary for microglial development and homeostasis. Our primary goal was to retain the microglial phenotype in cultured primary microglia, by mimicking the input normally present in the CNS. For this purpose we have tested and developed specific culturing conditions, which are able to restore the *in vivo* phenotype in primary microglia. We used qPCR and microarray analysis to compare the gene expression in our cultured primary microglia to the expression levels seen in freshly isolated microglia. Furthermore we were interested in the functioning of microglia in relation to neurodegenerative disease, specifically AD. Microglial-specific activity such as phagocytosis (of amyloid-beta) and responses to inflammatory stimuli were tested in freshly isolated microglia and cultured primary microglia. Firstly, we found that the native microglial phenotype is indeed restored by culturing primary microglia with a specific mix of factors important for microglial development and functioning. We also show that this effect is time-dependent. Regarding the functioning of the newly generated in vivo-like primary microglia, we expect these cells to function in a similar fashion as freshly isolated microglia and thus to better resemble microglia cells inside the brain. The culturing conditions developed in our lab make it possible to better analyze and understand the functioning of microglia in neurodegenerative disease. Our main focus is on the role of microglia in antibody-mediated clearance of amyloid-beta plaques, to improve the development and *in vitro* testing of possible new therapeutic antibodies for AD.

Disclosures: H. Cynis: None. **S. Barendrecht:** None. **B. Hietel:** None. **R. Eichentopf:** None. **S. Schilling:** F. Consulting Fees (e.g., advisory boards); Probiodrug AG. **H.U. Demuth:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Probiodrug AG. F. Consulting Fees (e.g., advisory boards); Probiodrug AG. **D.C. Wagner:** None.

Poster

295. Biology of Microglia

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Program#/Poster#: 295.15/O1

Topic: B.12. Glial Mechanisms

Support: NIH Grant MH106330

Title: Microglial-specific gene expression in adult prefrontal cortex following perinatal exposure to high fat diet

Authors: *B. L. SMITH¹, S. E. MCKEE², E. S. WOHLEB¹, T. M. REYES¹ ¹Dept. of Psychiatry and Behavioral Neurosci., Univ. of Cincinnati, Cincinnati, OH; ²Dept. of Pharmacol., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Impulsivity is a key behavioral feature of many mental health disorders, such as substance abuse, attention-deficit/hyperactivity disorder, autism, and bipolar disorder. The prefrontal cortex (PFC) is critical for impulse control. Maternal malnutrition in the form of high fat diet (HF) increases impulsivity in the adult offspring and changes PFC gene expression of targets involved in reward, inflammation, and epigenetics. Using this maternal malnutrition model for impulsivity in mice, we sought to identify cell type specific molecular mediators that could contribute to this behavioral phenotype. Maternal HF diet increases markers of neuroinflammation in the offspring, but more work is needed to understand the exact consequences of this proinflammatory effect for the offspring into adulthood. To this end, we isolated microglia from the PFC of the adult male (M) and female (F) offspring that were either exposed to maternal HF or control diet (CD) throughout gestation and lactation. Because stress exposure can enhance neuroimmune responses and alter PFC-mediated behaviors, we included an acute restraint challenge prior to tissue collection (8 groups total, n = 6/group: M CD unstressed, F CD restraint, F HF unstressed, F HF restraint). Finally, we performed high throughput RT-qPCR to measure

microglial gene expression of 32 targets, including various cytokines, chemokines, epigenetic markers, and receptors expressed on microglia that are putatively involved in stress and PFC-mediated behaviors.

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Poster

295. Biology of Microglia

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Topic: B.12. Glial Mechanisms

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Guangdong Innovative and Entrepreneurial Research Team Program 2013S046

Shenzhen Peacock Plan

Title: Repopulated microglia are solely derived from the proliferation of residual microglia, not from de novo progenitor cells

Authors: ***B. PENG**¹, Y. HUANG², S. XIONG², G. QIN³, G. HU², J. WANG², F. SUN², Y. LIANG⁴, Z. XU², K.-F. SO⁶, T.-F. YUAN⁷, Y. RAO⁵

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Abstract: Studies found new-born microglia replenished the whole brain after selective elimination of microglia (>99%) in adult mice. Immunohistochemical evidences suggested that repopulated microglia were differentiated from *de novo* progenitors expressing Nestin in the brain, which raised the possibility that the cross-lineage differentiation occurs in the mature brain. However, the origin of repopulating microglia has been hotly debated. In the present study, we investigated the origin of repopulating microglia by fate mapping. We first excluded that repopulated microglia were from blood cells. We then identified that repopulated microglia were not differentiated from Nestin-positive cells. Next we demonstrated all new-born microglia were derived from the proliferation of surviving microglia (<0.90%). Moreover, we confirmed that the whole brain transcriptome were not largely altered among stages of microglial progenitor cells were not existed in the adult brain.

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Poster

295. Biology of Microglia

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Topic: B.12. Glial Mechanisms

Support: NIH Grant NS094137

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Title: Translational profiling of microglia reveals artifacts of cell sorting

Authors: *J. D. FRYER¹, S. KANG²

¹Neurosci., Mayo Clin. FL, Jacksonville, FL; ²Neurosci., Mayo Clin., Jacksonville, FL

Abstract: Microglia are the resident innate immune population of the central nervous system that constantly survey and influence their local environment. Transcriptomic profiling has led to significant advances in our understanding of microglia in several disease states, but tissue dissociation and purification of microglia is known to lead to cellular activation. Here we use RiboTag translational RNAseq profiling to demonstrate that commonly used cell sorting methods lead to a fundamental alteration of the microglial transcriptome, with several transcripts that can be used to mark artifacts of isolation. Microglial RiboTag RNAseq profiling after peripheral immune challenge with lipopolysaccharide demonstrates unique transcriptional targets that are not evident using cell sorting methodology. Finally, we applied our technique to reveal novel shared and distinct pathways when comparing microglial transcriptomes after peripheral challenge with bacterial or viral mimetics. This study has broad implications for approaches that examine microglial transcriptomes in normal and pathological states.

Disclosures: J.D. Fryer: None. S. Kang: None.

Poster

295. Biology of Microglia

Location: Halls A-C

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Topic: B.12. Glial Mechanisms

Support: NSF Grant 1557971

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NIH Grant NS099973-01

Title: Noradrenergic modulation of microglial dynamics and synaptic plasticity

Authors: *R. STOWELL, A. MAJEWSKA

Univ. of Rochester, Rochester, NY

Abstract: Microglia, the innate immune cells of the central nervous system (CNS), respond rapidly and dynamically to homeostatic perturbations of the CNS milieu. In the healthy unperturbed brain, microglial processes make frequent contacts with neurons at synapses, impacting synaptic remodeling and turnover of dendritic spines. However, it remains unclear what receptors and signaling pathways govern microglial surveillance and synapse monitoring. Noradrenaline is a powerful signal that can affect many aspects of synaptic function and plasticity. Because microglia express high levels of β_2 adrenergic receptors (AR) compared to other cell types in the brain, we asked whether noradrenergic tone could alter microglial behavior with respect to synapses through β_2 AR signaling. To test this hypothesis we have manipulated β_2 AR signaling pharmacologically using the following agents: Nadolol (blood brain barrier (BBB) impermeant β AR antagonist), Clenbuterol (BBB permeant β_2 AR agonist), and ICI 118-551 (BBB permeant β_2 AR antagonist). We paired nadolol with clenbuterol to stimulate β_2 ARs centrally without concomitant peripheral stimulation. We then evaluated changes in basic microglial physiology through a combination of *in vivo* two-photon microscopy and immunohistochemical staining for Iba-1, a microglia-specific protein. We have found that stimulation of β_2 AR signaling *in vivo* reduces microglial motility and pseudopodia formation and causes microglia to assume a less ramified morphology. We also found that stimulation of β_2 ARs leads to impaired microglial responsiveness to focal tissue injury. These experiments show that β_2 AR signaling can affect microglial physiology and immune responses. Our next question was if these changes in basic microglial function could impact microglial interactions with neurons and functional experience-dependent plasticity. Using intrinsic optical signal imaging we have shown that pharmacological manipulation of β_2 AR signaling impairs ocular dominance plasticity in the visual cortex during the visual critical period in mice. We have shown that microglia are directly involved in this impairment through cre-mediated excision of β_2 AR

specifically in microglia. Based on our results we believe that β_2 AR signaling serves important roles in modulating microglial physiology and our future experiments will begin to address how the endogenous ligand, norepinephrine, is involved in mediating the effects observed. These results and future findings will improve our understanding of the signaling mechanisms that govern microglial interactions with synapses and how they impact activity-dependent synaptic modifications.

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Poster

295. Biology of Microglia

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Topic: B.12. Glial Mechanisms

Support: NIH R21AA021260

Title: Alcohol, exercise, and sex differences in microglial activation

Authors: *E. A. BARTON¹, C. BAKER¹, J. L. LEASURE²

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Abstract: Alcohol use disorders (AUDs) are associated with damage to corticolimbic brain regions. As the prevalence AUDs increases, it is necessary to find effective treatments. Exercise is a low-cost adjunctive treatment option that has been investigated. While it provides numerous health and cognitive benefits, the interactive effects of alcohol and exercise on the brain remain largely unexplored. Additionally, our current data suggest sex differences in basal microglia activation states as well as the microglial response to binge alcohol and exercise, as well as the potential sex differences in neural immune function.

Our lab utilizes a short-term (4-day) animal model of binge alcohol exposure that results in coroticolimbic damage consistent with damage seen in the clinical population of chronic alcohol user. In this model, rats are gavaged with ethanol (25% w/v) every 8 hours for 4 days. Control animals receive an isocaloric control diet also by gavage. Using this model we have demonstrated a loss of granule cells in the dentate gyrus of the hippocampus that persisted 35 days following the last dose of alcohol in female rats that is not seen in males. Voluntary wheel running, however, recovered the observed cell loss. This exciting finding supports the idea of using exercise as an adjunctive treatment for AUDs. However, when we examined microglia in the medial prefrontal cortex (mPFC) of the same animals, we found a drastically altered microglial response following binge and exercise that resulted in a staggering decrease in microglia cell number. Emerging evidence suggests alcohol exposure may be a priming stimulus

for microglia, meaning it potentiates microglia to launch an exaggerated response to a second stimulus. Indeed, a morphological analysis of microglia revealed a subpopulation of larger, thicker cells consistent with morphological descriptions of priming. These primed microglia were found in all female animals regardless of experimental condition, however, the binge exercise group had the most. Additionally, all females (regardless of binge or exercise) show marked OX-42 and MHCII (both markers of early microglia activation and priming), which is comparably limited in males, suggesting a basal sex difference in microglia expression profiles. Furthermore, the female binge exercise group shows the highest expression of both markers. This sex difference in microglia expression profiles and morphology may contribute to female vulnerability to alcohol-induced neural damage.

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Poster

295. Biology of Microglia

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 295.20/O6

Topic: B.12. Glial Mechanisms

Support: NIH DA034185

Title: Microglial-specific myd88 signaling impacts extinction of morphine cpp and adult hippocampal neurogenesis

Authors: *P. D. RIVERA^{1,2}, R. HANAMSAGAR^{1,2}, M. KAN³, P. TRAN¹, D. STEWART⁴, M. GUNN³, S. BILBO^{1,2,4}

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Abstract: It is now appreciated that drugs of abuse - such as morphine - promote a neuroimmune response from glia, primarily microglia, which may play an important role in mediating addiction-like behaviors. For example, opiate administration in rodents increases the microglial surface marker CD11b and neuro-immune signaling molecules such as IL-1B and TNFa within discrete regions of the CNS involved in reward. Additionally, addiction-like behaviors such as conditioned place preference (CPP) can be prevented using pharmacological blockades and genetic knock-outs of signaling molecules responsible for pro-inflammation from microglia (i.e. Toll-like receptor 4 and MyD88). However, the specific role of microglial pro-inflammatory signaling on morphine CPP has yet to be determined. We therefore developed a novel transgenic mouse in which pro-inflammatory signaling (i.e. MyD88-dependent pathway) specifically in microglia (i.e. CX3CR1-Cretg/0 x MyD88f/f, Cretg/0) is ablated within the CNS. Microglial isolations from Cretg/0 verified diminished MyD88 gene expression in the CD11b+ cells while CD11b- cells, Cre0/0 x MyD88f/f, and WT controls maintain MyD88. A battery of behavioral tests on young and aged Cretg/0 mice revealed similar levels of anxiety and social interaction relative to Cre0/0, indicating normal mouse behavior. Next, 4-month-old Cretg/0 and Cre0/0 mice were trained under the morphine (3 mg/kg) CPP paradigm to assess the role of microglial-specific pro-inflammatory signaling. Both Cretg/0 and Cre0/0 groups acquired morphine CPP, however the Cretg/0 group had prolonged extinction and enhanced reinstatement compared to Cre0/0 controls. Given that adult hippocampal neurogenesis is involved in extinction learning, dentate gyrus doublecortin (DCX) was then examined. Interestingly, Cretg/0 mice had decreased DCX relative to Cre0/0 mice after morphine CPP. To determine if microglia are involved in the morphine CPP induced decrease in adult neurogenesis, CD68+, a lysosomal marker found in microglia, DCX+ cells were also assessed. Taken together, our results suggest that microglial-MyD88 signaling impacts extinction learning and adult neurogenesis and may have a protective role in addiction-like behaviors.

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Poster

295. Biology of Microglia

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Topic: B.12. Glial Mechanisms

Support: NIH grant NS060017

NMSS RG1507

Texas A&M Univ.

Title: The role of microglial Stat3 signaling in early postnatal brain development

Authors: B. BARTH¹, H. LU¹, S. KIM¹, A. BOWLING¹, D. MICHAUD¹, K. KONGANTI², J. CAI¹, W. GRIFFITH³, *J. LI¹

¹Vet. Integrative BioSci, ²Inst. for Genome Sci. and Society, Texas A&M Univ., College Station, TX; ³Neurosci. and Exptl. Therapeut., Texas A&M Hlth. Sci. Ctr., Bryan, TX

Abstract: Recent studies have suggested that microglia play critical roles in maturation and refinement of neural networks during postnatal brain development. Although it is known that microglia arise from the yolk sac and colonize the CNS during early embryogenesis and undergo a second wave of expansion during the first two weeks of postnatal life in rodents, it is less clear what intrinsic signals regulate the postnatal microglia development and how disrupted microglial

function at this developmental stage impacts on normal brain development. Here we report that signal transducer and activator of transcription 3 (Stat3) signaling plays a key role in microglial survival and activation in postnatal developing brain. $CX_3CR1^{CreER/+}$: Stat3^{flox/flox}: R26-tdTomato conditional mice were generated to achieve inducible and selective ablation of the phosphorylation site containing exon 22 of the *Stat3* gene in microglia after birth (P2-P4). Expression of tdTomato was used to evaluate the efficacy of tamoxifen-induced Crerecombination and to visualize Stat3 mutant microglia. Immunohistochemistry was used to analyze brain tissues collected from mice at different postnatal developmental stages. Our data showed clear morphological differences between activated microglia at P7-P10 and ramified microglia at P28-30. Cell counting analysis revealed significant decrease of microglial cell population in the Stat3 mutant mice (P7 and P10) in comparison to littermate controls. Interestingly, this loss of microglia during the first 1-2 weeks was largely recovered by P30. However, the transient decrease of microglia at P7-P10 was associated with increased innate immune responses including interferon signaling, complement pathways and cytokine/chemokine production as determined by RNA-sequencing analysis of acutely isolated microglia at P7. Dystrophic dendrites of cortical pyramidal neurons were found in the mutant mice when analyzed at P30. The functional consequence of microglial Stat3 inactivation is being examined in adult mice and our preliminary data suggest behavioral alterations in the conditional Stat3 mice as compared with littermate controls. Together, our data demonstrate that Stat3 signaling plays an essential role in microglial survival and activation in the postnatal developing brain and suggest that disruption of Stat3 signaling may elicit long-term neurological effects.

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Poster

296. Oligodendrocyte and Schwann Cells Development and Myelination

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Topic: B.12. Glial Mechanisms

Support: NIH Grant R21NS087511

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Title: Lifelong myelin plasticity along single cortical axons

Authors: *R. A. HILL, A. LI, J. GRUTZENDLER Neurol., Yale Univ. Sch. of Med., New Haven, CT Abstract: Unlike any other cell in the cerebral cortex, myelinating oligodendrocytes are continuously generated in the adult. Little is known about how new cells integrate into the preexisting neural architecture and whether the structure and distribution of myelin internodes along single axons is fixed throughout life. Using high resolution label-free and fluorescence in vivo optical imaging with transgenic fate mapping we discovered distinct patterns of lifelong oligodendrocyte generation occurring in parallel with changes in myelin internode deposition, length, and distribution along single axons. Myelin formation was largely a result of oligodendrocyte generation and occurred on both previously partially myelinated and unmyelinated axons. The distribution of internodes and total myelin coverage of single axons progressed up to two years of age demonstrating that these processes continue to evolve into late adulthood in the mouse neocortex. These findings reveal novel forms of lifelong myelin-dependent plasticity with broad implications for flexibility within adult cortical circuits.

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Poster

296. Oligodendrocyte and Schwann Cells Development and Myelination

Location: Halls A-C

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Topic: B.12. Glial Mechanisms

Support: Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB)

Title: Nanoscale alignment of the periodic subcortical cytoskeleton of axon and glia at nodes of Ranvier

Authors: *E. D'ESTE, D. KAMIN, F. BALZAROTTI, S. W. HELL Max Planck Inst. For Biophysical Chem., Goettingen, Germany

Abstract: The ultrastructural organization of nodes of Ranvier has been investigated mainly by electron microscopy due to their small size (~1 μ m). This technique, however, provides limited molecular specificity. On the other hand, the resolution power of conventional light microscopy impedes the identification of substructures formed by antibody labelled proteins. Here, we exploit Stimulated Emission Depletion (STED) super-resolution microscopy to analyze the nanoscale organization of axonal and glial markers at the nodes of Ranvier of teased sciatic nerve fibers from mice. STED nanoscopy permits to achieve resolutions of few tens of nanometers without compromising on the molecular specificity. We found that the ~190 nm periodic subcortical actin/spectrin lattice that has been previously described in cultured neurons is present all along myelinated axons. Indeed, this lattice is continuous at the transition between paranodes and nodal gaps, where betaII spectrin and betaIV spectrin are present, respectively. In the latter compartment, sodium and potassium channels (Kv7.2) alternate and also follow the

~190 nm periodicity, which, however, appears less regular for adhesion molecules such as neurofascin-186 and nrCAM.

Paranodes are characterized by a tight interaction between the axon and glial cells. In these regions, both axonal (Caspr) and glial (neurofascin-155) adhesion molecules exhibit the same quasi-one-dimensional order, and the axonal subcortical cytoskeleton has a periodic arrangement. In the paranodal loops, the glial cytoskeletal protein Ankryin B features this same periodic organization. Notably, we observed a high degree of interdependence between the position of the axonal and glial proteins, hence suggesting the presence of mechanisms that finely align the cytoskeleton of the axon with the one of the glial cells.

Taken together, our observations suggest a master role for the periodic subcortical cytoskeleton in the organization of nodes of Ranvier and pave the way for a deeper investigation of these structures.

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Poster

296. Oligodendrocyte and Schwann Cells Development and Myelination

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Rachleff Endowment

Title: Oligodendrocyte myelination: Axon size may be all that matters

Authors: *S. R. MAYORAL¹, A. ETXEBERRIA², C. J. SHIN³, C. S. YIAN³, J. R. CHAN² ¹Neurol., Univ. of California San Francisco Dept. of Neurol., San Francisco, CA; ²Neurol., UCSF, San Francisco, CA; ³Chem. and Biomed. Engin., Nanyang Technological Univ., Singapore, Singapore

Abstract: Myelination in the CNS is thought to occur via two distinct, but coupled processes. First, highly proliferative and migratory oligodendrocyte precursor cells (OPCs) differentiate

into mature, post-mitotic oligodendrocytes (OLs). Second, OL membrane extensions concentrically wrap around selected axons forming myelin. OL differentiation and myelination occur in a spatially and temporally stereotyped manner throughout development, which implies that environmental cues are at play in their regulation. There is increasing evidence that dynamic neuronal signaling involving neuronal activity and transcriptional control plays a role in regulating these processes, but it is unclear whether this is the case in regions where there is synchronous and robust differentiation and myelination within a short timeframe as in the early development of white matter tracts like the optic nerve. By eliminating dynamic neuronal signaling in the optic nerve our findings indicate that (1) OL differentiation and myelination are indeed coupled processes where myelination will occur upon differentiation if provided with a permissive substrate, and (2) dynamic neuronal signaling is not required for the majority of OL differentiation during early white matter development. We propose a model in which OL differentiation is largely regulated by extrinsic, non-neuronal factors during development, but myelination is sensitive to the biophysical properties of axonal diameter.

Disclosures: S.R. Mayoral: None. A. Etxeberria: None. C.J. Shin: None. C.S. Yian: None. J.R. Chan: None.

Poster

296. Oligodendrocyte and Schwann Cells Development and Myelination

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 296.04/P1

Topic: B.12. Glial Mechanisms

Support: Danish Research council, YDUN

Danish Society for multiple sclerosis

Title: Selection of CNS axons for myelination is negatively regulated by EphA4 and RhoA signaling

Authors: *L. S. LAURSEN¹, M. HARBOE¹, J. TORVUND-JENSEN², K. KJAER-SORENSEN³

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Abstract: During development of the central nervous system not all axon are myelinated, and each axon may have a distinct myelination pattern, combined with the oligodendrocytes ability to myelinate up to 50 different axons this suggest that local signals between the oligodendrocyte process and the axon determine whether a myelin sheath can be formed. However, our current knowledge about the axonal signals involved in regulating this process is very limited. We here identify EphA4 as the receptor responsible for ephrinA-1 induced inhibition of process

extension in oligodendrocytes, and show that Ephrin-A1-EphA4 interaction activates a RhoA-Rock-myosin-II signaling cascade. Inhibition of signaling at different levels of this pathway regulates myelination by increasing the number of myelin sheaths formed by each oligodendrocyte. In contrast, activation of EphA4 reduces the number of internodes formed per oligodendrocyte. Combined this suggest that activation of the EphA4-RhoA pathway by axonal ephrin-A1 negatively regulate the formation of the stable axo-glia interaction required for formation of a myelin sheath.

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Poster

296. Oligodendrocyte and Schwann Cells Development and Myelination

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Topic: B.12. Glial Mechanisms

Support: CNPq

FAPERJ

CAPES

Title: Pentose pathway activation in M leprae-infected Schwann cells leads to reduced lactate generation and nerve damage

Authors: *B. S. MIETTO¹, K. G. C. VASCONCELLOS¹, B. JUNQUEIRA¹, R. C. A. MEDEIROS¹, S. L. G. ANTUNES¹, M. C. V. PESSOLANI¹, E. N. SARNO², F. A. LARA² ¹Leprosy Dept., Fiocruz, Rio De Janeiro, Brazil; ²Leprosy Dept., Fiocruz, Rio de Janeiro, Brazil

Abstract: Leprosy is a chronic infectious disease caused by the intracellular pathogen *Mycobacterium leprae (M. leprae)*, which disturbs peripheral nerve integrity, impairing axonal and myelin function. There is growing evidence supporting that the molecular basis of Leprosy neuropathy could be located in infected Schwann cells. Of importance, Schwann cells are deeply involved in a wide range of axonal behavior including the maintenance of axonal and myelin integrity. Our group recently demonstrated that *M. leprae* was able to modulate Schwann cells glucose metabolism for its own benefit (Medeiros et al., 2016). Our results indicated that infected human Schwann cells increased glucose uptake with a concomitant upregulation in glucose-6-phosphate dehydrogenase (G6PDH) activity, the key enzyme of the oxidative pentose pathway. We also observed a two-fold reduction in lactate release from infected cells when compared to control ones. We also observed mitochondria shutdown in infected Shwann cells *in vitro* and mitochondrial swelling in myelinated and unmyelinated axons from Leprosy patients.

Here, we advance these findings by showing that neurons treated with M. leprae-infected Schwann cell-derived supernatant had a marked impairment in neurites outgrowth and complexity. In addition, ML was able to enhance the degradation of myelin contents in infected Schwann cells *in vitro*, and nerve biopsies harvested from patients with Leprosy neuropathy have augmented expression of mRNA for several autophagy genes (*ATG5, ATG7, BECLIN1 and LC3B*) as compared with control non-Leprosy nerves. In short, our results points to the notion that low levels of lactate content along with the presence of toxic mediators in ML-infected Schwann cells-derived supernatant are able to disturb neuronal integrity and growth responses. In addition, ML infection accelerated myelin degradation by up-regulating autophagy pathways *in vitro* and *in vivo*. Collectively, these data indicates that the pentose phosphate pathway is paramount for *M. leprae* infection success in Schwann cells, leading to a reduction in lactate generation, and that infected Schwann cells are detrimental to nerve function.

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Poster

296. Oligodendrocyte and Schwann Cells Development and Myelination

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Topic: B.12. Glial Mechanisms

Support: NIH Grant R01 NS065218

Charles and Johanna Busch Memorial Funds at Rutgers University

Title: Nectin-like 2 (Necl-2) cell adhesion molecule is a negative regulator of Schwann cell myelination

Authors: *P. MAUREL, M.-S. CHEN, C. HEFFERNAN, M. RODRIGUEZ Dept. of Biol. Sci., Rutgers - The State Univ. of New Jersey, Newark, NJ

Abstract: Axo-glial interactions are critical for Schwann cell genesis, myelination and domain organization of myelinated fibers. We previously characterized three members of the Nectin-like (Necl) cell adhesion molecules family as mediators of the axo-glial interaction along the internode, in the Peripheral Nervous System (PNS). Necl-4 is expressed by the myelinating Schwann cells whereas its ligand Necl-1 is expressed by the axon to be myelinated. Necl-2 expressed by both cell types, is also a ligand for Necl-1, although at a lower affinity than the Necl-1/Necl-4 pair. As we and others have shown that Necl-4 is needed for PNS myelination, it was assumed that Necl-1 would also be pro-myelinating. Our work has now shown that Necl-1 is

in fact inhibitory to myelination. We therefore extended our analysis to Schwann cell-expressed Necl-2, the other ligand for Necl-1. Using loss and gain of function approaches and the *in vitro* model of PNS myelinating, we demonstrate that Necl-2 is also inhibitory to the formation of myelin segments by Schwann cells. Our findings suggest that Necl-2 employs at least two mechanisms to prevent myelination. One is the down-regulation by Necl-2 of the promyelinating Akt signaling molecule. This is most dramatically observed upon axonal contact. The second mechanism is a dysregulation of small GTPases Rac1 and RhoA in Schwann cells, leading to dramatic changes in Schwann cell morphology and stress fibers organization. The stress fibers are almost completely absent in Schwann cells that do not express Necl-2, whereas the over-expression of Necl-2 induces a 3-fold increase in the number of lamellipodia formed by a single Schwann cell. We are currently evaluating how Schwann cell- axon interaction is perturbed by the absence or over-expression of Necl-2 in Schwann cells. Both Necl-1 and Necl-2 are expressed at birth, onward. However their developmental pattern of expression is in opposition. Necl-2 is strongly expressed during the first week post-nataly (onset of myelination) then its levels dramatically drop down, whereas the expression of Necl-1 increases over time. Our data suggests a new concept that Necl-1 and Necl-2 put a "negative clamp" on the promyelinating properties of Necl-4, at different time points of the myelination process.

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Poster

296. Oligodendrocyte and Schwann Cells Development and Myelination

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P30 Core HD03352

MBTG (NIH) T32 GM07215

SciMed GRS

Title: Reversing polycomb repression in schwann cells after nerve injury

Authors: *P. T. DUONG^{1,2}, K. H. MA^{2,2}, J. P. SVAREN^{3,2} ²Waisman Ctr., ³Dept. of Comparative Biosci., ¹Univ. of Wisconsin-Madison, Madison, WI

Abstract: The peripheral nervous system relies on Schwann cells to provide metabolic support, and insulating myelin that wraps the axons. Schwann cells also play a role in coordinating the regenerative response when peripheral nerves sustain injury. Schwann cells are a potential

therapeutic target as they have an ability to stimulate the functional recovery and restoration of damaged nerves. Underlying this regeneration potential, Schwann acquire a new state by transdifferentiating into repair cells. In this mode of reprogramming, a number of previously unexpressed genes become expressed after injury and are vital for axon regeneration. Our preliminary data have pinpointed epigenomic pathways that are responsible for the reprogramming of Schwann cells to their repair state after nerve injury. We found many regeneration genes are dually associated with repressive histone H3K27 methylation (H3K27me3) and H2A ubiquitination (H2AmUb) in mature nerve prior to injury. These histone modifications are formed by Polycomb Repressive Complex 2 (PRC2) and PRC1 respectively, which either independently or in conjunction repress transcription. ChIP profiling revealed significant loss of H3K27me3 and H2AmUb modifications on several genes that become activated upon nerve injury, such as sonic hedgehog and glial-derived neurotrophic factor (Shh and Gdnf). Furthermore, we have investigated H3K27 demethylases JMJD3/KDM6B and UTX/KDM6A as the putative mechanisms that facilitate the reversal of histone methylation on H3K27. Previous studies have shown JMJD3 induction at 4 days post injury (Gomez-Sanchez et al, 2013), and we find also that UTX appears to be upregulated in 1 and 5 post injury. These data support the hypothesis that reversal of polycomb repression by removal of H3K27 methylation is required for Schwann cell reprogramming after nerve injury. Analysis of Schwann cell-specific knockouts of these demethylases will decipher this early step of the regeneration timeline and test whether reversal of Polycomb repression is a requirement in initiating the injury response.

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Poster

296. Oligodendrocyte and Schwann Cells Development and Myelination

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 296.08/P5

Topic: B.12. Glial Mechanisms

Support: NIH Grant R01NS100464

Title: Schwann cell metabolism: Is glycolysis favoured to support axons?

Authors: G. DELLA FLORA NUNES¹, *M. FELTRI², Y. POITELON¹, E. HURLEY¹, M. PATEL³, L. WRABETZ¹, *M. FELTRI^{2,1}

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Abstract: The energetic requirement of the nervous system is extremely high in proportion to its size. In addition, some axons of peripheral neurons extend over 1m in humans, raising the question of how their metabolic needs are attained at regions distant from the neuronal cell body. A natural candidate to perform metabolic support functions is Schwann cells (SCs), the glia of the peripheral nervous system. As they ensheath both myelinated and non-myelinated axons along their length, SCs could provide metabolites to fuel mitochondrial activity in axons. Furthermore, partial oxidation of substrates might be favored in SCs to fulfill the need of carbons for lipid biosynthesis to produce and maintain myelin. However, the metabolic properties of SCs are poorly understood. In order to investigate the metabolic needs of SCs, we first bred mice expressing floxed Pdha1 gene and CNPase-Cre to generate Pdha1f/f; CnpCre/+ mice and Pdha1f/f littermate controls. The Pdha1f/f; CnpCre/+ animals lack pyruvate dehydrogenase complex (PDC) activity in SCs. PDC is responsible for connecting the glycolysis with the TCA cycle trough the conversion of pyruvate to acetyl-CoA. Surprisingly, there was no significant change in myelination, nerve conduction velocity or motor performance in Pdha1f/f; CnpCre/+ mice at 1 month. In addition, results were corroborated and extended with Pdha1f/f; P0-Cre mice, which did not show any morphological, behavioural or functional alteration up to 10 months. Taken together, these results indicate that PDC function might be dispensable in SCs. A particularly intriguing hypothesis is that SCs satisfy their energetic requirement via glycolysis and secrete partially oxidised metabolites, such as lactate, to be used by axonal mitochondria. We are currently investigating the SC intermediary metabolism using the Seahorse Xp Flux Analyzer. Moreover, we are characterizing mitochondrial location and morphology in Schwann cells throughout development using the photo-activatable mitochondria (PhAM) mouse, which allows fluorescent labeling of mitochondria in SCs upon recombination mediated by P0-Cre. Understanding the energetic adaptations of SCs could reveal new targets for drug development and medical strategies aiming to restore nerve function or prevent axon damage in demyelinating diseases.

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Poster

296. Oligodendrocyte and Schwann Cells Development and Myelination

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Topic: B.12. Glial Mechanisms

Support: NMSS FG 1927-A-1

Title: AMPA receptor signaling regulates the fate of oligodendrocyte progenitors

Authors: *A. AGARWAL¹, L. CHAKRAVARTI², K. SPARKS², A. MENON², D. E. BERGLES³

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Abstract: Oligodendrocyte precursor cells (OPCs) express Ca²⁺ permeable AMPA receptors and form synapses with unmyelinated axons throughout the CNS, but the role of this signaling in vivo remains undefined. The primary obstacle to defining the role of this neuron-glial cell signaling is that OPCs express the same complement of receptors as neurons, precluding pharmacological manipulations. To overcome this limitation, we developed a conditional knockin mouse line in which the formation of functional AMPA receptors can be prevented cell autonomously by expressing a dominant-negative GluA2 subunit (EGFP-dnGluA2), in which a glutamine to arginine substitution was made in the pore region. EGFP-dnGluA2 was targeted to the ROSA26 locus with a conditional allele to enable cell-type specific expression. To evaluate whether dnGluA2 subunits are expressed at levels sufficient to modulate AMPA receptor function in OPCs, we bred these mice to NG2-CreER/+ mice. Double transgenic offspring were injected with 4-hydroxytamoxifen (4HT) at various developmental stages and analyzed 2-20 weeks later. Tetrameric AMPA receptors containing this subunit are non-conducting and OPCs expressing dnGluA2 lack AMPA receptor-mediated EPSCs. Fate tracing revealed that blocking AMPA receptor function in OPCs in vivo enhanced their survival and rate of proliferation during development, and reduced their differentiation into oligodendrocytes (OLs). OPCs lacking functional AMPA receptors were also morphologically less complex and extended shorter processes. To investigate the role of this signaling in controlling the behavior of OPCs following demyelination, mutant and control mice were either fed with 0.2% cuprizone or subjected to unilateral focal demyelination by injecting 1.0% lysolecithin into the corpus callosum. The number of OPCs expressing dnGluA2 was significantly enhanced in demyelinated lesions in both conditions, indicating that recruitment of OPCs is normally reduced by AMPA receptor signaling. However, fate tracing revealed that the effect of AMPA receptor blockade on OPC differentiation was distinct in the two contexts - OL generation was reduced following cuprizone-induced demyelination, which lacks an adaptive immune response, and enhanced following lysolecithin-induced demyelination, which is associated with infiltration of peripheral immune cells. These results indicate that the effect of AMPA receptor signaling in OPCs is highly context dependent, either promoting or inhibiting lineage progression depending on the environment and perhaps the pattern of activity exhibited by the axons they form synapses with.

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Poster

296. Oligodendrocyte and Schwann Cells Development and Myelination

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Topic: B.12. Glial Mechanisms

Support: SINAPSE Seed Fund R-175-000-121-733

GSK R-179-000-003-592

Title: Optogenetic, electrical and electromagentic stimulation for subcellular induction of neuron activity in myelination studies *In vitro*

Authors: *A. BLASIAK, D. WONG, N. V. THAKOR, J. YOO, I. YANG Singapore Inst. for Neurotechnology, NUS, Singapore, Singapore

Abstract: Objective: Neuron activity modulates myelination and is considered a therapeutic target for treating demyelination diseases. Neuron activity induction can be applied to a whole cell *in vitro*, but is cumbersome *in vivo*. Alternatively, the stimulation can be applied focally, i.e. to the distal axons or to the somata only. Our objective was to develop a platform to study the mechanisms of activity-dependent myelination after focal neuron stimulation. Materials and Methods: Primary neurons were cultured in a two-chamber (somatic and axonal) device such, that the primary oligodendrocyte precursor cells (OPCs) plated in the axonal chamber could interact exclusively with the distal axons. This device was integrated with novel stimulators electrical, optogenetic, and electromagnetic (ESTIM, OSTIM and MSTIM, respectively) - for inducing neuron activity via the stimulation in the somatic (SomaSTIM), axonal (AxonSTIM) or both chambers (WholeSTIM). The effects on neuron activity and myelination were assessed by calcium imaging, cAMP level measurements, and through quantification of OPCs differentiation markers and morphology. **Results:** We integrated the culture device with platinum electrodes to apply ESTIM. Calcium firing and increased cAMP level showed that the stimulation successfully induced neuron activity. Interestingly, an indirect stimulation of axons (SomaSTIM), direct stimulation of axons and OPCs (AxonSTIM), and direct stimulation of the whole system (WholeSTIM) had the same effects on myelination. To achieve subcellular OSTIM we: (i) successfully induced neuronal expression of channelrhodopsin; (ii) modified the device material so it blocked the light, but remained biocompatible; (iii) integrated the device with custom build programmable LED array. In contrast to ESTIM, OSTIM exclusively stimulated neurons and not OPCs. Nevertheless, the effects of SomaSTIM, AxonSTIM and WholeSTIM on myelination were the same. Subcellular MSTIM does not require modification of neuronal expression or the culture device but it requires generation of pulsatile magnetic field spatially restricted to the size of one chamber. The stimulator and its effects on myelination are being characterized. Conclusions: The cellular mechanisms of ESTIM and OSTIM are not the

same. However, the consistency in their results strongly suggests that focal stimulation is sufficient to increase axon myelination. SomaESTIM and OSTIM results further imply that the activity-dependent myelination does not require direct stimulation of OPCs. MSTIM platform has the biggest translational potential as it is the least invasive out of the three presented neuron stimulation methods.

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Poster

296. Oligodendrocyte and Schwann Cells Development and Myelination

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Topic: B.12. Glial Mechanisms

Support: NSF Graduate Research Fellowship

Title: Diverse patterns of myelination along individual axons in the adult cerebral cortex

Authors: *C. CALL, D. E. BERGLES

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Abstract: Axons in the cerebral cortex exhibit diverse patterns of myelination, with some axons devoid of myelin, some exhibiting discontinuous patches of myelin, and others continuous myelin that is interrupted only by nodes of Ranvier. It is unclear when these distinct patterns are established, whether different classes of neurons exhibit consistent myelination and if these patterns are stable over time or modified by experience. To define the distinctive characteristics of cortical axon myelination in the adult brain, we examined the distribution of myelin along single axons in layer I of somatosensory cortex. In this region, a wide array of axons from different cell types comingle within the same microenvironment, such that resident oligodendrocytes are provided a diverse set of myelination targets. Thus, differences in myelination are likely to reflect axonal differences in oligodendrocyte preference and myelin stability. Using confocal laser scanning microscopy in horizontal sections of flattened cerebral cortex, we measured the distribution of myelin internodes along individual corticocortical, thalamocortical, and interneuron axons for up to 2-3 mm in adult (5 months old) mice. For the cell types that were myelinated (parvalbumin+ interneurons, neurexophilin-4+ layer 6b/7 neurons, and neurons from ventromedial and posterior thalamic nuclei), the distribution of myelin was highly variable between different axons of the same cell type, as well as across different branches of a single axon. These results suggest that although cell identity can bias myelination of a given axon, other factors (e.g. activity or local environment) appear to influence the pattern of myelination that is established.

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Poster

296. Oligodendrocyte and Schwann Cells Development and Myelination

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Topic: B.12. Glial Mechanisms

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NMSS RG 4748-A-13

NMSS 1962-A-1

Title: Axonal domain components are transported independently in separate vesicles

Authors: *Y. BEKKU¹, J. L. SALZER^{1,2}

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Abstract: The trafficking and targeting of proteins to distinct sites underlies the exquisite, polarized organization of neurons. Myelinated axons are further organized into a series of functionally and biochemically distinct domains, i.e. the nodes, paranodes, juxtaparanodes, and internodes. This organization provides a striking example of the differential targeting of proteins to subdomains of the axon and is essential for effective saltatory conduction. Of particular interest are the nodes of Ranvier, which are enriched in a multimeric complex of voltage gated Na⁺ channels form and their associated beta subunits, the neuronal cell adhesion molecules (CAMs) neurofascin (NF) 186 and NrCAM, and the cytoskeletal protein ankyrin G.We previously reported that NF186 and other axonal CAMs redistribute to the node and to other domains from pre-existing pools of diffusely distributed proteins on the axon surface (Zhang et al., Neuron 2012). In contrast, ion channels and ankyrin G require transport to the node during myelination. The slow replenishment of all nodal components in mature myelinated fibers also depends on transport. We have now directly investigated how these proteins are transported within the axon prior to and after myelination. To this end, we live-imaged neurons engineered to express combinations of proteins labeled with distinct fluorescent tags. In general, each nodal protein is anterogradely transported in separate vesicles except for NrCAM and NF186, which are co-transported in the same vesicles. Likewise, CAMs destined for other axonal domains are independently transported in distinct vesicles. In contrast, there is significant co-transport of retrogradely transported proteins presumably during recruitment into a common endocytic vesicle. These patterns of transport did not change with myelination. Taken together with our earlier study, these results support a model in which nodal component are sorted to separate
vesicles and transported independently to the axon rather than as a preformed complex; upon myelination, they assemble at the node via a combination of local recruitment (CAMs) and de novo transport (channels and cytoskeletal proteins).

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Poster

297. Amyloid-Beta Tau Interaction

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Amyloidosis Research Committee, surveys and research on special disease

Longevity Science Committee of the Ministry of Health and Welfare of Japan

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Title: Abeta oligomers in synaptic lipid rafts induce phosphorylated tau and impair signal transduction

Authors: *T. KAWARABAYASHI, S. NARITA, K. SATO, T. NAKAMURA, Y. SEINO, M. HIROHATA, N. NAKAHATA, M. SHOJI Hirosaki Univ. Grad. Sch. of Med., Hirosaki, Japan

Abstract: Lipid rafts are crucial platform in $A\beta$ production and aggregation, leading to the formation of neurotoxic $A\beta$ oligomers in the brain. Recent findings suggest that lipid rafts are pathological signaling platforms where $A\beta$ oligomers exert synaptic failure and neurotoxicity. We examined whether $A\beta$ oligomers induce phosphorylated tau and impair signal transduction in

lipid rafts from synaptosome of Tg2576 mice. Tg2576 mice and nontransgenic littermates (4, 6, 12, and 22 month old, Tg n=18, NonTg n=18) were used. Synaptosome fraction was separated by Ficoll gradient fractionation. Lipid rafts fraction was separated from synaptosome by sucrose gradient fractionation. In Tg2576 mice, the amount of phosphorylated tau and Fyn increased in synaptosome, and also in lipid rafts of synaptosome with accumulation of A β oligomers. Accumulation of phosphorylated tau in lipid rafts of synaptosome began at 6 month when the behavioral disturbance appears in Tg2576 mice. A β oligomers may induce phosphorylated tau and Fyn-NMDA receptor signal transduction pathways in lipid rafts of synapse.

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Poster

297. Amyloid-Beta Tau Interaction

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 297.02/Q1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R21AG051946

NIA/NIH R01AG051386

UCLA Easton Center grant JV-68800

Title: Extracts from AD synapses accelerate In vitro tau propagation

Authors: *E. E. MIYOSHI¹, T. BILOUSOVA¹, M. ALAM², J. J. CAMPAGNA², C. J. ELIAS², A. HATAMI², D. FAKHRUTDINOV¹, V. JOHN², K. H. GYLYS¹ ¹Sch. of Nursing and Mary S. Easton Ctr. for Alzheimer's Dis. Res., ²Drug Discovery Lab, Dept. of Neurol. and Mary S. Easton Ctr. for Alzheimer's Dis. Res., UCLA, Los Angeles, CA

Abstract: The microtubule-associated cytoskeletal protein tau has been considered primarily axonal in healthy brains, while in Alzheimer's (AD) the tau gradient shifts toward somatodendritic compartments where it accumulates as intracellular aggregates. Tau is now generally thought to be released into the extracellular space from synaptic terminals, facilitating transynaptic spreading across neural circuits, but this process is poorly understood. Our previous work has shown that C-terminal truncated tau is abundant in synaptic terminals, and that tau and exosomes are released by depolarization from Alzheimer's disease synaptosomes. In the present work, synaptosomes were prepared from cryopreserved cortical (A7) samples from AD cases, from A β -independent tauopathy cases, and from cognitively normal aged control cases. The Tau RD P301S FRET biosensor system was used to evaluate the capability of human synaptosome-derived extracts for seeding tau aggregation. P-2 (crude synaptosome) extracts were added to the

biosensor cell media, and after 48 hours, cells were analyzed for FRET fluorescence using an ImageStream flow cytometer. Extracts from normal (n=2) and tauopathy cases (n=3) generated little FRET signal (<5% positive); in contrast, samples from two AD cases propagated tau aggregation, generating FRET signal in 43% and 28% of the populations examined (10,000 cells/sample). These results are consistent with the hypothesis that oA β localized to AD synapses accelerates tau propagation in this *in vitro* system. Using this system, clarification of synaptic tau release mechanisms in human samples can provide important insights with respect to disease progression and tau immunotherapies.

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Poster

297. Amyloid-Beta Tau Interaction

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Support: APA Dissertation Research Award

Phi Kappa Phi Love of Learning Award

GMU Osher Lifelong Learning Institute (OLLI)

Title: Characterization of a novel APP/tau transgenic mouse model of Alzheimer's disease

Authors: *S. L. LIPPI, M. L. SMITH, T. T. DIMOPOULOS, J. M. WOOD, A. BOOTH, J. M. FLINN

George Mason Univ., Fairfax, VA

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder that consists of the accumulation of amyloid and tau proteins in the brain, causing deficits in learning, memory, and behavior. Mouse models of AD highlight the role of amyloid, but tau is often less studied. Dyshomeostasis of trace metals in the brain, including Zinc (Zn) plays important roles in the progression of AD-pathology and remediation may serve as an area of future therapeutics. The current study utilizes a novel double transgenic mouse bred to express mutated human amyloid precursor protein (hAPP) and mutated human tau (P301L). Offspring are the result of a J20 x rTg4510 cross (Jackson Lab); this is the first mouse to be made from these parent mice. Half of the mice were given 10 parts per million (ppm) Zn in their drinking water and behavior was assessed at 3.5 months. Previous research shows that Zn supplementation can impact behavior in Tg mouse models of AD. Three and a half months represents an early time point in disease

progression, when we can assess Zn supplementation on behavior early in the disease. Behaviors were assessed through the following tests: open field (OF), elevated zero maze (EZM) and Barnes Maze. Preliminary data show significant differences of genotype in time spent in the center of the OF and the percent time in the open arms of the EZM. Mice carrying mutations in both hAPP/tau (double) and only tau spent more time in the open arms of the EZM and spent less time in the center of the OF compared to those animals with no mutations (wildtypes). A significant genotype x water interaction was also seen with number of head dips in the EZM. Significant differences in spatial memory were seen in the Barnes Maze; animals carrying mutations in only tau and both hAPP/tau showed increased latencies to escape and find the target hole compared to wildtype animals and those with only the presence of the CaMKIIa promoter. Behavior will also be assessed at 7 months to examine changes as a result of increased cellular pathology and long-term Zn supplementation.

Disclosures: S.L. Lippi: None. M.L. Smith: None. T.T. Dimopoulos: None. J.M. Wood: None. A. Booth: None. J.M. Flinn: None.

Poster

297. Amyloid-Beta Tau Interaction

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 297.04/Q3

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Impairments in activities of daily living and wheel running behavior in a novel APP/tau mouse model of Alzheimer's disease

Authors: *M. L. SMITH, *M. L. SMITH, S. L. P. LIPPI, J. M. WOOD, T. T. DIMOPOULOS, C. M. HERNANDEZ, J. M. FLINN George Mason Univ., Fairfax, VA

Abstract: Alzheimer's Disease (AD) is characterized by the formation of beta-amyloid plaques and hyperphosphorylated tau tangles which slowly damage the brain. Zinc (Zn) is a biometal that can alter progression and remediation of AD. This novel mouse model includes both amyloid and tau pathology, and examines how they impact disease progression. J20 (hAPP) and rTg4510 (tau) mice were obtained from Jackson Laboratory and bred to produce APP/tau offspring. Mice were split into groups and received either 10 parts per million Zn in their drinking water, or standard laboratory water. Animals were tested at 3.5 months of age in activities of daily living including nesting, burrowing, and circadian wheel running behavior. Preliminary data show animals having both hAPP/tau perform significantly worse in both nesting and burrowing, as early as 3.5 months of age. The animals' wheel running activity was assessed for one week. Differences were seen in activity between genotypes. These non-cognitive tasks are important to study because the brain accumulates amyloid plaques and tau tangles, which may be impacting these behaviors years before diagnosis of AD. This study attempts to allow early detection of AD by measuring innate behaviors, which may offer insight into early detection, diagnosis, and treatment. No metal differences were seen at 3.5 months, in contrast to results in EZM (see linked poster, Lippi et. al). Behavior will also be assessed at 7 months of age to examine the effects of long term zinc exposure.

Disclosures: M.L. Smith: None. S.L.P. Lippi: None. J.M. Wood: None. T.T. Dimopoulos: None. C.M. Hernandez: None. J.M. Flinn: None.

Poster

297. Amyloid-Beta Tau Interaction

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 297.05/Q4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Prosetta

Title: A common upstream target? Novel small drug-like molecules decreasing toxic Abeta species and tau phosphorylation simultaneously

Authors: *V. R. LINGAPPA¹, A. MÜLLER-SCHIFFMANN², D. DEY¹, S. SELVARAJAH¹, V. ASUNDI¹, C. KORTH²

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Abstract: Background

Alzheimer's Disease (AD) is characterized by senile plaques consisting of the A β peptide and neurofibrillary tangles formed by hyperphosphorylated tau. Aggregation of Abeta and tau play key roles in progression of AD, but, so far, a compelling molecular scenarios connecting both proteins has not been established.

We hypothesize that many enzymes involved in biochemical pathways don't just work together, but are physically associated, and their association does not come about spontaneously, but rather is catalyzed, by transient multi-protein complexes (MPCs) we term assembly machines. In our view, A β aggregates and tau hyperphosphorulation are both downstream consequences of aberrant assembly machine action and should be correctable by targeting the previously unappreciated assembly machines.

Over the last decade, we have established a library of 300 small molecules targeting the assembly machines that catalyse viral capsid formation, termed the Hit-finder collection. By virtue of the diversity of viral structure, the Hit-finder collection is a promising starting point for discovery of assembly machine modulators able to alter events in the pathogenesis of AD.

Objectives

To screen the Hit-finder collection for compounds interfering with both oligomerization of $A\beta$

and phosphorylation of tau, simultaneously.

Methods

We generated cell models that secrete either monomeric or toxic oligomeric and N-terminal elongated monomeric A β forms, and cell lines that express mutant tau. For drug target identification we used drug resin affinity chromatography (DRAC) followed by mass spectrometry.

Results

We identified a subset of chemotypes that, after structure-activity relationship optimization, reduced the secretion of toxic $A\beta$ species at low nanomolar concentrations. DRAC revealed at least 8 proteins, some of which had already been described in the context of AD thus supporting the validity of our working hypothesis and screening approach. The most potent molecules also reduced total monomeric $A\beta$ load without affecting gamma-secretase activity, or APP expression. Interestingly, some of the most active compounds against $A\beta$ also reduced the phosphorylation of tau at specific sites in a dose dependent manner.

Conclusion

Novel compounds act against MPC assembly machines upstream of $A\beta$ and tau aggregation and counter their misassembly into aggregates and tangles. The exact nature of these upstream MPC assembly machines is beginning to be revealed and will advance our understanding of AD cellular pathology.

Disclosures: V.R. Lingappa: A. Employment/Salary (full or part-time):; all Time Employee of Prosetta. **A. Müller-Schiffmann:** None. **D. Dey:** A. Employment/Salary (full or part-time):; FTE Prosetta. **S. Selvarajah:** A. Employment/Salary (full or part-time):; FTE Prosetta. **V. Asundi:** A. Employment/Salary (full or part-time):; FTE Prosetta. **C. Korth:** 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.; roject in part funded by Prosetta, but own salary independentt.

Poster

297. Amyloid-Beta Tau Interaction

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 297.06/Q5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant EY022358

Title: Exogenous tau produces sectorial degeneration in the murine retinal projection

Authors: *M. A. SMITH¹, E. S. PLYLER¹, G. N. WILSON³, C. M. DENGLER-CRISH², S. D. CRISH⁴

²Pharmaceut. Sci., ¹Northeast Ohio Med. Univ., Rootstown, OH; ³Kent State Univ., Akron, OH; ⁴Pharmaceut. Sci., NEOMED, Rootstown, OH

Abstract: Retinal pathologies are suggested to be some of the earliest changes in chronic neurodegenerations such as Alzheimer's disease (AD). Glaucoma, a group of chronic optic neuropathies that make up the leading cause of blindness worldwide, has recently been shown to have epidemiological and mechanistic similarities to AD. Along these lines, several lab groups have reported somatodendritic accumulation of the microtubule associated protein tau with advancing pathology in common mouse models of glaucoma. Axonal and trans-synaptic transport of tau is postulated to play a large role in the onset and progression of several neurodegenerations, yet the mechanisms remain unknown. We investigated whether application of tau to the retina would produce glaucomatous pathologies in normal mice. We injected fibrillized Alexa Fluor-tagged recombinant human tau (FITau) into the vitreal chamber of C57BL/6J mouse eyes. After two weeks we found extensive astrogliosis, microglial activation, and loss of the retinal ganglion cell synaptic markers in subcortical visual structures corresponding to the injected eyes. Furthermore, these projections exhibited distinct sectorial patterns of pathology in the superficial superior colliculus (SC). Retinotopic sectors of SC exhibiting normal distribution of neural and glial markers were adjacent to areas with extreme gliosis and reduced synaptic marker immunoreactivity—mimicking the sectorial axonopathy seen in glaucoma and diabetic retinopathy. These pathological changes in SC were accompanied by increased levels of the FlTau, indicating that it was taken up and transported by retinal ganglion cells. Additionally, we saw increased gliosis and reduced synaptic markers in the primary visual cortex contralateral to injected eyes. Fluorescence-tagged tau label was also present in this region, a finding that suggests trans-synaptic transport occurred and produced pathology. We conclude that intraocular administration of tau induces neurodegeneration that mimics sectorial pathologies in other diseases affecting the retina and its projection. Furthermore, given its accessibility, simplicity, and straightforward connectivity, we believe that the primary visual system may be a useful and accessible model for studying tau-related mechanisms of neurodegeneration.

Disclosures: M.A. Smith: None. E.S. Plyler: None. G.N. Wilson: None. C.M. Dengler-Crish: None. S.D. Crish: None.

Poster

297. Amyloid-Beta Tau Interaction

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 297.07/Q6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant EY022358

Prevent Blindness Fellowship for Female Scholars in Vision Research

Title: Ocular amyloid-beta induces sectorial gliosis in the distal retinal projection

Authors: *E. S. PLYLER¹, M. A. SMITH¹, C. M. DENGLER-CRISH², S. D. CRISH³ ²Pharmaceut. Sci., ¹Northeast Ohio Med. Univ., Rootstown, OH; ³Pharmaceut. Sci., NEOMED, Rootstown, OH

Abstract: The anterior visual system (AVS; retina, optic nerve, and optic tract) is a common site of neurodegeneration in blinding disorders and in more widespread degenerative conditions of the central nervous system (CNS) such as multiple sclerosis. Intriguingly, amyloid-beta (A β), a neurotoxic protein relevant to Alzheimer's disease (AD), was recently identified in post-mortem retinal tissue of AD patients as well as in the visual system of certain animal models of AD. Moreover, the retina and other parts of the AVS has been implicated as some of the earliest sites of pathology in AD, with amyloidosis occurring before the degeneration of hippocampus and entorhinal cortex that characterizes this disorder. Amyloid-β aggregation and tau accumulation were recently reported to occur in age-related macular degeneration, diabetic retinopathy, and glaucoma. Given that the AVS is accessible, simple, and topographically mapped, it is an ideal system for studying mechanisms of, and interventions targeting, neurodegeneration. We exploited the accessibility of this system to study the effects of AB on axonopathy and neuroinflammation, two processes heavily implicated in CNS degenerations. To conduct these studies, we injected fluorophore-tagged, fibrillized Aß into the vitreal chamber of C57BL/6J mice and used immunofluorescent techniques to stain brain, nerve, and retinal tissue after a 2week survival period. Within two weeks, amyloid fibrils were evident throughout the retina along with hypertrophied astrocytes in the nerve fiber layer. We also found pronounced astrogliosis and microglial activation in major retinal targets in the brain, including the lateral geniculate nucleus and superior colliculus. Even though our injection procedure exposed the entire retina to Aβ oligomers, gliosis was sectorial, i.e. occurring in discrete retinotopic regions, with surrounding areas appearing normal. This parallels the pattern of gliosis and degeneration in glaucoma and diabetic retinopathy, two blinding disorders where abnormal Aß aggregation has been implicated. In contrast with our previous findings in mouse models of glaucoma, Aβinduced collicular gliosis preceded axonal transport deficits and overt axon or cell body loss. Overall, our findings suggest a direct role for retinal amyloidosis in AD and other chronic neurodegenerations, emphasizing the importance of studying the AVS as it relates to a widerange of neurodegenerative disorders.

Disclosures: E.S. Plyler: None. M.A. Smith: None. C.M. Dengler-Crish: None. S.D. Crish: None.

Poster

297. Amyloid-Beta Tau Interaction

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 297.08/Q7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Research start-up funds to CMD-C

Title: Retinofugal degeneration in Alzheimer's disease: Evidence of axonal transport deficits in aged 3xtg mice

Authors: *C. M. DENGLER-CRISH¹, M. A. SMITH², A. J. SCHULLER³, E. S. PLYLER², S. D. CRISH⁴

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Abstract: The scope of structures damaged in Alzheimer's disease (AD) is rapidly expanding beyond the classic cortical and hippocampal associated with the hallmark cognitive and memory deficits of this disorder. Of these expansions, none may show more promise in helping us understand the pathogenesis and progression of degeneration, as well as develop much needed biomarkers and interventions, than the visual system. In other disorders affecting retinal ganglion cells (RGCs), such as glaucoma and diabetic retinopathy, axonal transport deficits and axonopathy precede cell body loss. Using neuronal tract tracing, immunofluorescence, and capillary-based western blotting techniques on a Wes Simple Western platform, we examined the integrity of the retinofugal projection in 3xtg mice, a widely-used model that develops agerelated, progressive AD-like neuropathology. Aged 3xtg mice (10+ months) showed loss of CTB transport from the retina to the superior colliculus (SC) and this was not seen in young (3 mo.) 3xtg or age-matched background-specific (B6129SJ) and C57BL6J controls. This transport loss occurred in "sectors" of the retinotopic SC similar to the pattern of degeneration seen in mouse glaucoma models. RGC counts did not differ between young and old 3xtg mice or from controls and nearly 100% of RGCs in 3xtg retina took up and packaged CTB for transport. Intraocular pressure (a major risk factor for glaucoma) was not elevated in 3xtg mice as a function of age or as compared to controls. Quantification of phosphorylated tau (ptau) load in retina, optic nerve (ON) and retinorecipient SC using capillary electrophoresis showed significantly increased ptau-231 burden in ON and SC, but not retina, at 10 mo. Overall, these findings are indicative of axonal transport deficits and tauopathy in the retinal projection of a progressive, pathological model of Alzheimer's disease. This underscores the utility of the anterior visual system in expanding our approach to studying and understanding neurodegenerative disease.

Disclosures: C.M. Dengler-Crish: None. M.A. Smith: None. A.J. Schuller: None. E.S. Plyler: None. S.D. Crish: None.

Poster

297. Amyloid-Beta Tau Interaction

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 297.09/Q8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 4U54MD008621-04

1T34GM105550-01A1

Title: Mechanism of toxicity mediated by the interplay of tau, amyloid- β and α -synuclein

Authors: K. S. INGRAM¹, E. WIAFE², B. FORBES², T. LEWTER², S. TATE², M. GUERRERO-MUNOZ³, *D. L. CASTILLO-CARRANZA²

¹Minority Men's Hlth. Initiative / Hampton Univers, Hampton, VA; ²Minority Men's Hlth., ³Dept. of Chem. Engineering, Sch. of Engin. and Technol., Hampton Univ., Hampton, VA

Abstract: Alzheimer's disease (AD) is the most common type of dementia caused by a mechanistic relationship between misfolded protein tau and amyloid- β . Previous literature has disclosed evidence that acknowledges the role of α -synuclein as a third key toxic component involved in the disease. Although a crosstalk between these abnormal proteins have been suggested, the mechanism of toxicity remain unclear. Dysfunctional tau is key in most types of dementia. However, while amyloid- β promotes tau missorting from the axon to the cell body and dendrites, tau mediates the detrimental effects of amyloid- β . This suggests amyloid- β and tau act synergistically to promote neurodegeneration. Interestingly, recent studies in mice have suggested that an increase in α -synuclein levels depend upon synergy between amyloid- β and tau. However, amyloid-beta aggregates alone do not promote cognitive decline; and neither dysfunctional tau nor α -synuclein reproduce the full effects of AD. Thus, dissecting the relationship between abnormal proteins tau and amyloid- β is pivotal in understanding the initial events that may drive α -synuclein dysfunction and the subsequent neurodegenerative process that characterizes AD. Errant proteins tau, amyloid- β , and α -synuclein are known to form pre-fibrillar aggregates called oligomers (multiples of monomeric proteins). These are considered the toxic entity of the disease and have been found within synaptic compartments in AD brains. We previously showed that oligomers of tau and α -synuclein exist in the same aggregates, forming hybrid oligomers in Parkinson's disease cases, providing evidence of co-occurrence of asynuclein and tau into their most toxic forms. Herein, we investigated the synergistic relationship of these oligomeric species that is essential in identifying the mechanisms of toxicity in AD. Using stable cells lines, we found that both, amyloid- β and α -synuclein oligomers potentiate tau oligomers toxicity and accelerates neuronal death. Our findings provide evidence of the mechanism by which amyloid- β enhances the harmful effects of tau, thus, contributing to disease progression.

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Poster

297. Amyloid-Beta Tau Interaction

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 297.10/Q9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CONICYT PFB 12/2007

FONDECYT 1160724

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FONDECYT 3140395

Title: Amyloid beta and Tau neuropathology increases with aging in a natural model of Alzheimers Disease (Octodon degus)

Authors: *C. B. LINDSAY¹, D. S. RIVERA², P. CISTERNAS³, N. C. INESTROSA⁴ ¹Pontificia Univ. Catolica De Chile, Santiago, Chile; ²Pontificia Univ. Católica de Chile, Santiago, Chile; ³CARE-UC, Pontificia Univ. Católica de Chile, Santiago, Chile; ⁴Ctr. For Aging and Regeneration (CARE), P. Catholic Univ. of Chile, Santiago, Chile

Abstract: Alzheimer's disease (AD), one of the most disabling syndromes in the adult population and the most common form of dementia in the world, has not cure or even an effective treatment. AD is characterized the presence of senile plaques of amyloid beta $(A\beta)$ peptide and neurofibrillary tangles of phosphorylated *tau* protein in the brain that trigger neuronal loss and consequently a severe cognitive deficit which then precludes the entire life. In the present work, we explore the hippocampus of different groups of young and old Octodon degus (O. degus), an animal model for the study of non-genetic AD maintained in a clear acrylic aquarium (50x35x23 cm) under standard conditions; with food and water ad libitum, without exercise or environmental enrichment. AD hallmarks were observed by biochemical and histological approaches finding almost 3-fold higher A β_{1-42} and A β_{1-40} peptide levels (≈ 20 pg/ml in young animals and ≈ 60 pg/ml in old animals), at least 2 times increased levels of Aβoligomers and the presence of A β aggregates called senile plaques in different areas of the hippocampus and cortex of old O. degus compared to young ones (up to 6 plaques per 300µm² of hippocampus in old animals and no plaques in young animals). Furthermore, up to 3-fold increased levels of phosphorylated *tau* protein at different epitopes related to AB and microtubule dissemble (Thr231, Ser235, Ser202 and Thr205) were observed in the old animals. In agreement, the presence of *tau* protein aggregates were found in the old O. degus group compared to the

young group (from 0 to 1 in young *O.degus* and up to 8 positive cells per field in old *O. degus*). Our data support the idea that *O. degus* present the main neuropathology of AD, and therefore is an optimal study model for this disease.

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Poster

298. Dementia: Proteinopathy and Pathology Other Than Abeta and Tau

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 298.01/Q10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG054025

NIH Grant NS094557

Title: Dipeptide repeat-containing protein oligomers in ALS and FTD

Authors: *M. CARRETERO MURILLO^{1,2}, S. A. MCALLEN^{1,2}, U. SENGUPTA^{1,2}, J.

RUDRA³, R. KAYED^{1,2}

¹Neurol., Univ. of Texas Med. Br. At Galveston, Galveston, TX; ²Mitchell Ctr. for Neurodegenerative Dis., ³Pharmacol. and Toxicology, Univ. of Texas Med. Br., Galveston, TX

Abstract: The C9*orf*72 hexanucleotide repeat expansion is one of the most common causes of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD); two disorders which display different protein aggregates. The C9*orf*72 hexanucleotide expansion is a structural polymorphism formed by a GGGGCC repeat region that impedes traditional transcription, and leads to several abortive transcripts. These transcripts can then undergo non-AUG dependent translation, leading to the production of several dipeptide repeat-containing proteins (DPRs) including GP, GA, GR, PA or PR. These DPRs form neuronal inclusions in patients with ALS and FTD, and several studies indicate that these inclusions are neurotoxic. Among the different dipeptides produced by the non-AUG translation, there are some such as GA, which have aggregative properties, as well as the tendency to sequester other proteins in the cell. Furthermore, with the increasing evidence that oligomeric species are the main cause of neurotoxicity in disorders such as Alzheimer's disease and Parkinson's disease, and with the ability of these DPRs to aggregate, we decided to investigate the formation of DPR oligomers. We demonstrate that some of these DPRs not only form oligomers, but also cross-seed with other

proteins, causing their gain in neuronal toxicity, and perhaps the loss of function of important molecular pathways. Using immunohistochemical, immunofluorescent, and immunoblot techniques, we show the formation of these oligomeric species, and their cross seeding *in vitro*. These results show that the aggregation of DPR is harmful to the cells, and may be acting via several mechanisms including direct toxicity by DPR oligomers alone and in combination with other oligomeric proteins. Thus, the ability to detect oligomeric DPR using specific antibodies has great potential to further our understanding of these diseases and aid in the development of targeted therapeutics.

Disclosures: M. Carretero Murillo: None. **S.A. McAllen:** None. **U. Sengupta:** None. **J. Rudra:** None. **R. Kayed:** None.

Poster

298. Dementia: Proteinopathy and Pathology Other Than Abeta and Tau

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 298.02/Q11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIGMS-GM60654

NCRR-RR003037

Title: Genetic modification of proteasome expression in Drosophila

Authors: *T. SCHMIDT-GLENEWINKEL¹, C.-H. YEH¹, M. E. FIGUEIREDO-PEREIRA² ¹Biol. Sci., Hunter Col. of CUNY, New York, NY; ²Hunter Col., New York, NY

Abstract: Unfolded, misfolded, mutant, and oxidatively damaged proteins are a severe and never ending threat to cell survival. The ubiquitin proteasome system (UPS) degrades these abnormal proteins to prevent protein aggregation and avoid disrupting cellular homeostasis. To investigate the proteotoxic effects induced by impaired UPS *in vivo*, we developed a Drosophila model with "tunable" proteasome dysfunction by regulating the expression of its d β 5 catalytic subunit through double-stranded RNA interference (RNAi). The duration and expression level of the d β 5 RNAi were controlled by an RU486 inducible act5C promoter. We first assessed the effects of long term downregulating d β 5 in adult male and female flies. D β 5 downregulation induced shorter lifespan, proteasome impairment, accumulation and aggregation of ubiquitinated (Ub)-proteins, locomotor dysfunction, and low resistance to oxidative stress in both fly genders. In females only, a potentially new 20S proteasome form exhibiting chymotrypsin-like activity was detected upon d β 5 downregulation. We next investigated the effects of short term (5 days) proteasome dysfunction. We controlled the duration and level of d β 5 downregulation via the conditional Gene-Switch binary system, by altering the length of RU486 administration. Our

results demonstrate that transient proteasome impairment negatively and irreversibly affects lifespan and locomotor activity. Moreover, short term proteasome impairment was sufficient to induce long lasting Ub-protein accumulation and aggregation that was not reversed upon proteasome recovery. In conclusion, we clearly show that transient proteasome impairment is critical to the formation of irreversible Ub-protein aggregates. Moreover, short term proteasome impairment in adult flies can have long term negative and irreversible consequences.

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Poster

298. Dementia: Proteinopathy and Pathology Other Than Abeta and Tau

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 298.03/Q12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG054025

NIH Grant NS094557

Title: TDP-43 oligomers in AD pathogenesis

Authors: *S. A. MCALLEN^{1,2}, M. CARRETERO MURILLO^{1,2}, U. SENGUPTA^{1,2}, R. KAYED¹

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Abstract: TDP-43 is a protein that binds to both DNA and RNA and is present mostly in the nucleus of the cell. This protein is required in many cell functions such as microRNA biogenesis, RNA splicing, and in neurons, TDP-43 has a much more critical role in synaptic plasticity and axonal transport. The essential role of TDP-43 was demonstrated in rodents when knockouts were embryonic lethal. The diseases frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are characterized by inclusion bodies that are formed when TDP-43 has been ubiquitinated and hyperphosphorylated into aggregates that are mislocalized into the cytoplasm. The role of TDP-43 in these diseases suggested that the protein is also involved in Alzheimer's Disease (AD). The primary pathology of AD is $A\beta$ senile plaques and Tau neurofibrillary tangles (NFT), though more recently TDP-43 aggregates have been shown in AD as well. A large amount of evidence suggests that Tau, a protein that is important to axonal transport and microtubule stability, forms oligomers that are the toxic entity in AD and other neurodegenerative diseases. Immunohistochemistry and biochemical analyses have shown that TDP-43 oligomers are present and interact with Tau and Aβ in AD cases and we utilize these techniques to further investigate these interactions. By using both generic and newly generated antibodies against TDP-43 oligomers in immunoblot and immunohistochemistry techniques, we

aim to establish the incidence of these oligomers in Alzheimer's Disease. We will investigate the relationship between TDP-43 aggregates and Tau in the oligomerization process and determine if TDP-43 and Tau form hybrid oligomers within the AD brain. Investigating the formation and interactors of TDP-43 oligomers in AD could lead to a better understanding of the role of cross-seeding in the formation, stability and toxicity of the different protein aggregates in AD and may lead to the development of disease therapies.

Disclosures: S.A. McAllen: A. Employment/Salary (full or part-time):; University of Texas Medical Branch. M. Carretero Murillo: None. U. Sengupta: None. R. Kayed: None.

Poster

298. Dementia: Proteinopathy and Pathology Other Than Abeta and Tau

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 298.04/R1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Consortium for Frontotemporal Dementia Research

NIH Grant F32 NS090678 Glenn/AFAR Postdoctoral Fellowship NIH Grant R01NS075487 NIH Grant P30NS47466

Bluefield Project to Cure FTD

Title: Restoration of progranulin to progranulin-deficient mice corrects lysosomal abnormalities

Authors: *A. E. ARRANT¹, D. E. UNGER¹, V. C. ONYILO¹, E. D. ROBERSON² ¹Neurol., Univ. of Alabama At Birmingham, Birmingham, AL; ²Neurol & Neurobio, UAB, Birmingham, AL

Abstract: Loss of function mutations in progranulin (*GRN*) are a major autosomal dominant cause of frontotemporal dementia (FTD), and likely cause disease through progranulin haploinsufficiency. Homozygous *GRN* mutations that produce complete progranulin deficiency cause the lysosomal storage disorder neuronal ceroid lipofuscinosis (NCL), revealing that progranulin is critical for proper lysosomal function. Impaired lysosomal function may also play a role in FTD due to *GRN* mutations (FTD-*GRN*), as brains from FTD-*GRN* patients exhibit elevated levels of lysosomal proteins. As in humans, progranulin-deficient mice (*Grn*^{+/-} and *Grn*^{-/-}) exhibit lysosomal abnormalities throughout the brain, with elevated levels of lysosomal proteins.

granules. In this study, we investigated whether brains from progranulin-deficient mice and FTD patients with *GRN* mutations exhibit similar lysosomal abnormalities, and tested whether restoration of progranulin to progranulin-deficient mice would reverse these abnormalities. $Grn^{+/-}$ and $Grn^{-/-}$ mice, as well as FTD-*GRN* patients, exhibited increased activity of β -Hexosaminidase A (HEXA), which was associated with increased protein and RNA levels of the enzyme. Brains from FTD-*GRN* patients and $Grn^{-/-}$ mice had reduced activity of β -glucocerebrosidase (GBA), an enzyme with previously reported abnormal trafficking in $Grn^{-/-}$ macrophages. $Grn^{-/-}$ mice also exhibited additional enzymatic changes not present in FTD-*GRN* samples. Restoration of progranulin to $Grn^{-/-}$ mice by intracranial injection of an adeno-associated viral (AAV)-progranulin vector normalized activity of lysosomal enzymes and reduced lipofuscin levels throughout the brain. These data indicate that lysosomal dysfunction due to progranulin deficiency is reversible, which supports use of progranulin-boosting therapies for FTD-*GRN* patients, particularly in the early stages of disease.

Disclosures: A.E. Arrant: None. D.E. Unger: None. V.C. Onyilo: None. E.D. Roberson: None.

Poster

298. Dementia: Proteinopathy and Pathology Other Than Abeta and Tau

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 298.05/R2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA AG031574

NIA AG017139

Title: Disruption of ion channel gradients in aged cognitively-impaired rats

Authors: *L. A. BEAN¹, T. F. MUSIAL¹, M. L. RUSSO¹, R. BORENSTEIN¹, S. A. MULLEN¹, G. D. AYALA¹, M. M. OH², J. F. DISTERHOFT³, D. NICHOLSON¹ ¹Neurolog. Sci., Rush Univ. Med. Ctr., Chicago, IL; ²Dept Physiol., Northwestern Univ. Med. Sch., Chicago, IL; ³Northwestern Univ. - Chicago, Chicago, IL

Abstract: Age-related memory changes are not homogenous within similar aged-groups. A few older people will not experience problems with cognitive function and retain a "youth-like" brain phenotype. However, most people will notice an increase in forgetfulness and take longer to learn and recall information, tasks that rely heavily on the hippocampus. Fortunately, symptoms of normal cognitive aging are not necessarily a harbinger of dementia; nevertheless if memory problems worsen, it may signal advancement into mild cognitive impairment, which is an intermediate stage between normal age-related memory changes and the more serious disabling

dementia. Therefore it is necessary to fully understand the neurobiology of aging to preclude the advancement of cognitive malfunction. The substrates involved in age-related cognitive decline are unknown. There is no major neuron or synapse loss in the hippocampus with this non-disease associated type of cognitive impairment. However, it has been proposed that voltage-signaling and/or Ca²⁺-signaling networks or interactomes within the neuron are disrupted leading to impairments in memory. We previously showed that single-dendrite gradients in synapse number and ion channels /receptors exist in young rats. For example, the number of synapses on an oblique dendrite decreased with distance from the main apical dendrite, and AMPAR expression increased while NMDAR expression decreased with distance from the soma. While immunolocalization of many ion channels and /or receptors is known within the young and aged hippocampus, little is known about the relative expression of these channels to one another. In this study, we employ multiple antibodies and high-resolution Array Tomography to visualize differences in ion channel gradients in electrophysiologically-characterized dendrites within the dorsal CA1 region of rats identified as being young (Y), aged-unimpaired (AU) or aged-impaired (AI) by two hippocampal-dependent memory tasks. We find disruption of ion channel interactomes involved in voltage and Ca^{2+} regulation in aged impaired animals. These experiments are necessary to identify targets for pharmacological approaches in preventing cognitive decline with age.

Disclosures: L.A. Bean: None. T.F. Musial: None. M.L. Russo: None. R. Borenstein: None. S.A. Mullen: None. G.D. Ayala: None. M.M. Oh: None. J.F. Disterhoft: None. D. Nicholson: None.

Poster

298. Dementia: Proteinopathy and Pathology Other Than Abeta and Tau

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 298.06/R3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA AG031574

NIA AG017139

Title: Alzheimer's disease-linked HCN channelopathy rescued via intrahippocampal infusion of carvedilol

Authors: *T. F. MUSIAL, L. A. BEAN, M. L. RUSSO, S. A. MULLEN, G. AYALA, D. A. NICHOLSON Neurolog. Sci., Rush Univ. Med. Ctr., Chicago, IL **Abstract:** In various transgenic mouse models of AD, we find that both supra- and sub-threshold voltage signaling, both of which are heavily influenced by passive and active dendritic properties, progressively deteriorate with age in hippocampal CA1pyramidal neurons. Utilizing functional, molecular, and ultrastructural imaging techniques, we identify the hyperpolarization-activated, cyclic nucleotide-gated (HCN) channel as a primary suspect responsible for this disrupted voltage signaling.

Calcium signaling abnormalities are thought to be a major agent involved in cellular dysfunction associated with AD. We sought to manipulate internal calcium signaling via the endoplasmic reticulum, and in doing so, elicit store depletion induced h-channel plasticity (SD-h plasticity) to re-establish HCN function in these altered neurons.

Delivering intrahippocampal infusions of carvedilol (a non-specific beta-adrenergic blocker with affinity to antagonize ryanodine receptors) in anaesthetized transgenic mice, we find that HCN channel function is indeed re-established using patch-clamp recordings of hippocampal slices post-infusion. In addition, some hippocampal sections were processed for pre-embedding immunogold electron microscopy to determine channel distribution after induction of SD-*h* plasticity. These results confirm the treatment increased insertion of the channel into plasma membranes as well as appearing to increase protein trafficking to the distal dendrites where these channels are critical for proper CA1 neuron dendritic computations.

Disclosures: T.F. Musial: None. L.A. Bean: None. M.L. Russo: None. S.A. Mullen: None. G. Ayala: None. D.A. Nicholson: None.

Poster

298. Dementia: Proteinopathy and Pathology Other Than Abeta and Tau

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 298.07/R4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIAAG031574

Title: Alterations in the failure rate of antidromic action potential generation in mouse models of Alzheimer's disease

Authors: *M. L. RUSSO, G. D. AYALA, S. A. MULLEN, D. NICHOLSON Neurolog. Sci., Rush Univ. Med. Ctr., Chicago, IL

Abstract: The substantial cognitive deficits experienced by patients with Alzheimer's disease (AD) have long been thought to be the product grey matter changes. However, recent work has indicated that disruptions in neural network connectivity may be attributable to changes in white matter (WM) integrity during AD progression. Diffusion tensor imaging (DTI) has revealed significant volume changes in the white matter of patients with early AD and mild cognitive

impairment (MCI). These changes are particularly robust in the parahippocampal gyrus and hippocampus, which are both highly susceptible to AD pathology. In addition, oligodendrocytes have exhibited sensitivity to the cytotoxic effects of amyloid beta (Aβ), the principle component of the characteristic AD plaques. Therefore, cognitive decline during MCI and AD may be, at least in part, due to disrupted connectivity that results from decreased integrity of WM. Given the primary function of myelin in facilitating rapid action potential (AP) propagation along axons via saltatory conduction, it is possible to examine physiological properties that depend on myelination of axons. In the present study, we employed whole cell patch clamp physiology to monitor AP generation in the axons of CA1 pyramidal neurons of wild-type and AD transgenic mice. Specifically, antidromic APs were generated by a stimulating electrode placed in the alveus, and successful AP generation and propagation was monitored using a somatic recording electrode. Two types of protocols were used to assess each neuron's ability to generate antidromic APs. The first consisted of two pulses spaced at varying frequencies from 2 Hz to 500 Hz, while the second consisted of a train of ten stimuli at frequencies between 10 Hz and 100 Hz. Our results have shown that failure rate is increased in the CA1 pyramidal neurons of aged AD mice, consistent with observed changes in the WM of AD patients and animal models. Interestingly, the increased failure rate appears to be more pronounced at specific stimulus frequencies, regardless of the number of pulses used. Overall, our findings support previously reported WM changes in AD, as well as provide physiological characterization of the consequences of these WM alterations in a specific portion of the hippocampal circuit.

Disclosures: M.L. Russo: None. G.D. Ayala: None. S.A. Mullen: None. D. Nicholson: None.

Poster

298. Dementia: Proteinopathy and Pathology Other Than Abeta and Tau

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 298.08/R5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: JZ101 is required for mitochondrial quality control

Authors: *L. CHAO, J. ZHANG Peking Union Med. Col., Beijing City, China

Abstract: Mitochondrial dysfunction is a prominent feature in many neurodegenerative diseases, such as Parkinson's disease (PD). Many tail-anchored (TA) proteins are located to the endoplasmic reticulum (ER) membrane. However, TA proteins may misplace on mitochondria rarely and affect mitochondrial normal function. Here, we identified a new mitochondrial out membrane protein named JZ101 which mediates the clearance of the TA protein on mitochondria. And, JZ101 overexpression enhanced the fusion of mitochondria, whereas JZ101 deficiency results in more fragmented mitochondria and impaired energy metabolism of

mitochondria, which could be rescued by restoring the expression of JZ101. Our study may provide a potential target for neurodegenerative diseases.

Disclosures: L. Chao: None. J. Zhang: None.

Poster

298. Dementia: Proteinopathy and Pathology Other Than Abeta and Tau

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 298.09/R6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Dr. Amantha Thathiah startup grant

Title: CRISPR/Cas9-mediated generation of a GPR3 knockin mouse

Authors: *Y. HUANG¹, C. FERGUSON², G. E. HOMANICS^{1,2,3,4}, A. THATHIAH^{1,4,5,6} ¹Neurobio., ²Anesthesiol., ³Pharmacol. and Chem. Biol., Univ. of Pittsburgh, Pittsburgh, PA; ⁴Univ. of Pittsburgh Brain Inst., Pittsburgh, PA; ⁵Pittsburgh Inst. for Neurodegenerative Dis., Pittsburgh, PA; ⁶KU Leuven Ctr. for Human Genet. and Leuven Inst. for Neurodegenerative Dis. (LIND), Leuven, Belgium

Abstract: Alzheimer's disease (AD) is the most common type of dementia and is characterized by the insidious degeneration of brain networks involved in memory and cognition. Accumulation and aggregation of the amyloid β (A β) peptide in the brain are pathological hallmarks of AD. Sequential cleavage of the amyloid precursor protein (APP) by the β - and γ secretases leads to generation of the AB peptide. We have shown that the orphan G proteincoupled receptor 3 (GPR3) plays an important role in regulation of the γ -secretase complex and Aβ generation in AD. However, investigation of the *in vivo* functions of GPR3 is challenging due to the lack of availability of suitable antibodies that recognize endogenous mouse GPR3. To circumvent this issue with other GPCRs, fluorescently and hemagglutinin (HA) labeled GPCR knockin (KI) mice have been generated to study the physiological and pathophysiological function of several GPCRs. Several genome editing systems, including transcription activatorlike effector nucleases (TALEN), clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein-9 nuclease (Cas9), and zinc finger nucleases (ZFNs) have been used to insert fluorescent proteins and HA tags to study receptor function. Among the three genome editing technologies, CRISPR/Cas9 is efficient, highly specific, and easier to design than TALEN and ZFNs. In the current study, we utilized a CRISPR/Cas9-mediated genome editing strategy to insert two HA tags into the murine Gpr3 gene. By co-injection of guide RNAs (gRNAs), Cas9 mRNA, and single-stranded DNAs (ssDNA) containing the HA tag sequence into the one-cell mouse embryos, we successfully introduced two HA tags into the N-terminus of the Gpr3 gene in the first generation founder mice. One founder has been bred and successful

germline transmission has been confirmed. The HA-GPR3 KI mice are currently being used to investigate the expression, localization, and binding partners of GPR3 *in vivo*. The protein expression pattern and localization of GPR3 in different cell types will be essential to understand the physiological roles of endogenous GPR3 and to evaluate potential therapeutic approaches targeting GPR3 in AD.

Disclosures: Y. Huang: None. **C. Ferguson:** None. **G.E. Homanics:** None. **A. Thathiah:** None.

Poster

299. LRRK2 Mechanisms, Targets, and Pathways

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 299.01/R7

Topic: C.03. Parkinson's Disease

Support: MJFF Grant 11604

Title: In silico simulation of LRRK2 and rab3 vesicle cycle interactions in Parkinson's disease

Authors: *L. E. VINCENT¹, J. W. RYAN², D. A. DODDS², A. D. LEE², B. BEHROUZ² ¹Neuropsychology, ²Neurosci., Neuroinitiative, Jacksonville, FL

Abstract: In 2004, two articles were published which suggested mutations in the leucine-rich repeat kinase 2 (LRRK2) gene cause autosomal dominant Parkinson's disease (PD). This gene encodes for the protein of the same name, which impacts vesicular trafficking within the cell through phosphorylation of a subset of rab GTPases. Effector proteins, in addition to guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) activate and deactivate rabs for their various tasks. Rab activation occurs when a GEF exchanges the GTPase's GDP for GTP, while inactivation occurs when a GAP initiates hydrolysis of GTP to GDP. A great number of GEFs and GAPs exist in the cell, which provides both specificity, and some redundancy, to this highly specialized system. At present, LRRK2 has been shown to phosphorylate 14 of these rabs: rab3A, rab3B, rab3C, rab3D, rab5A, rab5B, rab5C, rab8A, rab8B, rab10, rab12, rab29, rab35, and rab43. While phosphorylation of these rabs decreases their affinity for GEFs and GDIs, it is unclear how this may impact the downstream effects of the rab proteins. In this study, NeuroInitiative's, Simulation Environment for Experimental Design (SEED), was used to run in silico pathway models of rab3A, rab3B, rab3C, and rab3D, which play a role in vesicle trafficking and exocytosis. Data from baseline LRRK2 models was compared with mutated LRRK2 (G2019S) and knockout models.

Disclosures: L.E. Vincent: A. Employment/Salary (full or part-time):; NeuroInitiative. J.W. Ryan: A. Employment/Salary (full or part-time):; NeuroInitiative. D.A. Dodds: A.

Employment/Salary (full or part-time):; NeuroInitiative. **A.D. Lee:** A. Employment/Salary (full or part-time):; NeuroInitiative. **B. Behrouz:** A. Employment/Salary (full or part-time):; NeuroInitiative.

Poster

299. LRRK2 Mechanisms, Targets, and Pathways

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 299.02/R8

Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation

Title: Inhibition of LRRK2 kinase activity results in abnormal protein dynamics and reduces protein stability

Authors: *B. J. SANSTRUM, B. M. S. S. GOO, D. Z. Y. HOLDEN, D. D. DELGADO, N. G. JAMES

Cell and Mol. Biol., Univ. of Hawaii at Manoa, Honolulu, HI

Abstract: Mutations in the LRRK2 (Leucine Rich Repeat Kinase 2) gene have been linked to both sporadic and familial forms of Parkinson's disease (PD). Our lab has performed many fluorescence-based microscopy experiments to study the protein-protein interactions of LRRK2-EGFP in live cells. We have demonstrated that the active, membrane bound form of LRRK2-EGFP is predominantly a dimer due to self-association on the membrane. However, lack of knowledge about the mutations that alter the kinase function of this protein has resulted in limited targeted treatment options. The most common PD-linked LRRK2 mutation, G2019S, has been shown to increase the kinase activity of LRRK2 resulting in major deficits in endocytosis and vesicle trafficking. Because of this finding, current drug discovery efforts have been geared toward the optimization of LRRK2 kinase inhibitors as a potential disease modifying therapy. Controversially, previous studies have shown adverse side effects of LRRK2 kinase inhibitors in peripheral tissues and decreased LRRK2 protein concentration in mouse brain lysates treated at therapeutic doses. Since these studies were performed postmortem, little data have been shown in functional living systems. This project expanded past experiments by adding LRRK2 kinase inhibitors (100nM) to quantify their effects on LRRK2-EGFP function in a live dopaminergiclike neuronal system. We utilized 2-photon confocal microscopy and total internal reflection fluorescence microscopy (TIRF) to monitor protein dynamics and self-association of LRRK2-EGFP in the cytosol and at the membrane. Raster image correlation spectroscopy (RICS), photon counting histogram (PCH), and number and brightness (N&B) replicated the decreases in LRRK2 protein concentration but also indicated a novel increase in dimer and higher-order oligomers and a decreased diffusion rate in both wild type (WT) LRRK2-EGFP and G2019S LRRK2-EGFP expressing cells. This suggests an alteration in protein dynamics that is

contradictory to the intended function of kinase inhibitors. In order to characterize this functional abnormality in more therapeutically relevant models, we lowered the drug doses to IC50 concentration and conducted spatial intensity distribution analysis (SpIDA) on endogenously expressed protein. These methods noted similar alterations in oligomerization and concentration when compared to vehicle treated controls. Overall, these studies show striking results about the effects of LRRK2 kinase inhibitors on protein dynamics. Follow up studies are needed to determine the extent of these notable abnormalities and the efficacy of LRRK2 kinase inhibitors for novel PD therapy.

Disclosures: B.J. Sanstrum: None. B.M.S.S. Goo: None. D.Z.Y. Holden: None. D.D. Delgado: None. N.G. James: None.

Poster

299. LRRK2 Mechanisms, Targets, and Pathways

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation

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Spanish Ministry of Economy and Competitiveness (SAF2014-58653-R)

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Ministère de la Recherche et de la Santé (PHRC Convergence)

Inserm

Title: Parkinson's disease-associated mutations in LRRK2 cause centrosomal defects via Rab8a phosphorylation

Authors: *J. MADERO-PÉREZ¹, E. FDEZ¹, B. FERNÁNDEZ¹, A. J. LARA ORDÓÑEZ¹, M. BLANCA RAMÍREZ¹, P. GÓMEZ-SUAGA¹, D. WASCHBÜSCH², E. LOBBESTAEL³, V. BAEKELANDT³, A. C. NAIRN⁴, J. RUIZ-MARTÍNEZ⁵, A. AIASTUI⁵, A. LÓPEZ DE MUNAÍN⁵, P. LIS⁶, T. COMPTDAER⁷, J.-M. TAYMANS⁷, M.-C. CHARTIER-HARLIN⁷, A. BEILINA⁸, A. GONNELLI⁹, M. R. COOKSON⁸, E. GREGGIO⁹, S. HILFIKER¹ ¹Inst. Parasitology and Biomedicine López-Neyra,CSIC, Granada, Spain; ²Dept. of Exptl. Tumorbiology, WestfälischeWilhelms Univ. Münster, Münster, Germany; ³Lab. for Neurobio. and Gene Therapy, KU Leuven, Leuven, Belgium; ⁴Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT; ⁵Div. of Neurosciences, InstitutoBiodonostia-CIBERNED, San Sebastián, Spain; ⁶Med. Res. Council Protein Phosphorylation and Ubiquitylation Unit, Col. of Life Sciences, Univ. of Dundee, Dundee, United Kingdom; ⁷Ctr. de Recherche Jean-Pierre AUBERT Neurosciences et Cancer, Univ. Lille, Lille, France; ⁸Lab. of Neurogenetics, Natl. Inst. on Aging, Natl. Inst. of Hlth., Bethesda, MD; ⁹Dept. of Biology, Univ. of Padova, Padua, Italy

Abstract: Mutations in LRRK2 are a common genetic cause of Parkinson's disease (PD). LRRK2 interacts with and phosphorylates a subset of Rab proteins including Rab8a, a protein which has been implicated in various centrosome-related events. However, the cellular consequences of such phosphorylation remain elusive. Here, we show that pathogenic LRRK2 causes deficits in centrosomal positioning with effects on neurite outgrowth, cell polarization and directed migration. Pathogenic LRRK2 also causes deficits in centrosome cohesion which can be detected in peripheral cells derived from LRRK2-PD patients as compared to healthy controls, and which are reversed upon LRRK2 kinase inhibition. The centrosomal cohesion and polarity deficits can be mimicked when co-expressing wildtype LRRK2 with wildtype but not phosphodeficient Rab8a. The centrosomal defects induced by pathogenic LRRK2 are associated with a kinase activity-dependent increase in the centrosomal localization of phosphorylated Rab8a, and are prominently reduced upon RNAi of Rab8a. Our findings reveal a new function of LRRK2 mediated by Rab8a phosphorylation and related to various centrosomal defects.

Disclosures: J. Madero-Pérez: None. E. Fdez: None. B. Fernández: None. A.J. Lara Ordóñez: None. M. Blanca Ramírez: None. P. Gómez-Suaga: None. D. Waschbüsch: None. E. Lobbestael: None. V. Baekelandt: None. A.C. Nairn: None. J. Ruiz-Martínez: None. A. Aiastui: None. A. López de Munaín: None. P. Lis: None. T. Comptdaer: None. J. Taymans: None. M. Chartier-Harlin: None. A. Beilina: None. A. Gonnelli: None. M.R. Cookson: None. E. Greggio: None. S. Hilfiker: None.

Poster

299. LRRK2 Mechanisms, Targets, and Pathways

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 299.04/R10

Topic: C.03. Parkinson's Disease

Title: Rats carrying human LRRK2 G2019S mutation show dopaminergic vulnerability and gait abnormalities after peripheral LPS induced inflammation

Authors: *S. SUBRAMANIAM¹, E. HINGCO¹, N. NECKEL², J. WANG², R. MALHAS¹, R. MIRAMONTES¹, X. SU², M. S. FIANDACA¹, H. J. FEDEROFF¹ ¹Neurol., Univ. of California Irvine, Irvine, CA; ²Neurosci., Georgetown Univ. Med. Ctr., Washington DC, DC Abstract: Abstract: Leucine-rich repeat kinase (LRRK2) mutations are associated with autosomal dominantly inherited Parkinson's disease (PD). Genome-wide-association-studies (GWAS) indicate that the genetic locus containing the LRRK2 gene also presents a risk factor for sporadic PD. A mutation in the LRRK2 kinase domain, G2019S, is most common in familial and apparently sporadic forms of PD. Such a mutation increases the kinase activity by ~two-fold, with toxic effects confirmed in vitro and in vivo. Whether LRRK2 kinase activity is essential for its role in PD is still hotly debated. Despite involvement of LRRK2 in several neuronal pathways, including cytoskeletal dynamics, vesicular trafficking, mitochondrial function, and autophagy, its physiological function and role in PD pathology remains unclear. In the current rodent study, we induced peripheral inflammation via single intraperitoneal (ip) injection of lipopolysaccharide (LPS) to transgenic rats expressing the human G2019S mutation featuring the bacterial artificial chromosome (BAC) form of the entire human LRRK2 genome locus (BAChLRRK2-G2019S). BAC-hLRRK2-G2019S rats showed elevated levels of the pro-inflammatory cytokine IL-1ß in nigral tissue 90 days after a single LPS ip injection. In addition, 90 days after LPS administration there was loss of dopaminergic neurons in the substantia nigra and striatal dopaminergic neurites in BAC-hLRRK2-G2019S rats. Moreover, LPS treated BAC-hLRRK2-G2019S rats also exhibited gait abnormalities as early as 4 days after ip injection, which progressively worsened until day 60. Non-transgenic animals showed no pathobiology following LPS administration. In conclusion, these results suggest that LRRK2-G2019S carriers may be predisposed to excessive focal brain responses to a peripheral inflammatory stimulus, resulting in PD-like neurotoxicity and motoric deficits. To advance our investigations, we have generated novel rat BAC transgenic lines that express either hLRRK2-G2019S or hLRRK2-WT but lack endogenous rat LRRK2 (rLRRK2-/-) (BAC-hLRRK2-G2019S::rLRRK2-/-, BAC-hLRRK2-WT::rLRRK2-/-). These new transgenic lines will allow us to remove the contributory effect of endogenous LRRK2 thus yielding a precise model for future experiments. Using these animal models, we intend to more fully characterize mutant LRRK2 function as it relates to systemic and central neuroinflammation, nigrostriatal vulnerability and the potential to evaluate therapeutic options.

Disclosures: S. Subramaniam: None. E. Hingco: None. N. Neckel: None. J. Wang: None. R. Malhas: None. R. Miramontes: None. X. Su: None. M.S. Fiandaca: None. H.J. Federoff: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on a patent application.

Poster

299. LRRK2 Mechanisms, Targets, and Pathways

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 299.05/S1

Topic: C.03. Parkinson's Disease

Support: KHIDI Grant HI14C0093

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NRF Grant 2015R1A2A2A01003080

Yonsei University Future-leading Grant 2015-22-0055

Title: Parkinson's disease-linked LRRK2 positively regulates type 1 Interferon-induced inflammatory signaling and cytokine production through selective DSCR1 phosphorylation

Authors: H. PARK¹, K. HAN¹, *H. RHIM², K. CHUNG¹ ¹Yonsei Univ., Seoul, Korea, Republic of; ²Korea Inst. Sci. Tech. (KIST), Seoul, Korea, Republic of

Abstract: LRRK2 is a Ser/Thr kinase with mixed lineage kinase-like and GTPase domains, and controls neurite outgrowth and cell death. Mutations in *LRRK2* gene is known to cause autosomal dominant late-onset Parkinsonism. Several studies have also suggested that LRRK2 is involved in innate immune response signaling, but the underlying mechanism is unknown yet. Down syndrome candidate region 1 (DSCR1, also known as regulator of calcineurin 1 or RCAN1) protein was shown to upregulate type 1 interferon-induced inflammatory signaling by modulating several intracellular targets of interleukins in immune cells. Moreover, DCSR1 negatively affects neuronal cell death and survival through increased susceptibility to oxidative stress. In this study, we investigated any biochemical and functional link between LRRK2 and DSCR1, and their roles in type 1 interferon-mediated inflammatory signaling. At the meeting, our recent data concerning the action of LRRK2 on DCSR1 and DSCR1-mediated downstream inflammatory signaling will be presented, In conclusion, our data demonstrate that LRRK2 directly phosphorylates DSCR1 and consequently acts as a positive regulator of inflammatory signaling though DCSR1 modulation under type 1 interferon stimulation. This study further imply that LRRK2 positively regulates inflammatory responses in Parkinson's disease.

Disclosures: H. Park: None. K. Han: None. H. Rhim: None. K. Chung: None.

Poster

299. LRRK2 Mechanisms, Targets, and Pathways

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Program#/Poster#: 299.06/S2

Topic: C.03. Parkinson's Disease

Support: NINDS (R01 NS071251)

NINDS (P50NS094733 to JS)

Title: Age-dependent dopaminergic neurodegeneration and impairment of the autophagylysosomal pathway in LRRK-deficient mice

Authors: *Y. YUAN¹, E. GIAIME¹, Y. TONG¹, L. WAGNER¹, J. SHEN^{1,2} ¹Dept. of Neurol., Brigham and Women's Hosp., Boston, MA; ²Program in neuroscience, Harvard Med. Sch., Boston, MA

Abstract: LRRK2 mutations are the most common genetic cause of Parkinson's disease, but its normal physiological role in the brain is unclear. We previously generated LRRK2-/- mice and found no detectable phenotypes in the brain, but the kidney develops striking age-dependent PDlike change. To determine whether the absence of the phenotypes in the LRRK2-/- brain is due to the relative high expression of its functional homologue LRRK1 in the brain, we developed double knockout mice lacking both LRRK1 and LRRK2. Here we show that inactivation of LRRK2 and LRRK1 results in earlier mortality and age-dependent, selective neurodegeneration. Loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and noradrenergic neurons in the locus coeruleus is accompanied with increases in apoptosis, whereas the cerebral cortex and cerebellum are unaffected. Furthermore, selective age-dependent neurodegeneration is only present in LRRK-/- but not LRRK1-/- or LRRK2-/- brains, and is accompanied with increases of α -synuclein and impairment of the autophagy-lysosomal pathway. Quantitative electron microscopy analysis revealed age-dependent increases of autophagic vacuoles in the SNpc of *LRRK*-/- mice before the onset of dopaminergic neuron loss. These findings revealed an essential role of LRRK in the survival of dopaminergic neurons and the regulation of the autophagy-lysosomal pathway in the aging brain.

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Poster

299. LRRK2 Mechanisms, Targets, and Pathways

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 299.07/S3

Topic: C.03. Parkinson's Disease

Support: Support from Michael J Fox Foundation grant 11014

Title: Altered slow-wave sleep in the LRRK2 G2019S mouse model of Parkinson's disease

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Abstract: The leucine-rich repeat kinase 2 (LRRK2) mutation represents a common genetic cause of Parkinson's disease (PD). Although REM sleep behavior disorder is associated with sporadic PD and the development of synucleinopathies, REM disorders are less common in LRRK2 PD (Kestenbaum and Alcalay, 2017). Furthermore, the LRRK2 mutation is associated with altered cortical synaptic excitability, which could alter sleep-associated oscillatory activity. Consequently, we sought to determine if transgenic mice with the LRRK2 G2019S mutation expressed changes in behavioral and physiological markers of sleep and sleep depth. To investigate this question, we measured cortical electroencephalography (EEG) and cortical and subcortical local-field activity in 15 LRRK2 G2019S and 10 wild-type (WT) C57bl/6 male mice. Physiological and behavioral data (EMG and inertia measurement data) were acquired during periods of rest and active foraging behavior. Surface electrodes were implanted above somatosensory (S1) and visual cortex (V1). We observed that LRRK2 G2019S mice showed an increase in EMG activity during sleep and behavioral epochs when compared to WT mice (p < p0.0005, Kruskal-Wallis with Tukey post-hoc). Analysis of inertial and EMG activity also revealed that LRRK2 G2019S mice had shorter sleep bouts during post-foraging sleep (p < 0.005), shorter inter-sleep bout intervals in the pre-foraging sleep epoch (p < 0.05), and spent proportionally more time sleeping in the pre-sleep epoch (p < 0.001). Physiological measurements indicated that LRRK2 mice had higher power in high gamma band frequencies (80-120Hz) in their cortical EEG LFP (p < 0.001) and also expressed a significant increase in the power of sleep spindles relative to WT controls (p < 0.05, Student's t-test) in the S1 trunk region. In contrast, no difference in the distributions of peak spindle frequencies and durations was observed between LRRK2 G2019S and WT mice. Our results suggest that the LRRK2 G2019S mutation alters behavioral and physiological markers of the depth of slow-wave sleep.

Disclosures: J. Wiegand: None. K. Gies: None. M.J. Bartlett: None. T. Falk: None. S.L. Cowen: None.

Poster

299. LRRK2 Mechanisms, Targets, and Pathways

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 299.08/S4

Topic: C.03. Parkinson's Disease

Support: Reta Lila Weston Institute

Title: Divergent autophagic signature in G2019S-LRRK2 mutation compared to idiopathic PD cases

Authors: A. MAMAIS¹, C. MANZONI², I. NAZISH³, T. WARNER³, M. R. COOKSON¹, P. LEWIS², *R. BANDOPADHYAY⁴

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Abstract: The gene encoding for Leucine Rich Repeat Kinase 2 (LRRK2) has been linked to familial and sporadic/idiopathic forms of Parkinson's disease (iPD), as well as cancer, leprosy and Crohn's disease, making this gene a target for discovery therapeutics. Since it's discovery as a PD gene in 2004, LRRK2 has been associated with a wide range of cellular functions but the physiological roles of LRRK2 remain unclear. The most prevalent and pathogenic LRRK2 mutations in PD have been shown to affect cellular macroauthophagy in a variety of cellular models^{1,2}. Furthermore, recent *in vivo* studies have recapitulated an effect of LRRK2 in autophagy signalling^{3,4}. This raises the question whether differential autophagic activity is relevant to disease progression in PD patients harbouring LRRK2 mutations. To test this, we examined the levels of autophagic markers in the basal ganglia of G2019S LRRK2 PD postmortem tissue, in comparison to pathology-matched iPD. An increase in LC3-II levels was observed in iPD compared to controls but this was not the case for G2019S PD cases that showed similar levels to control cases. Furthermore, significantly lower levels of ULK1, a downstream effector of mTOR and AMPK involved in autophagosome generation, were observed in G2019S compared to iPD cases. An increase in p62 was observed in iPD but not reflected in G2019S cases while Lamp1 levels were found to be divergent in these cases compared to iPD and controls. Immunohistochemistry of p62 on brain tissue recapitulated a distinct signature for G2019S PD. Our data highlight a divergence of G2019S PD carriers in terms of autophagic response in affected brain regions compared to iPD, similar to what we have reported for alpha-synuclein solubility in these cases⁵. It is possible that the deregulation of autophagy and altered alpha-synuclein solubility in the LRRK2 G2019S cases are inter-linked. References:

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- 3. Tong, Y. et al. Molecular Neurodegeneration. 7, 2 (2012).
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Disclosures: A. Mamais: None. C. Manzoni: None. I. Nazish: None. T. Warner: None. M.R. Cookson: None. P. Lewis: None. R. Bandopadhyay: None.

Poster

299. LRRK2 Mechanisms, Targets, and Pathways

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 299.09/T1

Topic: C.03. Parkinson's Disease

Title: Phosphorylation of p53 by LRRK2 induces microglial TNFα-mediated neurotoxicity

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Abstract: Leucine-rich repeat kinase (LRRK2), a major causal gene of Parkinson's disease (PD), functions as a kinase. The most prevalent mutation of LRRK2 is G2019S. It exhibits increased kinase activity compared to the wildtype LRRK2. Previous studies have shown that LRRK2 can phosphorylate p53 at T304 and T377 of threonine-X-arginine (TXR) motif in neurons. Reduction of LRRK2 expression or inhibition of LRRK2 kinase activity has been shown to be able to alleviate LPS-induced neuroinflammation in microglia cells. In this study, we found that LRRK2 could also phosphorylate p53 in microglia model BV2 cells. Transfection of BV2 with phosphomimetic p53 T304/377D significantly increased the secretion of pro-inflammatory cytokine TNFα compared to BV2 transfected with p53 wild type after LPS treatment. In addition, conditioned media from these transfected cells increased the death of dopaminergic neuronal SN4741 cells. Moreover, such neurotoxic effect was rescued by co-treatment with the conditioned media and etanercept, a TNFa blocking antibody. Furthermore, TNFa secretion was significantly increased in primary microglia derived from G2019S transgenic mice treated with LPS compared to that in cells derived from their littermates. These results suggest that LRRK2 kinase activity in microglia can contribute to neuroinflammation in PD via phosphorylating p53 at T304 and T377 site.

Disclosures: D. Ho: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Y BIOLOGICS. **J. Eun:** None. **W. Seol:** None. **I. Son:** None.

Poster

299. LRRK2 Mechanisms, Targets, and Pathways

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 299.10/T2

Topic: C.03. Parkinson's Disease

Support: NINDS (R01NS071251)

NINDS (P50NS094733 to JS)

Title: LRRK1 and LRRK2 function in the kidney

Authors: *G. HUANG¹, L. WANGER¹, J. SHEN^{1,2}

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Abstract: Dominantly inherited missense mutations in leucine-rich repeat kinase 2 (LRRK2) are the most common genetic cause of Parkinson's disease. LRRK1 and LRRK2 belong to the Roco family and share a high degree of sequence similarity. Our previous findings showed that LRRK2-deficient mice display an age-dependent dysregulation of protein degradation pathways in the kidney, including striking α -synuclein accumulation and aggregation and increases of apoptotic cell death and oxidative damage. To investigate the normal physiological role of LRRK and the relative contribution of LRRK1 and LRRK2 in carrying out LRRK function, we generated LRRK-/-, LRRK1-/- and LRRK2-/- mice. Analysis of these mutant mice and wild-type controls at the ages of 6, 10 and 15 months ages showed age-dependent reduction of body weight in LRRK-/- mice beginning at 6 months. LRRK1-/- mice also showed significant reduction of body weight at 15 months but not at 6 and 10 months, whereas LRRK2-/- mice are unaffected. In the kidney of LRRK-/- mice, significant increases of apoptotic cell death were detected at 6 months and were further increased at 10 and 15 months of age, and apoptosis was also increased in the kidney of LRRK2-/- mice beginning at 10 months, whereas apoptosis in the LRRK1-/kidney was unaffected. We also observed age- and genotype-dependent accumulation and aggregation of α -synuclein, ubiquitinated proteins and p62 in the kidney of these mutant mice. The detailed results of these analyses in all four genotypic groups will be presented.

Disclosures: G. Huang: None. L. Wanger: None. J. Shen: None.

Poster

299. LRRK2 Mechanisms, Targets, and Pathways

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 299.11/T3

Topic: C.03. Parkinson's Disease

Support: Supported by a donation from Ms. Marilyn Bishop

Title: Decreased Lamp2 CSF concentrations in female Parkinson's disease patients with LRRK2 mutations

Authors: *D. A. LOEFFLER¹, A. C. KLAVER¹, M. P. COFFEY¹, J. O. AASLY², P. A. LEWITT³

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Abstract: Chaperone-mediated autophagy (CMA) is the principal pathway for clearing alpha synuclein, the main component of Lewy bodies in Parkinson's disease (PD). Lysosomeassociated membrane glycoprotein 2a (Lamp2a), the rate-limiting protein in CMA, is thought to be decreased in the PD substantia nigra. The effect of LRRK2 gene mutations, the most frequent cause of inherited PD, on Lamp2a levels is unknown. We used ELISA to measure total Lamp2 (isoforms Lamp2a, Lamp2b and Lamp2c) in CSF from patients with sporadic PD (sPD, n = 31), PD patients with LRRK2 gene mutations (LRRK2 PD, n = 20), and healthy similar-aged control subjects with or without LRRK2 mutations (LRRK2 CTL = 30, CTL = 27). The lowest median Lamp2 value was in the LRRK2 PD group; median values (pg/mL) were sPD = 333, LRRK2 PD = 127, CTL = 436, and LRRK2 CTL = 412. The difference between Lamp2 concentrations in LRRK2 PD and LRRK2 CTL subjects was statistically significant (p = 0.02). Gender effects may have influenced this result, because the LRRK2 PD group had a larger proportion of females (80%) than the other three groups (52% - 63%) and Lamp2 levels in the 67 females in this study (median = 247 pg/mL) were lower than the levels of this protein in the 41 males (median = 778 pg/mL) (p = 0.0002). An ANOVA was therefore performed on log-transformed Lamp2 concentrations which simultaneously modeled gender, diagnosis, and interaction effects. Evidence was found for differences between the eight "gender*diagnosis" groups (p < 0.0001); Tukey-Kramer multiple comparison testing revealed that the log-transformed Lamp2 levels in LRRK2 PD females were statistically different (lower) than those in each of the other gender*diagnosis groups except for sPD females. These results suggest that CSF concentrations of Lamp2 may be lower in females than in males, and may also be decreased in female PD patients with LRRK2 mutations. Further studies are required to determine the correlation between CSF and substantia nigra Lamp2 levels.

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Poster

299. LRRK2 Mechanisms, Targets, and Pathways

Location: Halls A-C

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Support: The Michael J. Fox Foundation

Consolidated Anti-Aging Foundation

Paul and Susan Hansen

Harold and Ronna Cooper Family

Orchard Foundation

Title: LRRK2 G2019S mutation modulates intracellular and ER calcium homeostasis in human iPSC-derived neurons

Authors: *J. A. KORECKA¹, S. TALBOT², S. M. DE LEEUW¹, E. F. FERRARI¹, A. M. MOSKITES¹, L. BARRET², D. C. DINESH¹, F. M. JODELKA³, A. J. HINRICH³, T. OSBORN¹, C. J. WOOLF², M. L. HASTINGS³, O. ISACSON¹, P. J. HALLETT¹ ¹Neuroregeneration Res. Inst., McLean Hospital/Harvard Med. Sch., Belmont, MA; ²F.M Kirby Neurobio. center, Children's Hosp. Boston., Boston, MA; ³Dept. of Cell Biol. and Anat., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: The Leucine-Rich Repeat Kinase (LRRK2) G2019S gain of function gene mutation is one of the most prevalent mutations contributing to Parkinson's disease (PD) pathogenesis. Using human induced pluripotent stem cell (iPSC)-derived neurons carrying the LRRK2 G2019S mutation, we and others have shown that the LRRK2 mutation, which increases LRRK2 kinase activity, alters axon outgrowth, intracellular trafficking, mitochondrial health and autophagy. We have also shown that this mutation contributes to an increased vulnerability of iPSC-derived neurons to PD-associated cell stressors and can be rescued by treatment with LRRK2 inhibitors (Cooper et al., 2012, Sci Transl Med. 2012, 4;4(141):141ra90.). Previously we described that human iPSC-derived neurons carrying the LRRK2 G2019S mutation challenged with the sarco/endoplasmic reticulum Ca²⁺ -ATPase (SERCA) uptake blocker thapsigargin (THP), exhibit an increase in depolarization-induced calcium influx and a modified calcium decay (interpreted as buffering capacity), when compared to neurons derived from healthy subject controls. Here, we show that endoplasmic reticulum (ER) Ca²⁺ levels, measured using an ER specific calciummeasuring organelle-entrapped protein indicator (Cepia-ER), are lower in iPSC-derived forebrain and midbrain dopamine neurons carrying the LRRK2 G2019S mutation compared to healthy subject controls. The lower level of ER calcium in LRRK2 G2019S neurons is still present even after THP-induced SERCA block. This phenotype was ameliorated by treatment with an antisense oligonucleotide that partially decreases LRRK2 levels. qPCR analysis of key ER Ca²⁺ channels and regulators, and membrane Ca^{2+} channels, known to regulate store operated calcium entry (SOCE), indicates altered expression in LRRK2 G2019S neurons. In summary, these data suggest that the LRRK2 G2019S mutation alters intracellular calcium homeostasis, which could contribute to PD-specific neuronal dysfunction. Further studies will identify specific targets of the ER homeostasis pathway affected by the LRRK2 G2019S mutation.

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299. LRRK2 Mechanisms, Targets, and Pathways

Location: Halls A-C

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Topic: C.03. Parkinson's Disease

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Yonsei University Future-leading Research Initiative of 2015 Grant 2015-22-0055

Title: Parkinson's disease-linked LRRK2 regulates epigenetic histone tail acetylation and neuronal apoptosis through HDAC3 phosphorylation and modulation of its activity and localization

Authors: W. SHIN¹, K. HAN¹, H. RHIM², *K. C. CHUNG¹ ¹Dept Systems Biol., Yonsei Univ., Seoul, Korea, Republic of; ²Korea Inst. Sci. Tech. (KIST), Seoul, Korea, Republic of

Abstract: Parkinson's disease (PD) is characterized by slow, progressive degeneration of dopaminergic neurons in the substantia nigra. The cause of neuronal death in PD is largely unknown, but several genetic loci, including LRRK2, have been identified. LRRK2 has GTPase and kinase activities, and mutations in LRRK2 are the major cause of autosomal-dominant familial PD. Histone deacetylases (HDACs) remove acetyl groups from lysine residues on histone tails, promoting transcriptional repression via condensation of chromatin. Here, we demonstrate that LRRK2 binds to and directly phosphorylates HDAC3 at Ser-424, thereby stimulating HDAC activity. Specifically, LRRK2 promoted the deacetylation of Lys-5 and Lys-12 on histone H4, causing repression of gene transcription. Moreover, LRRK2 stimulated nuclear translocation of HDAC3 via the phoshorylation of karyopherin subunit $\alpha 2$ and $\alpha 6$. HDAC3 phosphorylation and its nuclear translocation were increased in response to 6-OHDA treatment. LRRK2 also inhibited myocyte-specific enhancer factor 2D activity, which is required for neuronal survival. LRRK2 ultimately promoted 6-OHDA-induced cell death via positive modulation of HDAC3. These findings suggest that LRRK2 affects epigenetic histone modification and neuronal survival by facilitating HDAC3 activity and regulating its localization.

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Poster

299. LRRK2 Mechanisms, Targets, and Pathways

Location: Halls A-C

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Topic: C.03. Parkinson's Disease

Support: NIH Grant R24OD019803

NIH Grant T32GM750738

NIH Grant P510D011106

NIH Grant UL1TR000427

Title: CRISPR/Cas9 genomic editing of leucine-rich repeat kinase 2 (LRRK2) in marmoset stem cells

Authors: *S. C. VERMILYEA^{1,2}, A. BABINSKI², S. GUTHRIE², T. G. GOLOS^{2,3,4}, M. E. EMBORG^{2,5}

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Abstract: LRRK2 G2019S is the most common mutation associated with familial Parkinson's disease (PD). Located in the kinase domain of LRRK2, G2019S facilitates substrate access to the kinase thus increasing the catalytic rate of the enzyme. Patient-derived induced pluripotent stem cells present increased activated caspase-3, simplification of neurites and cell body accumulation of α -synuclein after dopaminergic differentiation of the A9 subtype. Although some transgenic rodent species overexpressing human LRRK2 G2019S exist, there have not yet been similar modeling strategies applied to nonhuman primates. Moreover, utilization of clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 for precision editing, models that more closely mimic natural disease progression are now more achievable. In an effort to validate the common marmoset as a candidate species for a genomic model of PD, we first chose to investigate in vitro cell modeling strategies. The p.G2019S mutation derives from a g.G6055A mutation within exon 41. Here we report utilization of CRISPR/Cas9 that recognizes the G6055 region of the marmoset LRRK2 genome. We have successfully isolated a marmoset embryonic stem cell clone that contains bi-allelic nucleotide deletions that lead to a truncated form of LRRK2 (tLRRK2). After the clone was expanded, we administered a dual-SMAD inhibition protocol that our group previously validated for differentiating marmoset stem cells to neurons. The wild type (WT) parental cell line was differentiated in parallel as a control for phenotypic comparison. On day 28 of differentiation, the cells were incubated in a maturation medium for two weeks. The cells were then lifted, quantified, and replated to cover slips at an equal density

for morphological analysis and fixed one week later. After immunostaining for βIII-tubulin, 30 different fields for parental and edited cell lines were imaged. The ImageJ plugin NeuriteQuant was used for objective quantification of neurite complexity. Preliminary results suggest that tLRRK2 in neurons leads to a significant increase in average neurite length, number of branches per cell and overall neurite length per cell when compared to WT controls. There was no significant difference in the number of cells evaluated, number of neurites per cell, branches per neurite length and branches per neurite. Our results demonstrate that LRRK2 may play a critical role in the level of neurite complexity in marmoset neurons, and that disrupting functional LRRK2 expression may have a reverse effect of the gain-of-function G2019S mutation phenotype observed in human neurons. We are currently evaluating LRRK2 G2019S marmoset cells.

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Poster

299. LRRK2 Mechanisms, Targets, and Pathways

Location: Halls A-C

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Topic: C.03. Parkinson's Disease

Support: NIG Grant R01 NS064934

NIG Grant R21 NS097643

Title: Pharmacodynamic profiles of LRRK2 kinase inhibitors in transgenic rats

Authors: *K. J. KELLY

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Abstract: Parkinson disease (PD) is the most common movement disorder and is pathologically characterized by the presence of Lewy bodies and the loss of dopaminergic neurons in the substantia nigra. In 2004, the genetic link between mutations in *leucine rich repeat kinase 2* (LRRK2) and PD were identified in familial PD cases. The G2019S mutation in the LRRK2 kinase domain is the most common pathogenic mutation and leads to a gain-of-function that increases LRRK2 kinase activity. Small molecule LRRK2 kinase inhibitors are sought that might block or return LRRK2 activity to normal. Although several LRRK2 kinase inhibitors have been developed, few successful chronic dosing regimens in rats have been reported. Outbred rats are the standard rodent model in most pre-clinical campaigns to identify clinical candidate molecules. Here, we evaluated several chronic dosing strategies for two LRRK2 kinase inhibitors, PF-360 and MLi2, in wild-type and hBAC G2019S-LRRK2 transgenic rats, both on
the Taconic Sprague Dawley outbred background. Our goal is to relate drug concentrations in the brain and plasma in reducing the abundance of autophosphorylated LRRK2, pS935-LRRK2, and total LRRK2 protein, in both the context of over-expressed mutant LRRK2 enzyme as well as wild-type LRRK2 protein in the rats. We developed a scalable and ultra-sensitive assay using protein capillary electrophoresis to assess these proteins from tissues and cerebral spinal fluid. Our data suggest that MLi2 and PF-360, despite both compounds binding to the LRRK2 ATP pocket, differentially affect total protein stability and show different sensitivities towards inhibition of the autophosphorylation pS1292 residue and the constitutively phosphorylated pS935 residue. We can demonstrate that successful long-term chronic dosing regimens are possible in the rat, tuned to different potencies with respect to LRRK2 inhibition.

Disclosures: K.J. Kelly: None.

Poster

300. Movement Disorders I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: C.04. Movement Disorders

Support: NIH R01 NS072446

Leblang Charitable Foundation

Title: Biochemical and behavioral outcome in AMN mice after intrathecal delivery of AAV9-hABCD1

Authors: *Y. GONG¹, F. LAHEJI¹, A. BERENSON¹, A. VOLAK¹, G. GAO³, X. O. BREAKEFIELD², C. MAGUIRE¹, F. EICHLER¹ ¹Massachusetts Gen. Hosp., Boston, MA; ²Molec Neurogenetics Unit, Massachusetts Gen.

Hosp., Charlestown, MA; ³Gene Therapy Ctr. of Univ. of Massachusetts, Boston, MA

Abstract: Adrenomyeloneuropathy (AMN) is the most common phenotype of X-linked adrenoleukodystrophy, a debilitating neurological disorder caused by mutations in the ABCD1 gene that encodes a peroxisomal ATP binding cassette transporter (ABCD1) responsible for transport of CoA-activated very long-chain fatty acids into the peroxisome for degradation. Close examination of the known Abcd1-/- mouse model of AMN reveals a 4-fold increase of C26:0 levels in spinal cord. Further we discovered that mechanical hypersensitivity of Abcd1-/- mice begins around 9 month of age, half a year prior to motor symptoms. In previous work we demonstrated that rAAV9-mediated ABCD1 gene transfer via intrathecal osmotic pump (IT pump) led to more uniform and widespread gene delivery to CNS with reduced leakage into the systemic circulation compared to either intravenous injection or intrathecal bolus (IT bolus)

injection. Here we report the biochemical and behavioral impact of intrathecal rAAV9-ABCD1 delivery. rAAV9 encoding ABCD1 (rAAV9-ABCD1) were delivered to Abcd1-/- mice intrathecally (IT) at spine region L4-L5 using either a gas-tight Hamilton syringe attached to a 33-gauge steel needle over 2mins or an osmotic pump over 24h, with PBS injections serving as sham control. Two weeks after injection, mice were sacrificed and tissue was collected for lipid analysis (C26:0 measurements). For behavioral testing another cohort of mice was similarly injected with rAAV9-ABCD1 at 5 months of age. Lipid analysis showed a 27% and 32% reduction in C26:0 of the spinal cord after rAAV9-ABCD1 IT pump and IT bolus injection (1X10¹¹gc/mouse) respectively. Importantly, after AAV9-ABCD1 delivery via IT pump we found a 2-fold improvement in the nominal force threshold compared to the PBS injected group. Behavioral testing after AAV9-ABCD1 IT bolus delivery is still pending. Meanwhile, no significant weight loss was detected after 1X10¹¹gc rAAV9-ABCD1 IT pump delivery compared to the PBS group. Even a 3-fold higher dose $(3X10^{11}gc/mouse)$ did not impact bodyweight, suggesting that intrathecal rAAV9-ABCD1 delivery is well tolerated. We conclude that rAAV9mediated ABCD1 gene transfer via intrathecal osmotic pump leads to biochemical correction and sensory improvements without associated toxicity. These findings may encourage further dose escalation and bode well for future clinical translation.

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Poster

300. Movement Disorders I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 300.02/T9

Topic: C.04. Movement Disorders

Title: The DNA repair histone H2AX controls motor learning and balance

Authors: *U. WEYEMI¹, B. D. PAUL², S. H. SNYDER³

¹Neurosci., Johns Hopkins Univ., Baltimore, MD; ²The Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; ³Johns Hopkins Univ. Sch. Med., Baltimore, MD

Abstract: Ataxia telangiectasia (A-T) is an autosomal recessive disorder caused by a mutated ATM gene, which is characterized by genomic instability and progressive cerebellar degeneration leading to loss of motor control. A crucial aspect of A-T disease progression may be the elevated level of reactive oxygen species (ROS). Interestingly, while ATM is best known for its function in DNA repair, it has recently been shown to have an important role in cellular ROS homeostasis. Our previous study revealed that oxidative stress mitigation reduces some of the A-T phenotypes. ATM drives efficient DNA repair through rapid phosphorylation of the

histone variant H2AX. H2AX was recently described to elicit several functions beyond DNA repair, including the sensing of oxidative stress. H2AX mice recapitulate several features of Atm knockout mouse including defective DNA repair, immunodeficiency and increased risk of lymphoma. However, little is known about a role for H2AX in oxidative stress and the deficits in motor balance observed in Atm knockout mouse. Moreover, the neurobehavioral deficits in the Atm knockout mice are not robust, and the mutants do not display cerebellar degeneration. Therefore, there is an urgent need for a surrogate model that more faithfully recapitulates the A-T phenotype. We have preliminary observations that mouse embryonic fibroblasts deficient for the histone H2AX exhibit substantial ROS. Of interest, expression of several genes involved in the physiologic response to oxidative stress is impaired in H2AX knockout cells. Our observations are consistent with evidence that H2AX-deficient cells exhibit enhanced cytotoxicity in response to exogenous and endogenous oxidants like hydrogen peroxide (H₂O₂) and L-buthionine-S,R- sulfoximine (BSO). Many studies have suggested a crucial role for oxidative stress in the key phenotypes observed in A-T. We explored the functional consequences of H2AX deficiency in relation to behavioral defects. Our preliminary observations indicate that H2AX knockout mice exhibit impaired motor performance. Our data provide the first evidence that deficiency of H2AX, one of the major ATM targets, results in increased oxidative stress and impaired motor coordination. Additional lines of investigation will include whether mitigation of the oxidative stress in vivo rescues mice for the observed deficits. Analyses of cerebellum as well as other motor balance-associated regions in the brain including the cortex and the striatum will help to substantiate whether the H2AX mouse is a heuristic model for A-T disease.

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Poster

300. Movement Disorders I

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Topic: C.04. Movement Disorders

Title: How extracellular α -synuclein affects the function of oligodendrocytes and the pathology of multiple system atrophy?

Authors: *S. KAJI, T. MAKI, N. UEMURA, R. TAKAHASHI Neurol., Kyoto Univ. Grad. Sch. of Med., Kyoto-Shi, Japan

Abstract: <u>Background</u> Multiple system atrophy (MSA) is one of the most refractory neurodegenerative diseases with no disease-modifying treatment. Since α -synuclein (α -syn), known as a neuronal protein, accumulates within oligodendrocytes (OLGs) even in the early stage of MSA, the interaction between α -syn and OLGs is believed to play a pivotal role in MSA pathogenesis. As oligodendroglial α -syn pathology eventually leads to neurodegeneration in MSA brains, the interpretation of how extracellular α -syn affects OLGs would aid the development of treatment for MSA. Our research aims to elucidate the oligodendrocyte condition in MSA and the influence of exogenous α -syn on OLG function.

<u>Method</u> To investigate the mRNA expressions of *SNCA* and genes for glial cell markers in MSA human brains, we performed quantitative PCR analysis. The frontal cortices of four MSA cases were examined, along with those of four control cases. For *in vitro* understanding of α -syn-induced OLG dysfunction, we prepared primary OLG culture. Mixed glial cells were obtained from postnatal day 1-2 rat, followed by isolation of oligodendrocyte precursor cells by overnight shaking. Seven days after maturation induction, OLGs were used for experiments. Bacterially expressed recombinant human α -syn was purified by ion exchange chromatography, followed by 3-7 days incubation with agitation at 37°C for the preparation of α -syn pre-formed fibrils (PFFs). OLGs were incubated with α -syn PFFs for 24 hours (for immunoblotting), or 72 hours (for qPCR).

<u>*Results*</u> Patient analysis revealed no significant difference of mRNA expressions of α -syn in MSA cases compared with controls. *MBP* and *NG2* mRNA expressions showed tendency to be higher in MSA cases than controls. Exogenous α -syn PFFs application caused remarkable decrease in the protein expressions of myelin basic protein (MBP). On the other hand, the *MBP* mRNA expression was significantly increased after α -syn PFFs application. *Discussion MBP* mRNA expressions were increased both in MSA cases and in α -syn-PFFs-

Discussion MBP InRNA expressions were increased both in MSA cases and in a-syn-PPPstreated primary OLG culture. Given that a previous study has shown the decrease of normal MBP and the emergence of degraded MBP in MSA human brains, the extracellular α -syn PFFs may trigger the degeneration of normal MBP and the compensatory increase of *MBP* mRNA expressions, which leads to the OLG dysfunction and neurodegeneration in MSA patients.

Disclosures: S. Kaji: None. T. Maki: None. N. Uemura: None. R. Takahashi: None.

Poster

300. Movement Disorders I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 300.04/T11

Topic: C.04. Movement Disorders

Support: MSA Coalition

Lizanell and Colbert Coldwell Foundation

Title: Preclinical assessment of an FTY720-derivative, FTY720-Mitoxy, as a potential novel therapy for multiple system atrophy (MSA)

Authors: G. VIDAL-MARTINEZ¹, B. YANG¹, I. SEGURA¹, J. VARGAS-MEDRANO¹, S. CHAPPARO¹, J. B. ARTERBURN², *H. SANDOVAL³, R. G. PEREZ¹ ¹Texas Tech. Univ. Hlth. Sci. Center- El Paso Campus, El Paso, TX; ²New Mexico State Univ., Las Cruces, NM; ³Texas Tech., El Paso, TX

Abstract: In the demyelinating neurodegenerative disorder, multiple system atrophy (MSA), α synuclein (α Syn) protein accumulates inside myelinating oligodendroglia (OLG), forming glial cytoplasmic inclusions (GCIs). In MSA patients, this causes OLG glial and neuron cell death, demyelination, and related parkinsonian, autonomic, and cerebellar dysfunction. MSA patients also develop constipation. MSA mice overexpressing a Syn in OLG cells also develop GCIs and MSA-like symptoms. We have shown that the FDA-approved drug FTY720, is protective in parkinsonian mice. Our novel FTY720-analogue, FTY720-Mitoxy, crosses the blood brain barrier and can protect OLGs and neurons in cell culture, in part by turning up trophic factor expression. We propose that FTY720-Mitoxy protects MSA mice against progressive α Syn pathology and associated behavioral loss. MSA mice and wild type littermates received placebo or FTY720-Mitoxy from 8.5-11 mo by Alzet osmotic pumps. We used rotarod to assess balance and coordination, fecal water content to measure constipation, and carmine red gavage to measure total gut motility from 5-11 mo. Tissues were collected to evaluate aSyn pathology in brain and gut. At 4-6 mo, control and MSA mice were similar on all tests. MSA mice developed motor deficits that worsened by 10-11 mo in placebo treated MSA mice. After 2.5 mo of FTY720-Mitoxy treatment, preliminary data suggest MSA mice had better movement performance, reduced constipation, and improved gastrointestinal transit time. aSyn evaluation is underway. Evidence that FTY720-Mitoxy can slow MSA dysfunction and a Syn pathology in MSA mice encourages Phase I safety trials of the drug.

Disclosures: G. Vidal-Martinez: None. B. Yang: None. I. Segura: None. J. Vargas-Medrano: None. S. Chapparo: None. J.B. Arterburn: None. H. Sandoval: None. R.G. Perez: None.

Poster

300. Movement Disorders I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 300.05/T12

Topic: C.04. Movement Disorders

Title: TDP-43 is a key factor in regulating oligodendroglial lineage biogenesis and myelination

Authors: *J. WANG, W. HO, S.-C. LING Natl. Univ. of Singapore, Singapore, Singapore **Abstract:** TDP-43 aggregates are pathological hallmarks for amyotrophic lateral sclerosis (ALS) and frontotemporal demenia (FTD). Intriguingly, TDP-43 proteinopathies are characterized by loss of its nuclear staining with concomitant cytoplasmic accumulation. Furthermore, TDP-43 aggregates are present in neurons, astrocytes and oligodendrocytes. Together the evidence suggests that TDP-43 mediated neurodegeneration may be caused by both loss- and gain-of-TDP-43 functions and the damages from non-neuronal cells. To address how glial TDP-43 dysfunctions contributes to ALS, we selectively deleted TDP-43 in mature oligodendrocytes in mice. Although mice with TDP-43 deleted in oligodendrocytes are born in Mendelian ratio and develop normally, they develop progressive neurological phenotypes leading to early lethality by 90-days of age accompanied by age-dependent reduced myelination, altered oligodendrocyte biogenesis, loss of motor neurons, astrogliosis and microgliosis in the spinal cords. Our data showed that TDP-43 is indispensable for the functions of mature oligodendrocyte and loss of oligodendroglial TDP-43 may be an integral part in ALS pathogenesis.

Disclosures: J. Wang: None. W. Ho: None. S. Ling: None.

Poster

300. Movement Disorders I

Location: Halls A-C

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Program#/Poster#: 300.06/U1

Topic: C.04. Movement Disorders

Support: MSA coalition

Title: Non-immunosuppressive FTY720 analogues increase brain derived neurotrophic factor (BDNF) in oligodendroglia

Authors: I. SEGURA-ULATE¹, T. BELCHER¹, G. VIDAL-MARTINEZ¹, *R. G. PEREZ² ¹Texas Tech. Hlth. Sci. Ctr., El Paso, TX; ²Biomed. Sci., Texas Tech. Univ. Hlth. Sci. Ctr. - El Paso Campus, El Paso, TX

Abstract: Multiple system atrophy (MSA) is a demyelinating disorder with α-synuclein (aSyn) protein buildup inside myelinating oligodendroglia (OLG) and brain neurons. MSA dysfunction includes loss of brain-derived neurotrophic factor (BDNF) in OLGs. Loss of BDNF in brain cells is reversed by FTY720, an FDA-approved multiple sclerosis drug. Phosphorylated FTY720 also causes immunosuppression by reducing blood lymphocytes, which may be undesirable for MSA patients. New FTY720-analogues, FTY720-C2 and FTY720-Mitoxy, are not phosphorylated but still increase BDNF in neurons. Thus, we hypothesize that novel FTY720-analogues increase BDNF in OLG cells without producing immunosuppressive effects. BDNF expression in OLN-93, an OLG cell line, was assessed at 12 and 24 hr by qPCR after FTY720-C2 and FTY720-Mitoxy, or

equimolar FTY720-C2/FTY720-Mitoxy or 10X higher doses. Blood lymphocytes were counted by microscopy of Wright's stained blood smears at 24 hr after treatments. FTY720, as a positive control, caused immunosuppressive loss of blood lymphocytes to 80% of baseline at 24 hr in mice. Equimolar FTY720-C2 and FTY720-Mitoxy did not alter lymphocyte counts; though 10X FTY720-Mitoxy caused a 10% non-immunosuppressive loss of blood lymphocytes. Moreover, FTY720-Mitoxy increased BDNF expression in OLN-93 cells at 12 and 24 hr, with less effect noted for FTY720-C2.Cumulative evidence suggests that FTY720-Mitoxy increases BDNF in neuronal and OLG cells, similar to FTY720, while reducing the risk of immunosuppression. This supports further evaluation of FTY720-Mitoxy as a potential therapy for MSA.

Disclosures: I. Segura-Ulate: None. **T. Belcher:** None. **G. Vidal-Martinez:** None. **R.G. Perez:** None.

Poster

300. Movement Disorders I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 300.07/U2

Topic: C.04. Movement Disorders

Title: Understanding the basis of CSMN vulnerability and degeneration using a proteomics approach

Authors: *M. C. SCHULTZ¹, J. KLESSNER¹, P. THOMAS^{2,3}, S. SANCHEZ¹, R. DAVIS², P. GOTTLIEB², N. KELLEHER^{2,3}, P. ÖZDINLER^{1,3,4}

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Abstract: Ubiquitin carboxy-terminal hydrolase L1 (UCHL1) is a deubiquitinase that plays a critical role in maintaining free ubiquitin levels in neurons. Mice that lack all UCHL1 function (UCHL1^{nm3419}, UCHL1-/-) display motor neuron defects and profound corticospinal motor neuron (CSMN) degeneration characterized by increased ER-stress, vacuolated apical dendrites, and spine loss. These findings show the importance of UCHL1 for CSMN health. We hypothesize that in the absence of UCHL1, UCHL1 interacting partners could lose appropriate modulation leading to improper protein recycling or mislocalization in the neuron, and this in turn could reveal in underlying mechanism of CSMN vulnerability and degeneration in motor neuron diseases including amyotrophic lateral sclerosis. Here, we used a UCHL1- immunoprecipitation coupled with bottom-up proteomics approach to reveal the proteins that interact with UCHL1 in the motor cortex. Our findings have shown that UCHL1 indeed interacts with numerous proteins in the motor cortex including collapsing response mediator protein-2 (CRMP2). CRMP2 is a CRMP family member responsible for neurite outgrowth, spine

maintenance and axon guidance. Our data demonstrates a novel interaction between UCHL1 and CRMP2 that could explain the pathology observed in CSMN devoid of UCHL1.

Disclosures: M.C. Schultz: None. **J. Klessner:** None. **P. Thomas:** A. Employment/Salary (full or part-time):; Proteomics Center of Excellence, Northwestern University, Evanston, IL 60208, Robert H. Lurie Cancer Center, Northwestern Universit. **S. Sanchez:** None. **R. Davis:** None. **P. Gottlieb:** None. **N. Kelleher:** None. **P. Özdinler:** None.

Poster

300. Movement Disorders I

Location: Halls A-C

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Program#/Poster#: 300.08/U3

Topic: C.04. Movement Disorders

Support: NIH Grant 1R56NS095965-01A1

Title: Prolonged striatal cholinergic activation and nicotine treatment similarly reduce L-dopainduced dyskinesias

Authors: ***T. BORDIA**, M. QUIK, X. PEREZ Ctr. for Hlth. Sci., SRI Intl., Menlo Park, CA

Abstract: L-dopa treatment in Parkinson's disease leads to involuntary hyperkinetic movements called L-dopa-induced dyskinesias (LIDs). We recently showed that selective activation of striatal cholinergic interneurons reduces LIDs via nicotinic (nAChR) and muscarinic (mAChR) acetylcholine receptors. Notably, the extent of reduction was similar to that observed previously with nicotine treatment. We therefore probed the cholinergic mechanisms leading to LIDs reduction with nicotine treatment. Choline acetyltransferase (ChAT)-Cre mice were unilaterally lesioned with 6-hydroxydopamine to induce parkinsonism, injected with AAV5-ChR2-eYFP in the dorsal striatum and implanted with optical cannulas. After stable virus expression, mice were rendered dyskinetic using L-dopa and stimulated. As previously, short optical cholinergic interneuron stimulation (0.005 s ON/ 0.5 s OFF) applied for 2 h after L-dopa injection enhanced LIDs, while longer pulse stimulation (1 s laser on/ 0.5 s laser off) reduced LIDs. The mice were then divided into two groups, with one receiving saccharin and the other saccharin-containing nicotine (300 µg/ml, ramped over a two-week period) with L-dopa treatment continued. As expected, there was a reduction in LIDs in the nicotine-treated group. In addition, the nAChR antagonist mecamylamine decreased LIDs in saccharin-treated but not nicotine-treated mice. By contrast, the mAChR antagonist atropine increased LIDs expression in nicotine but not saccharin-treated mice. Short pulse stimulation increased LIDs in both saccharin and nicotine treated mice. While this effect was not blocked by mecamylamine in either treatment groups, atropine prevented the increase in LIDs in saccharin-treated mice and further augmented LIDs in nicotine-treated mice. These data suggest a pro-dyskinetic role for mAChRs, which is further potentiated with nicotine-treatment. We next investigated whether longer cholinergic interneuron stimulation still decreases LIDs after nicotine treatment. No change in LIDs was observed in the nicotine-treated mice with longer cholinergic activation. These data together with the lack of effect of mecamylamine in nicotine-treated mice suggest that nAChR desensitization is a common cohesive mechanism for LIDs reduction. The atropine-mediated increase in LIDs expression regardless of stimulation durations in nicotine-treated mice may indicate a regulatory relationship between desensitized nAChRs and mAChRs to exacerbate LIDs. Overall, these results highlight a critical role for decreased striatal cholinergic signaling arising from nAChR desensitization in reducing LIDs.

Disclosures: T. Bordia: None. M. Quik: None. X. Perez: None.

Poster

300. Movement Disorders I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 300.09/U4

Topic: C.04. Movement Disorders

Support: NIDA Intramural Funds

Title: Targeting hypersensitive corticostriatal terminals and the dopamine D₄ receptor in restless leg syndrome

Authors: *C. R. QUIROZ¹, G. YEPES¹, M. SANCHEZ-SOTO¹, H. YANO¹, X. GUITART¹, W. REA¹, N. S. CAI¹, V. CASADÓ-ANGUERA², V. CASADÓ², R. P. ALLEN³, C. J. EARLEY³, S. FERRÉ¹

¹Medications Discovery Br., Natl. Inst. on Drug Abuse Intramural Res. Program, Baltimore, MD; ²Univ. of Barcelona, Barcelona, Spain; ³Neurol., Johns Hopkins Univ. Dept. of Neurol. and Neurosurg., Baltimore, MD

Abstract: The objective of the study was to demonstrate alterations in the sensitivity of corticostriatal glutamatergic terminals in the rodent with brain iron deficiency (BID), a pathogenetic model of Restless Legs Syndrome (RLS), and to determine if these terminals constitute a significant target for drugs effective in RLS, including dopamine receptor agonists (pramipexole and ropinirole) and $\alpha 2\delta$ ligands (gabapentin). The methods included an in vivo optogenetic-microdialysis approach in the rat and in vitro dynamic Bioluminescence-Resonance-Energy-Transfer techniques in transfected cells, to determine the biochemical profile of the putative dopamine D2-like receptor subtypes present in corticostriatal glutamatergic terminals. The results demonstrate hypersensitivity of corticostriatal glutamatergic terminals (lower frequency of optogenetic stimulation to induce glutamate release) in rats with BID as compared

with those with normal brain iron levels. Both hypersensitive and control glutamatergic terminals were significant targets for pramipexole, ropinirole and gabapentin, which significantly counteracted optogenetically induced glutamate release. Using selective antagonists, a significant involvement of D4 and D2 receptor subtypes on the effects of the dopamine receptor agonists was determined with the in vivo and in vitro approaches. Specifically, the results suggest the involvement of D4-D2S receptor heteromers. Significant differences on the efficacy of pramipexole and ropinirole, that depended on receptor heteromerization, were observed between D4.4 and D4.7, the products of polymorphic variants of the human D4 receptor gene. The interpretation of these results is that: i) hypersensitivity of corticostriatal glutamatergic terminals can be a main mechanism involved in the pathogenesis of RLS symptoms; ii) these terminals are already constituting targets for drugs effective in RLS; iii) selective D4 receptor agonists should provide a more efficient treatment with less secondary effects.

Disclosures: C.R. Quiroz: None. G. Yepes: None. M. Sanchez-Soto: None. H. Yano: None. X. Guitart: None. W. Rea: None. N.S. Cai: None. V. Casadó-Anguera: None. V. Casadó: None. R.P. Allen: None. C.J. Earley: None. S. Ferré: None.

Poster

300. Movement Disorders I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 300.10/U5

Topic: C.04. Movement Disorders

Title: Assessment of haloperidol-induced tremulous jaw movement in rats using loose restraint and a video recording system

Authors: R. HODGSON, *E. LATONUMMI, A. J. NURMI, L. KOISTINEN Charles River Discovery, Kuopio, Finland

Abstract: Chronic administration of haloperidol to rats induces a tremulous jaw movement, also referred to as vacuous chewing, characterized by involuntary rapid downward deflections of the lower jaw. These jaw movement have been widely used as a model of the motor complications associated with Parkinson's disease; anti-parkinsonian drugs reliably reverse the jaw movements. More recently, haloperidol-induced jaw movements have been used as a model of antipsyhotic-induced extrapyrimadal syndrome (EPS); promising treatments for EPS, such as adenosine A2A receptor antagonists reliably reverse haloperidol-induced tremulous jaw movements. The model has a significant value for the advancement of novel chemical entities both to help derisk putative antipsychotics in development, or to assess the efficacy of novel candidates designed to reverse antipsychotic-induced extrapyramidal syndrome. While the rat model has much higher throughput than the Cebus monkey model, which is the gold standard animal model of EPS, a high-throughput execution of the model has not yet been developed. The most challenging

barrier to increasing the throughput of the model is that it requires a live human observer to score the moments and it is difficult to video the behavior because the observer must "bob and weave" with the rats in order to maintain a line of sight to the jaw. Here we developed a system to loosely restrain the rats during video recording forcing the animals to face the camera. Because the animals are treated with haloperidol, their movement is already pharmacologically limited and we find that the loose restraint is sufficient to maintain the directionality of the animals allowing for video recording and post-hoc recording of the jaw movements. Moreover, using post hoc video recording allows for scoring by more than a single observer to allow for confirmation of the findings by an independent observer. Here we show that, using this video recording system, we achieve reliable recordings and measurement of the haloperidol-induced jaw movements, and their reversal with the selective A2A receptor antagonist istradefylline. This system provides a novel way to execute the haloperidol jaw movement assay much higher throughput, and reliability way than has been previously reported.

Disclosures: R. Hodgson: None. E. Latonummi: None. A.J. Nurmi: None. L. Koistinen: None.

Poster

300. Movement Disorders I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 300.11/U6

Topic: C.04. Movement Disorders

Title: Novel viral vector-mediated rat model of multiple system atrophy

Authors: ***D. J. MARMION**¹, M. H. SAVOLAINEN¹, Y. CHU¹, D. KIRIK³, R. J. MANDEL⁴, T. MCCOWN^{5,7}, S. J. GRAY^{5,6}, J. H. KORDOWER^{2,8}

¹Dept. of Neurolog. Sci., ²Dept Neurol Sci., Rush Univ. Med. Ctr., Chicago, IL; ³Dept. of Exptl. Med. Sci., Lund Univ., Lund, Sweden; ⁴Univ. Florida Coll Med. Univ. Florida Brain Inst., Gainesville, FL; ⁵Gene Therapy Ctr., ⁶Dept. of Ophthalmology, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; ⁷Dept. of Psychiatry, Univ. of North Carolina, Chapel Hill, NC; ⁸The Van Andel Inst., Grand Rapids, MI

Abstract: Multiple system atrophy (MSA) is a rare, progressive neurodegenerative disorder with an uncertain pathophysiology, in which α -synuclein preferentially accumulates in oligodendrocytes rather than neurons. Aggregated α -synuclein is thought to elicit changes in oligodendrocyte function, such as reduced neurotrophic support and demyelination. To date, only transgenic murine models of MSA exist, which lead us to develop a novel viral vector-based model of MSA by over expressing α -synuclein in oligodendrocytes using a novel oligotrophic adeno-associated virus (AAV) vector, Olig001. As proof-of-concept, rats received unilateral injections of the Olig001 vector expressing GFP stereotaxically in the striatum. After 4-weeks,

rodents were sacrificed and histological methods were used to assess the specificity of the viral vector. Using unbiased stereology, our data showed $126,574 \pm 11,304$ GFP-positive cells in the striatum, and a volume of GFP transduction to cover 3.87 ± 0.609 mm³ throughout the rostral-caudal extend of the striatum. Immunofluorescence double labeling showed 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.

Additional cohorts of rats were injected with either Olig001-GFP or Olig001- α -syn in the striatum. 5-months following injection, histological analysis revealed widespread transgene expression throughout the striatum in both GFP and α -syn injected animals. α -syn expression, indicated by LB509 and phosphorylated Serine-129 immunoreactivity, revealed widespread inclusions in the corpus callosum and striatum, which were shown to be resistant to proteinase K digestion. These pathological inclusions were found to be localized in oligodendroglia, and seem to elicit pathological changes similar to early MSA. Marked demyelination was observed throughout the white matter of α -syn- but not GFP-injected animals. Within the timeframe of this study, no overt neurodegeneration or depletion of striatal dopamine or its metabolites were observed. Taken together, our data indicate that viral vector-mediated overexpression of human α -synuclein in oligodendroglia recapitulates pathology observed in early MSA. Longer time points are needed to fully evaluate the potential of this model to recapitulate neurodegeneration and behavioral deficits seen in MSA.

Disclosures: D.J. Marmion: None. **M.H. Savolainen:** None. **Y. Chu:** None. **D. Kirik:** None. **R.J. Mandel:** None. **T. McCown:** None. **S.J. Gray:** None. **J.H. Kordower:** None.

Poster

300. Movement Disorders I

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Program#/Poster#: 300.12/U7

Topic: C.04. Movement Disorders

Support: FAPESP: 2013/16168-8

FAPESP - 2014/06892-3

CNPq - 300552/2013-9

Title: Combined therapy with tempol (4-Hidroxy-TEMPO) and human mesenchymal stem cells from adipose tissue: Neuroprotection, glial reactivity attenuation, and immunomodulation in SOD1-G93A transgenic mice

Authors: *G. CHIAROTTO, M. V. DE CASTRO, A. S. S. DUARTE, Â. C. M. LUZO, A. L. OLIVEIRA

Univ. of Campinas, Campinas, Brazil

Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the selective loss of the upper motoneurons in the cerebral cortex, and the lower motor neurons in the brainstem and spinal cord. Among the hypotheses to explain the neurotoxic mechanism that triggers neuronal death are the oxidative stress, inflammation, and glutamate excitotoxicity. However, the precise mechanisms which lead to such scenario remain unknown. Stem cell therapy is a promising alternative for treating ALS based on the fact that such cells target active areas of degeneration, promoting immunomodulation and local release of various neurotrophic factors. Thus, the present work aimed to evaluate the success of combined therapy with the antioxidant tempol (4-hidroxy-TEMPO) and human mesenchymal stem cells (hMSC) obtained from adult adipose tissue. Such treatment was performed in SOD1-G93A transgenic mice, used as a model for ALS. Treatment with tempol (50mg/kg) and hMSC was initiated in 70 days old mice (pre-symptomatic stage). Tempol was given orally (gavage) on alternate days up to 100 days of life when the animals were sacrificed for spinal cord analysis. Cell therapy was carried out in 70 days old subjects through the tail vein, at the concentration of 1×10^5 cells. Data evaluation encompassed Nissl staining, for evaluation of neuronal survival and immunohistochemistry to evaluate astrogliosis, microglial reaction, and preservation of synapses. qRT-PCR was used for evaluation of pro-inflammatory cytokines, neurotrophic factors, and iNOS gene expression. The combined treatment of tempol and hMSC decreased astrocyte activation by 30% and microglial reaction by 40%. These results were paralleled with downregulation, by 50%, of IL1B and TNFa mRNA levels. Also, hMSC treatment led to downregulation of iNOS gene expression at lumbar spinal cord ventral horn. Overall, spinal cord microenvironment immunomodulation, by the combined therapy described herein, prevented motoneuron loss by 90% in 100 days old mice, which was coupled with a trend of preservation of synaptic circuits at the motor nucleus (lamina IX of Rexed). In turn, the association of hMSC and tempol administration may be considered as a promising therapy for attenuating the progression of ALS.

Disclosures: G. Chiarotto: None. M.V. De Castro: None. A.S.S. Duarte: None. Â.C.M. Luzo: None. A.L. Oliveira: None.

Poster

300. Movement Disorders I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 300.13/U8

Topic: C.04. Movement Disorders

Title: Optimizing patient-specific cellular neurodegenerative disease model based on human iPSc derived neuronal differentiation

Authors: *M. FANG¹, Z. HU², Y. YANG³

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Abstract: Researches concerning human brain disease are hampered by the difficulties of obtaining live specimens. The induced pluripotent stem (iPS)-derived and differentiated cells allow researchers to examine disease onset and progression directly in a human culture model. Creating isogenic iPS cell lines conquer problems of variability in human genomes; also it is much easier to employ genome-editing technique, thus performing therapeutic drug screens on a specific human genetic background. Shinya Yamanaka played his magic using 4 genes to do reprogramming and generate embryonic-like iPS cells, which were supposed to herald a medical revolution while they are transforming biological research instead. Our previous collaborative study have identified a dominantly inherited heterozygous variant c.1064G>A (p.G355D) in ATAD3A by using whole-exome sequencing in a mother presenting with hereditary spastic paraplegia (HSP) and axonal neuropathy and her son with dyskinetic cerebral palsy. Results show that overexpression of the mutant ATAD3A fragments the mitochondrial network and induces lysosome mass. Furthermore, altered dynamics of the mitochondrial network and increased lysosomes in patient fibroblasts and neurons derived through differentiation of patientspecific induced pluripotent stem cells were observed. These alterations were verified in patient fibroblasts to associate with upregulated basal autophagy through mTOR inactivation, which resembling starvation. Similarly, iPSC generated from patient carrying Usher syndrome causing mutation MYO7A were first differentiated to neurons, mitochondrial dynamic status were checked. And a bipolar disorder family has been identified, whole exome sequencing approached, fibroblasts and reprogramming iPSC were generated from the affected members, mitochondrial balance has been explored in patient neuronal differentiated cell model. The patient-specific iPSCs would provide disease pathogenesis as well as enable the evaluation of drug and patient classification of different types of neurodegenerative diseases.

Disclosures: M. Fang: None. Z. Hu: None. Y. Yang: None.

Poster

300. Movement Disorders I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 300.14/U9

Topic: C.04. Movement Disorders

Support: NRF-2016M3A9B4915706

Title: Neurological manifestation of Wilson disease as a distinct disease subgroup

Authors: *B. LEE¹, G. SEO², Y.-M. KIM², G.-H. KIM¹, H.-W. YOO² ¹Asan Med. Ctr., Seoul, Korea, Republic of; ²Asan Med. Ctr. Children's Hosp., Seoul, Korea, Republic of

Abstract: Wilson disease (WD) is an inborn error of copper metabolism (OMIM #277900) caused by the recessive genetic defects in the P-type copper-transporting ATPase. WD is one of the most common inherited metabolic disorders with the frequency of 1 in 30,000 in the Korean population. WD manifests as hepatic dysfunction or neurological manifestations that include tremor, dysarthria, ataxia, rigidity, dyskinesia, cognitive impairment and mood disturbances. In a previous study of a large Korean WD cohort, we found that the clinical courses of WD patients with neurological manifestations are more indolent and less favorable than those with hepatic dysfunction and without neurological manifestations. In the current study, we investigated the biochemical and molecular genetic features of WD with neurological manifestations to identify more evidences that these WD patients' group is a distinct phenotypic or genetic group among WD patients. We evaluated the detailed biochemical profiles and genotypic characteristics between WD patients with neurological manifestations and without neurological manifestations in 384 Korean WD patients. Of note, all the biochemical profiles including the levels of platelet, liver enzymes, serum copper and ceruloplasmin were different among these two groups, and these differences were still significant even among the patients with liver cirrhosis. Regarding the correlation with genotypes, the mutation types or common mutations including p.Arg778Leu, p.Ala874Val, p.Asn1270Ser, p.Lys838SerfsX35 and p.Leu1083Phe, were not differentially distributed between the two groups. However, the low frequency of mutations in the transduction or ATP-hinge domain, were significantly noted in patients with neurological manifestations. The results of the current study provide more evidence that WD patients with neurological manifestations hold a distinct phenotypic and or genotypic subgroup of WD.

Disclosures: B. Lee: None. G. Seo: None. Y. Kim: None. G. Kim: None. H. Yoo: None.

Poster

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Topic: C.04. Movement Disorders

Support: National Natural Science Foundation of China 81130021

NIH grant NS036232

Title: PRRT2 function and mutations in the pathogenesis of Paroxysmal Kinesigenic Dyskinesia

Authors: *Y. PAN^{1,2}, Y. YANG^{1,2}, Q. LIU^{1,2}, Y. TIAN², W. CHEN², K. YANG², X.-J. LI¹, S. LI^{1,2}, B. TANG² ¹Human Genet., Emory Univ. Sch. of Med., ATLANTA, GA; ²Dept. of Neurol., Xiangya Hospital, Central South Univ., Changsha, China

Abstract: PKD (Paroxysmal Kinesigenic Dyskinesia) is a rare autosomal dominant inherited paroxysmal movement disorder, which is characterized by recurrent, brief attacks of abnormal involuntary movements induced by sudden voluntary movements. Our previously study had identified that mutations in the *PRRT2* (proline-rich transmembrane protein 2) gene are associated with PKD and that the 'c.649dupC' missense mutation is the major causative mutation. However the underlying pathophysiological mechanism of PRRT2 mutations is still unclear. We found that PRRT2 started to express from embryonic day 18 and showed a markedly increased expression after postnatal stage till adulthood. In situ hybridization and immunoblotting results showed that PRRT2 is widely distributed in the whole brain, with high levels in the hippocampus, cortex, cerebellum, and thalamus. Immunofluorescence of brain slices revealed that PRRT2 was specifically expressed in neuron, but not in astrocytes. Consistent with the synaptic distribution of PRRT2, co-immunoprecipitation and GST pull-down assay demonstrated the interaction between PRRT2 and SNAP25 (synaptosomal-associated protein 25). We also found that the interaction sites in PRRT2 and SNAP25 are the cytoplasmic domain of PRRT2 and linkage domain of SNAP25, respectively. In addition, we have generated a Prrt2 knockout mouse model and the mutation (c.649DupC) knock-in mouse model to compare their motor related behaviors, neural plasticity, and epilepsy susceptibility in order to investigate the role of *PRRT2* mutations in the pathology of PKD.

Disclosures: Y. Pan: None. Y. Yang: None. Q. Liu: None. Y. Tian: None. W. Chen: None. K. Yang: None. X. Li: None. S. Li: None. B. Tang: None.

Poster

300. Movement Disorders I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 300.16/U11

Topic: C.04. Movement Disorders

Support: NIH Grant R01 NS058487

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NIH Grant T32 NS082168

Title: Functional activity within the visuomotor system predicts severity of essential tremor

Authors: *D. B. ARCHER¹, W. T. CHU¹, J. W. CHUNG¹, R. G. BURCIU², S. A. COOMBES¹, A. WAGLE SHUKLA¹, D. E. VAILLANCOURT³ ²Dept. of Applied Physiol. and Kinesiology, ³Applied Physiol. and Kinesiology, ¹Univ. of Florida, Gainesville, FL

Abstract: Abstract

Essential tremor (ET) is a neurological disease with dysfunction in the cerebello-thalamo-motor cortical pathway. It is unclear if other pathways in the brain also contribute to tremor in ET. ET patients demonstrate visuomotor control deficits, which suggests that the visuomotor pathways could play a significant role in tremor amplitude in ET. Here, we directly manipulated the visual feedback error signal during an fMRI grip force task in 19 ET patients and 19 controls, and hypothesized that an increase in visual feedback magnification would exacerbate tremor in the 4-12 Hz range in ET. We also hypothesized that this increase in tremor would be accompanied with dysfunction within the cerebello-thalamo-motor cortical and visuomotor pathways. Magnifying visual feedback led to reductions in force error in ET and a control group, whereas only ET tremor was exacerbated by increases in visual feedback. ET had hyperactivity within primary motor cortex, and hypoactivity within cerebellar lobules I-IV. Further, ET patients had a reduced blood oxygenation level dependent (BOLD) response in several cerebello-thalamomotor cortical and visuomotor regions including inferior and superior parietal lobules, primary motor cortex, middle temporal gyrus, and cerebellar lobule VI. Approximate entropy of the BOLD signal showed the ET patients had irregular approximate entropy in the left superior parietal lobule, supplemental motor area, extrastriate area, and right somatosensory cortex. Multiple regression models identified that two regions in the visuomotor pathway, in addition to cerebellar lobules I-IV, were predictive of the clinical severity of tremor irrespective of visual feedback level, including the extrastriate visual areas and inferior parietal lobule. These findings point to novel evidence that in addition to the cerebellar-thalamo-cortical pathway, the visuomotor pathway is a significant predictor of tremor amplitude in ET.

Disclosures: D.B. Archer: None. **W.T. Chu:** None. **J.W. Chung:** None. **R.G. Burciu:** None. **S.A. Coombes:** None. **A. Wagle Shukla:** None. **D.E. Vaillancourt:** None.

Poster

300. Movement Disorders I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 300.17/U12

Topic: C.04. Movement Disorders

Title: Creating volitional movement in cerebral palsy

Authors: *K. N. HUGGINS¹, M. TURNER²

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Abstract: Development of complex movement patterns (e.g. walking) requires the coordination of momentum and acceleration in space with no fixed points of reference. Functional movement builds upon experiences of moving in space from the neonatal period forward. An infant, having no experience with movement in a gravitational field, creates unique system mechanics in relation to gravity while lacking the cognitive ability to willfully transfer might against mass. An infant with a medical condition at birth or during the early stages of development will miss opportunities to lay the foundation for complex movement. Cerebral palsy results from a brain insult during the time of life between birth and age 5. Current therapies and standard of care are aimed at symptom management with no concerted effort to organize the nervous system and enable habilitation through neuroplasticity. Constraints during this developmental period either internal or external lead to system shut down or long-term restrictions. Here, the authors describe a method to improve the efficiency and frequency of volitional movement in children with movement disorders. Utilizing specific types of movement, the authors replicate early developmental period experience to organize the nervous system and facilitate movement following developmental delays. Changes in movement patterns and system mechanics are outward expressions of underlying changes in the brain and are followed by changes in vision, sensory processing and other concomitant delays.

Disclosures: K.N. Huggins: None. M. Turner: None.

Poster

301. Motor Neuron Disease: Animal Models I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 301.01/V1

Topic: C.05. Neuromuscular Diseases

Support: NS079348

Title: Human superoxide dismutase 1 (SOD1) enters the nucleus and binds chromatin in neurons of G93A-SOD1 transgenic mice

Authors: *B. KIM, L. J. MARTIN Pathology, Div. of Neuropathology, John Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that causes gradual degeneration and elimination of neurons that control voluntary muscles. Some familial ALS is linked to mutations in copper, zinc superoxide dismutase 1 (SOD1). This enzyme

destroys free superoxide radicals in the cytosol but mutant forms are believed to acquire an adverse property. However, underlying therapeutically relevant mechanisms on how the mutations in SOD1 cause neurodegeneration are unknown. Here, we used transgenic mice expressing human G93A-SOD1 to test the hypothesis that SOD1 has a nuclear presence that becomes abnormal during disease. By subcellular fractionation and immunoblotting, human SOD1 (hSOD1) in mouse brain and spinal cord was detected in cytoplasmic and nuclear fractions, hSOD1 was found chromatin bound. Immunofluorescence demonstrated that the nuclear localization of hSOD1 was exclusive to motor neurons and interneurons, not to glia such as astrocytes and oligodendrocytes. This hSOD1 in neurons showed three different localization patterns: higher level of hSOD1 in the nucleus, in the cytoplasm, or the protein being equally distributed in both compartments. Furthermore, compared to pre-symptomatic G93A-SOD1 transgenic mice, symptomatic mice showed overall decreased numbers of motor neurons and interneurons in the spinal cord but the expression patterns of hSOD1 and the relative percentages of neurons in each pattern remained comparable. Our findings suggest that degeneration of the vulnerable neurons in ALS is cell autonomous and nuclear hSOD1 and chromatin interactions could be important in the pathogenesis of the disease.

Disclosures: B. Kim: None. L.J. Martin: None.

Poster

301. Motor Neuron Disease: Animal Models I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 301.02/V2

Topic: C.05. Neuromuscular Diseases

Support: Target ALS

Title: Increasing urate levels may delay disease onset in the SOD1 G93A mouse model of amyotrophic lateral sclerosis

Authors: *E. GRANUCCI¹, K. E. GLAJCH¹, K. TSIORAS², K. A. MUELLER¹, A. M. DIOS¹, Y. XU¹, R. BAKSHI¹, X. CHEN¹, S. PAGANONI¹, M. A. SCHWARZSCHILD¹, E. KISKINIS², G. SADRI-VAKILI¹ ¹Massachusetts Gen. Hosp., Charlestown, MA; ²Northwestern Univ., Chicago, IL

Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive loss of upper and lower motor neurons resulting in impaired motor function, paralysis, and eventually death by respiratory failure. Currently there are no therapies for the treatment of ALS, indicating a clear need for the development of novel treatment options. Although the exact mechanisms involved in the onset and progression of the disease remain unknown an increase in oxidative stress has been implicated. Thus, an improved understanding

of oxidative stress-induced motor neuron death may lead to mechanism-based therapies. One of our major endogenous defenses against oxidative stress is urate, an antioxidant and byproduct of purine metabolism. Recent findings have highlighted the neuroprotective potential of urate in neurodegenerative diseases such as Parkinson's disease (PD), Huntington's disease, Alzheimer's disease (AD) and ALS. Specifically, urate was shown to be a strong molecular predictor for both reduced risk (PD, AD) and favorable progression (PD, ALS, HD). Furthermore, urate elevation was found to be neuroprotective in pre-clinical PD models. However, little is known about the neuroprotective effects of urate in ALS. Here we assessed the neuroprotective effects of increasing urate levels in the SOD1 G93A mouse model of ALS. To increase CNS urate levels, mice with a mutation in the gene (UOx) encoding urate oxidase, enzyme responsible for urate metabolism, were crossed with transgenic (Tg) SOD1 G93A mice to generate Tg SOD1/UOx^{-/-} mice and littermate controls. We assessed body weight, neurological score, motor function, disease onset and progression. Our results demonstrate that elevated urate levels are associated with a twenty-day delay in the onset of hind limb paresis in the Tg SOD1/UOx^{-/-} mutant mice compared to Tg SOD1/UOx+/+ or wild-type littermates. Ongoing studies are assessing the effects of increased urate on motor neuron counts and neuromuscular junction integrity. In addition, in order to validate the potentially beneficial effects of urate in the context of human patient motor neurons we assessed its ability to protect mutant SOD1 and isogenic control motor neurons in vitro. We found that pretreatment of patient motor neurons with urate significantly protected against glutamate and oxidative stress-induced toxicity. Together these findings demonstrate that, similar to PD, increasing urate levels may provide therapeutic benefits in ALS and support ongoing translational studies of urate elevation in patients with ALS.

Disclosures: E. Granucci: None. K.E. Glajch: None. K. Tsioras: None. K.A. Mueller: None. A.M. Dios: None. Y. Xu: None. R. Bakshi: None. X. Chen: None. S. Paganoni: None. M.A. Schwarzschild: None. E. Kiskinis: None. G. Sadri-Vakili: None.

Poster

301. Motor Neuron Disease: Animal Models I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 301.03/V3

Topic: C.05. Neuromuscular Diseases

Title: Behavioral and histological evaluation of SOD1 (G93A) and Profilin 1(PFN1 G118V) mouse models of motor neuron disease

Authors: *I. MORGANSTERN¹, N. ROBERTS¹, E. SABATH¹, D. HAVAS¹, L. THIEDE¹, M. KIAEI², T. HANANIA¹

¹Behavioral Pharmacol., Psychogenics, Tarrytown, NY; ²Pharmacol. and Toxicology, Dept. of Neurology, Dept. of Geriatrics, Univ. of Arkansas for Med. Sci., Little Rock, AR

Abstract: The present study was aimed at identifying behavioral tests that are most sensitive to the emergence of behavioral/neurological deficits in SOD1-G93A (SOD1) mice in a cross comparison with the newly proposed hPFN1G118V (PFN1) (Fil et al., 2016) mouse model of motor neuron disorder. The test battery consisted of commonly used metrics such as rotarod, open field and more complex proprietary algorithm-based behavioral platforms such as NeuroCube ® and SmartCube ®. In addition to behavioral assessment, histological evaluation of neuroinflammation in the spinal cord will also be presented. The behavioral findings demonstrate similarities in progressive muscle weakness and a decline in motor coordination in both models of motor neuron disease with a later onset and slower progression noted with PFN1 animals. Additionally, sophisticated algorithm-based systems determined a strong phenotype effect in both the PFN1 and SOD1 mice at very early ages. Our NeuroCube ® analyses identified early changes (8-12 weeks) related to gait geometry/ dynamics in both SOD1 and PFN1 mice, which progressed over time. Similarly, using SmartCube® technology we identified distinct behavioral changes as early as 6-7 weeks of age in SOD1 mice and 12-14 weeks for PFN1 mice. Histological assessment demonstrated reactive gliosis of both astro- and microglia in the spinal cord of end-stage disease SOD1 and PFN1 mice. Quantification of GFAP and Iba1 immunoreactivity revealed parallels in both mouse models in addition to distinct patterns of microglial activation. In summary, we demonstrate similar neurological/motor function deficits as well as glial cell activation in SOD1 and PFN1 mice, both exhibiting clinically-relevant attributes of Amyotrophic Lateral Sclerosis (ALS), with more advanced computer vision systems identifying distinctive behavioral patterns and discriminating the phenotype at very early disease stages in both models. This earlier period of disease identification presents a valuable model for early intervention and improved assessment of potential therapeutic approaches for ALS.

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Poster

301. Motor Neuron Disease: Animal Models I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 301.04/V4

Topic: C.05. Neuromuscular Diseases

Title: Longitudinal measurements of neurofilament light in the SOD1^{G93A} mouse model of amyotrophic lateral sclerosis

Authors: *B. L. BURGESS¹, D. L. BAKER², S. L. DOMINGUEZ³, A. DATWANI², F. L. YEH¹

¹Biomarker Develop., ²Bioanalytical Sci., ³Neurosci., Genentech, South San Francisco, CA

Abstract: Introduction: Neurofilaments have emerged as an important biomarker for neurodegenerative disorders, especially for diseases, such as amyotrophic lateral sclerosis (ALS) and multiple sclerosis. Neurofilament light (NF-L), medium (NF-M) and neurofilament heavy (NF-H) expression is largely restricted to neurons where they serve as intermediate filaments to lend structural support for the cell. NF-L levels in cerebrospinal fluid (CSF) and blood have been demonstrated to have diagnostic and prognostic value in ALS; however, additional studies are required to help guide interpretation of NF-L biomarker data in an interventional trial. To gain a greater understanding of the factors that govern peripheral levels of this biomarker we sought a suitable preclinical model of neurodegeneration that also exhibited elevated NF-L in blood. Transgenic mice carrying the ALS-associated mutant form of human superoxide dismutase 1 ^{G93A} (SOD1^{G93A}) recapitulate several aspects of ALS pathology and were therefore evaluated as a possible preclinical model to investigate the biology of NF-L in blood. Methods/Materials: Plasma was collected from SOD1^{G93A} transgenic (26-31 copies) mice and non-transgenic littermates (n=4 per group) at early (9 wks), mid (11 & 14 wks) and late stages (16 wks) of the disease. NF-L was measured using the Quanterix Simoa NF-L immunoassay with a bovine NF-L calibrator. **Results:** Plasma NF-L was elevated 15-fold in the SOD1^{G93A} positive animals over SOD1^{G93A} negative littermates at 9 weeks of age. NF-L continued to rise sharply in SOD1 ^{G93A} transgenic animals to 23-fold over controls at 11 weeks and 28-fold by 14 weeks, but appeared to plateau by 16 weeks at 30-fold over control. **Discussion** We observed that NF-L progressively increases in the plasma of SOD1^{G93A} mouse model of ALS. Previous characterization of this strain has shown that denervation of neuromuscular junctions in the gastrocnemius muscle occurs prior to 9 weeks without significant loss motor neurons in the spinal cord. These results demonstrate that *SOD1*^{G93A} mice can be used as a model system to investigate the mechanistic origins of peripheral levels of NF-L in ALS and the relationship between plasma NF-L and the development of motor neuron pathology.

Disclosures: B.L. Burgess: A. Employment/Salary (full or part-time):; Genentech. **D.L. Baker:** A. Employment/Salary (full or part-time):; Genentech. **S.L. Dominguez:** A. Employment/Salary (full or part-time):; Genentech. **A. Datwani:** A. Employment/Salary (full or part-time):; Genentech. **F.L. Yeh:** A. Employment/Salary (full or part-time):; Genentech.

Poster

301. Motor Neuron Disease: Animal Models I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 301.05/V5

Topic: C.05. Neuromuscular Diseases

Support: Australian NHMRC APP1065884

Motor Neurone Disease Research Institute of Australia Grant-in-Aid

Title: Altered regulation of upper motor neuron synaptic hyper-excitability by TrkB receptor signaling in the SOD1G93A mouse model of amyotrophic lateral sclerosis

Authors: J. PRADHAN¹, P. G. NOAKES^{1,2}, *M. C. BELLINGHAM³ ¹Sch. of Biomed. Sci., ²Queensland Brain Inst., ³Univ. of Queensland, Brisbane, Australia

Abstract: Brain Derived Neurotrophic Factor (BDNF) is well recognized for its neuroprotective functions, via activation of its high affinity receptor, tropomysin related kinase B (TrkB). However, BDNF/TrkB signalling has also been shown to cause detrimental effects on motor neurons in amyotrophic lateral sclerosis (ALS), via enhancement of glutamate excitotoxicity, causing hyper-excitation and ultimately motor neuron death. Furthermore, direct inhibition of TrkB receptors has been shown to protect motor neurons from these toxic insults. However, the effects of TrkB receptor modulation on neurotransmission and synaptic hyper-excitability in cortical pyramidal neurons that are vulnerable in ALS remain to be determined. We have investigated the effects of TrkB receptor modulation on excitatory synaptic activity in presymptomatic mice over-expressing a human superoxide dismutase 1 G93A mutation (SOD1G93A) and their wild type (WT) littermates (P21-P27). Whole cell patch clamp recordings of spontaneous excitatory postsynaptic currents (sEPSCs) were made from layer V pyramidal neurons (LVPNs) of the motor cortex in acute cortical brain slices. The TrkB receptor agonist, 7,8 dihydroxyflavone (10µM), significantly enhanced sEPSC frequency by 131% (pvalue 0.046, n=8) in WT LVPNs; however, 7,8 dihydroxyflavone did not evoke a significant change in sEPSC frequency in SOD1G93A LVPNs (n=4). The TrkB receptor antagonist ANA-12 (10nM) significantly reduced sEPSC frequency by 40% (p-value 0.011, n=4) in SOD1G93A LVPNs, but did not significantly reduce sEPSC frequency in WT LVPNs (n=6). There was no change in sEPSC amplitude after application of the TrkB agonist or antagonist for LVPNs of either genotype. These results clearly demonstrate that there is an up-regulation in TrkB receptor-mediated enhancement of sEPSC frequency in LVPNs of the motor cortex of presymptomatic SOD1G93A mice, presumably by

enhanced endogenous BDNF. Our results suggest that modulation of TrkB receptor signalling may be important in early disease pathogenesis in ALS.

Disclosures: J. Pradhan: None. P.G. Noakes: None. M.C. Bellingham: None.

Poster

301. Motor Neuron Disease: Animal Models I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 301.06/V6

Topic: C.05. Neuromuscular Diseases

Support: ERC grant n°340429

Title: Inhibitor of differentiation 2 (ID2) as a modifying factor of oligodendrocyte dysfunction in the SOD1G93A mouse model

Authors: C. EYKENS¹, C. JENSEN¹, A. IAVARONE², L. VAN DEN BOSCH¹, *W. L. ROBBERECHT³

¹VIB, KULeuven, Leuven, Belgium; ²Columbia Univ., New York, NY; ³Univ. Hosp Gasthuisberg, 3000 Leuven, Belgium

Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder defined by axonal retraction accompanied by selective upper and lower motor neuron degeneration. Patients suffer from progressive muscle atrophy and will typically die within 2 to 5 years after disease onset as no effective treatment is currently available. In 90% of ALS cases the etiology is unknown, the remaining 10% of ALS patients have a family history of disease. Dominant mutations in C9orf72, SOD1, TDP43 and FUS are the most frequent causes of this inherited form. Although traditionally viewed as a motor neuron disease, damage developed within nonneuronal supporting cells, such as oligodendrocytes, is crucial to motor neuron dysfunction in ALS. A cycle of oligodendrocyte death and replacement precedes the onset of motor neuron death and symptoms in ALS mice. Newly formed oligodendrocytes appear to be immature and dysfunctional in ALS, consistent with impaired oligodendrocyte differentiation. Motor neurons consequently loose an important source of both structural and trophic support, potentially contributing to axonal injury and motor neuron loss. In order to improve this differentiation process, we selectively removed Inhibitor of differentiation 2 (ID2) from oligodendrocyte progenitor cells (OPCs) of pre-symptomatic SOD1^{G93A} ALS mice using a conditional, tamoxifen-inducible mouse model. ID2 is a basic helix-loop-helix (bHLH) transcription factor that impairs differentiation of oligodendrocytes in vitro and was identified as a disease modifier in the ALS zebrafish model established in our lab. Our results showed that oligodendrocytic ID2 deletion was not able to delay disease onset of ALS mice, nor did it prolong survival. At disease end-stage, no significant improvement of oligodendrocyte function could be observed, possibly explaining the negative outcome on disease progression. It remains to be clarified whether ID2 ablation confers a transient effect on oligodendrocyte differentiation or function *in vivo* at earlier stages of disease.

Disclosures: C. Eykens: None. **C. Jensen:** None. **A. Iavarone:** None. **L. Van Den Bosch:** None. **W.L. Robberecht:** None.

Poster

301. Motor Neuron Disease: Animal Models I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 301.07/V7

Topic: C.05. Neuromuscular Diseases

Support: University of Queensland

Motor Neurone Disease Research Institute of Australia (MNDRIA) Bob Delaney MND Research Grant to STN

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University of Queensland Centennial Scholarship to RL

Research training program (RTP) scholarship to RL

Title: Assessing skeletal muscle bioenergetics *In situ* in amyotrophic lateral sclerosis: Characterizing metabolic perturbations in a SOD1 mouse model

Authors: *R. LI¹, S. T. NGO^{1,2,3,4}

¹The Univ. of Queensland, SBMS, Brisbane, Australia; ²Queensland Brain Inst., ³Ctr. for Clin. Res., The Univ. of Queensland, Brisbane, Australia; ⁴Dept. of Neurol., Royal Brisbane & Women's Hosp., Brisbane, Australia

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that primarily affects upper and lower motor neurons, causing progressive muscle wasting and paralysis. Systemic metabolic changes including rapid weight loss and impaired glucose and lipid handing have been observed in ALS patients and mouse models of ALS. While less is known about the mechanisms that contribute to metabolic abnormalities in ALS, recent evidence suggests that impairments in skeletal muscle metabolism may contribute to disease progression. In order to characterize alterations in skeletal muscle bioenergetics in ALS, we developed a novel method to assess real-time mitochondrial fuel utilization in intact extensor digitorum longus (EDL) muscle fibre bundles from the SOD1^{G93A} (SOD1) mouse model of ALS and littermatched wild type (WT) mice. In the presence of carbonyl cyanide-ptrifluoromethoxyphenylhydrazone (FCCP), EDL fibre bundles of mid-stage and end-stage SOD1 mice showed increased maximal capacity when utilizing pyruvate as a substrate, indicating that the mitochondrial oxidation capacity increased with the disease progression. Next, we determined the metabolic flexibility of muscle by assessing the ability of muscle fibres to oxidize a particular substrate (e.g. fatty acid) with or without the presence of alternative substrates (e.g. glucose or glutamine). By inhibiting pyruvate, long-chain fatty acid or glutamate transporters, we showed decreased glucose oxidation dependence and concomitant increased fatty acid oxidation dependence in EDL fibres of mid-stage SOD1 mice. Although we found increased intramuscular lipid accumulation in EDL muscle sections of mid-stage and end-stage SOD1 mice, the intact EDL fibres of end-stage SOD1 mice failed to oxidize stored intracellular lipids for energy after

the inhibition of pyruvate and glutamine utilization. Altogether, our study is the first to use realtime assessment of cellular bioenergetics to demonstrate a transition of fuel preference towards fatty acid oxidation in intact skeletal muscle of SOD1 mice. In line with the previous evidence of higher energy demands in muscle of SOD1 mice, our data suggest that enhanced mitochondrial oxidation, especially fat oxidation, may occur as a positive adaptive response to maintain the energy balance of skeletal muscle in ALS.

Disclosures: R. Li: None. S.T. Ngo: None.

Poster

301. Motor Neuron Disease: Animal Models I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 301.08/V8

Topic: C.05. Neuromuscular Diseases

Support: MIUR (SIR project RBSI14B1Z1) to Milanese Marco

Title: Knocking-down metabotropic glutamate receptor type 5 (mGluR5) in the SOD1^{G93A} mouse ameliorates ALS disease hallmarks

Authors: M. MILANESE¹, T. BONIFACINO¹, F. PROVENZANO¹, E. GALLIA¹, L. CATTANEO¹, A. PULITI², C. USAI³, F. CONTI⁴, *G. BONANNO⁵

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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder due to loss of upper and lower motor neurons (MNs). The mechanisms of neuronal death are largely unknown, thus prejudicing the successful pharmacological treatment. ASL is a multifactorial disease and one major cause for MN degeneration in ALS is represented by glutamate(Glu)-mediated excitotoxicity, sustained by glial uptake reduction (1), abnormal Glu release (2), and altered Glu receptor function (3). We reported that activation of Group I metabotropic Glu receptors (mGluR1, mGluR5) at glutamatergic spinal cord nerve terminals produces abnormal Glu release in the widely studied SOD1^{G93A} mouse model of ALS (4). We also reported that halving mGluR1 expression in the SOD1^{G93A} mouse had a positive impact on survival, disease onset and a number of cellular and biochemical clinical features of ALS (5). We generated here SOD1^{G93A} mice with reduced expression of mGluR5 (SOD1^{G93A}mGluR5^{-/+}) by crossing the SOD1^{G93A} mutant mouse with the mGluR5 heterozigous Grm5^{-/+} mouse. SOD1^{G93A}mGluR5^{-/+}

mice, and delayed pathology onset. These effects were associated to enhanced number of preserved MNs in the lumbar spinal cord; decreased expression of GFAP and CD11, as markers of astrocyte and microglia activation, respectively; reduced cytosolic free Ca²⁺ concentration and normalized Glu release in SOD1^{G93A}mGluR5^{-/+} mouse spinal cord synaptosomes. Unexpectedly, when studying disease progression by measuring the RotaRod task, the hind limbs extension reflex and gait impairment scores, only male SOD1^{G93A}mGluR5^{-/+} mice showed improved motor skills vs. SOD1^{G93A}mice, while SOD1^{G93A}mGluR5^{-/+} females did not. These results demonstrate that a lower constitutive level of mGluR5 has a significant positive impact in mice with ALS and support the idea that blocking Group I mGluRs may represent a potentially effective pharmacological approach to the disease. Rothstein, J.,D. et al 1995. Ann. Neurol. 38, 73-84; (2) Milanese, M., et al 2011. J. Neurochem. 116, 1028-42; (3) Van Den Bosch, et al 2000. J. Neurol. Sci. 180, 29-34. (4) Giribaldi et al 2013. Neuropharmacol. 66:253-63; (5) Milanese et al 2014. Neurobiol. Dis. 64:48-9.

Disclosures: M. Milanese: None. **T. Bonifacino:** None. **F. Provenzano:** None. **E. Gallia:** None. **L. Cattaneo:** None. **A. Puliti:** None. **C. Usai:** None. **F. Conti:** None. **G. Bonanno:** None.

Poster

301. Motor Neuron Disease: Animal Models I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 301.09/V9

Topic: C.05. Neuromuscular Diseases

Support: ALS Finding a Cure Foundation

Title: Symptom severity in the acute phase following repeat mild TBI predicts early disease onset in the SOD1^{G93A} ALS rat model

Authors: *G. M. THOMSEN, N. CHO, N. DHILLON, M. ALKASLASI, P. HARO-LOPEZ, O. SHELEST, G. BARMPARAS, E. LEY Cedars-Sinai Med. Ctr., West Hollywood, CA

Abstract: Objectives: Populations prone to sustaining traumatic brain injury (TBI), including veterans and professional contact-sport athletes, might be at a higher risk of developing such neurodegenerative disorders as chronic traumatic encephalopathy (CTE) and amyotrophic lateral sclerosis (ALS). Currently, there is only a loose correlation between trauma and ALS, however, genetically predisposed animal models, such as the SOD1^{G93A} ALS rat that recapitulates the human condition, allow us to closely study this link.

We recently developed a novel model of repeat TBI associated with sustained motor deficits. Rats administered multiple TBI exhibit brain atrophy and tauopathy consistent with the TBIlinked neurological disease CTE. These sustained deficits and pathology have not been observed in other rodent TBI models that deliver one-time, or repeat, *unilateral* insults. Using this model to more accurately represent injuries sustained by populations such as professional athletes and veterans, we further explored the link between repeat TBI and the initiation of premature onset of ALS in SOD1^{G93A} rats.

Methods: SOD1^{G93A} rats were administered repeat TBI starting at (pre-symptomatic) postnatal day 60, whereby a closed-skull, bilateral controlled cortical impact (CCI) injury was delivered once weekly for 5 weeks. Uninjured SOD1^{G93A} sham controls were exposed to anesthesia only. TBI rats were classified as having "mild" or "severe" symptoms based on rotorod performance following the last injury. Rats were assessed weekly for motor function and followed to disease endpoint.

Results: SOD1^{G93A} rats administered repeat TBI that were observed to have initial severe rotorod deficits following their final TBI, exhibited forelimb paralysis significantly earlier than their SOD1^{G93A} sham counterparts (median onset: 150 vs 169 days, respectively p<.0001). Earlier disease onset translated into shortened survival in SOD1^{G93A} TBI rats relative to SOD1^{G93A} sham controls (median survival: 168 vs 179 days, p=.038), with no observed gender differences. Interestingly, rats exhibiting only mild initial rotorod deficits did not show exacerbation of disease.

Conclusions: We demonstrate using a SOD1^{G93A} rat model of ALS that recurrent TBI can lead to earlier disease onset. Our findings have important implications for those who are genetically predisposed to neurodegenerative diseases and suggest that for these individuals, exposure to austere environments or participation in sporting teams that have a high incidence for repeat concussions should be carefully considered.

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Poster

301. Motor Neuron Disease: Animal Models I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 301.10/V10

Topic: C.05. Neuromuscular Diseases

Title: Studying neuroprotective mechanisms in the SOD1-G93A mouse model of Amyotrophic lateral sclerosis

Authors: *S. FIGUEROLA SANTAMONICA¹, F. DE LORENZO¹, M. SENDTNER², M. H. VOUTILAINEN¹, M. SAARMA¹ ¹Inst. of Biotech., Univ. of Helsinki, Helsinki, Finland; ²Univ. Wurzburg, Wuerzburg, Germany

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive degeneration of motor neurons (MN) leading to muscle atrophy, paralysis and

eventually death of the patients within 1-3 years from onset of symptoms. To date, there is no curative treatment for ALS.

Motoneuron degeneration in ALS is caused by a complex interplay between multiple pathogenic processes. Molecular processes such as mitochondrial dysfunction, accumulation of SOD1 and TDP-43 protein aggregates, oxidative stress and Endoplasmic reticulum (ER) stress have been shown to contribute to dysfunction and death of MNs in ALS.

The aim of this study was to characterize the SOD1-G93A mouse model of ALS and study pathological mechanisms associated in the SOD1-G93A mouse model. The progression of the disease in SOD1-G93A mice was evaluated by body weight changes, signs of disease and behavioral tests such as rotarod, grip strength and multirod system. At the disease endpoint, mice were sacrificed and samples from spinal cord, gastrocnemius muscle and motor cortex were collected for the molecular analyses of unfolded protein response (UPR) pathways. SOD1 mice showed reduced body weight, survival and motor deficits as compared to wild type

littermates. In addition, MN number was reduced and UPR pathways activated in spinal cord of these mice.

In the poster, we will report the effects of new molecule in SOD1-G93A mouse model.

Disclosures: S. Figuerola Santamonica: None. F. De Lorenzo: None. M. Sendtner: None. M.H. Voutilainen: None. M. Saarma: None.

Poster

301. Motor Neuron Disease: Animal Models I

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Topic: C.05. Neuromuscular Diseases

Support: W81XWH-13-1-0416

R01 NS072428-04

Title: SOD1^{G93A} astrocytes induce toxicity in motor neurons via a DR6-mediated pathway in ALS model

Authors: *V. MISHRA¹, V. LE VERCHE¹, D. B. RE², K. POLITI¹, P. RINCHETTI¹, M. J. ALVAREZ³, A. CALIFANO³, F. LOTTI¹, S. PRZEDBORSKI^{1,4} ¹Motor Neuron Ctr., ²Dept. of Envrn. Hlth. Sci., ³Dept. of Systems Biol., Columbia Univ., NY, NY; ⁴Dept. of Neurology, Pathology and Cell Biol., Columbia Univ., New York, NY

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease caused by loss of motor neurons in the brain and spinal cord. Among the 10% familial cases of ALS, substitution in superoxide dismutase-1 (SOD1) gene at position G93 is one of the most

common mutations found in ALS. Previous results in the lab have shown that conditioned media from SOD1^{G93A}, but not SOD1^{WT} expressing astrocytes were able to induce toxicity specifically in primary and embryonic stem cell-derived motor neurons (ES-MNs). We combined proteomics and genomics approaches to understand how astrocyte conditioned media (ACM) exposure induces motor neuron death and to identify pivotal players driving the death phenotype. Death Receptor 6 (DR6) is one of the candidate genes, whose activity is upregulated in motor neurons treated with SOD1^{G93A} ACM. DR6 is a member of Tumor Necrosis Factor receptor superfamily (TNFRSF) family of receptors that has been shown to be involved in cell death signaling pathway and immunomodulation in various in vitro cancer cell models. Additionally, the role of DR6 in neurite growth is well established but its role in neuronal cell death pathway is still unclear. We hypothesize that DR6 might be one of the upstream signaling molecules involved in initiation of motor neuron cell death. Indeed, cell culture-based experiments showed that DR6 KO motor neurons show protection against toxicity induced by SOD1^{G93A} expressing astrocytes compared to WT motor neurons. To further verify these results in animal models, we used DR6 knockout mice and viral transduction of motor neurons to assess the consequences of reduced DR6 levels on the disease phenotype of ALS mice. We performed a panel of morphological assays to determine the effect of reduced DR6 signaling on motor neuron survival and neuromuscular junction (NMJ) innervation. SOD1^{G93A} mice with DR6 knock-down exhibited improvement in motor neuron survival (P120) compared to SOD1^{G93A} alone but, no difference was detected in NMJ denervation at this time. In-spite of modest improvement in motor neuron counts, there was minimal effect on disease onset and survival in SOD1^{G93A} mice with reduced DR6 levels. Functional recovery evaluated by behavioral testing showed transient improvement in grip strength. Current efforts are focused on establishing an inducible ES-derived model to study the molecular pathways that lead to motor neuron-specific cell death by DR6 activation. This study will provide an insight into the possible mechanisms involved in the signaling cascade leading to motor neuron death, and in combination with NMJ preserving compounds, might lead to new therapies for preventing or delaying ALS.

Disclosures: V. Mishra: None. V. Le Verche: None. D.B. Re: None. K. Politi: None. P. Rinchetti: None. M.J. Alvarez: Other; DarwinHealth, Inc. NY. A. Califano: None. F. Lotti: None. S. Przedborski: None.

Poster

301. Motor Neuron Disease: Animal Models I

Location: Halls A-C

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Topic: C.05. Neuromuscular Diseases

Support: NIH NS069616

NIH NS098228

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Title: Towards syncing polytherapy to the multi-factorial dynamics of the SOD1 G93A ALS pathology

Authors: A. LEE¹, S. TRAVAGLINO¹, T. KITTEL¹, *C. S. MITCHELL² ¹Biomed. Engin., Georgia Inst. Technol., Atlanta, GA; ²Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA

Abstract: An expansive range of preclinical ALS treatments have been evaluated in SOD1 G93A mice with limited success. Given temporal dynamics are crucial to homeostasis, we hypothesize that the perceived failure of previous preclinical and clinical treatments may be due to the fact they targeted only one factor or were not optimally timed to the corresponding ALS disease stages, where the pathophysiology is dynamically ever-changing and has been found to be mathematically unstable. That is, ALS may require combination treatments or "polytherapy" initiated in sync with the corresponding target dynamics associated with each pathophysiologically distinct disease stage; such therapies may not necessarily target the initiating mechanistic perturbation, but rather, compensatory regulatory system dynamics capable of restoring homeostasis to halt the spread of motoneuron death. The long-term goal of the present work is to devise a multi-faceted polytherapy strategy that considers ALS dynamics at each disease stage and is clinically translatable. To this end, we first assess the efficacy of individual preclinical treatments targeting specific categories of ALS pathophysiology as a function of time, disease stage, and assessment modality (e.g. survival, general health, muscle function, time of onset). The approach consists of performing a multi-treatment meta-analysis of data transcribed from over 3,800 peer-reviewed studies to compare the individual effects of all 9 ALS ontological pathophysiology treatment targets (e.g. apoptosis, axonal transport, cellular chemistry, energetics, excitability, inflammation, oxidative stress, proteomics, and systemic) over 7 temporal stages throughout the in vivo SOD1 G93A mouse lifespan (on average, about 150 days). A sub-set of data, which experimentally examined the simultaneous or serial usage of two or more pathophysiological treatment categories is compared to the at-large traditional individual categorical treatments. Our presented results illustrate the best treatment targets at each temporal disease stage and their quantified efficacy as a function of assessment modality. Secondly, we present updated computational models to continuously assess inter-relationships and regulatory compensation needed to maintain motoneuron homeostasis in the physiological wild type system and re-establish post-onset homeostasis in the pathological ALS system. The latter models provide a foundation for in silico identification and testing of promising ALS polytherapy strategies precisely timed to maximally target pathology dynamics.

Disclosures: A. Lee: None. S. Travaglino: None. T. Kittel: None. C.S. Mitchell: None.

Poster

301. Motor Neuron Disease: Animal Models I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 301.13/V13

Topic: C.05. Neuromuscular Diseases

Title: Point mutations within Tardbp differentially affect TDP-43 functions

Authors: *A. ACEVEDO AROZENA^{1,2}, T. RICKETTS², P. SIVAKUMAR³, H. OLIVEIRA², V. PLAGNOL³, K. LO³, J. HUMPHREY³, E. M. C. FISHER³, P. FRATTA³ ¹Unidad de Investigación, Hosp. Universitario De Canarias, La Laguna, Spain; ²MRC Mammalian Genet. Unit, Harwell, Oxfordshire, United Kingdom; ³UCL, London, United Kingdom

Abstract: We generated and fully characterised two novel mouse models of TDP-43 proteinopathies, developed using *N*-ethyl-*N*-nitrosurea (ENU) mutagenesis, each containing a single substitution within the coding region of the mouse endogenous *Tardbp* gene. The first mutation (**F210I**) lies within the second RNA recognition motif (RRM2). The second mutation (**M323K**) occur within the C-terminal glycine rich domain where the majority of ALS/FTD causing mutations are found. However, none of the mutations have been reported in human patients. Both models have been extensively studied through behavioural and histopathology analysis. At the molecular level, RNA sequencing was used to examine alternative splicing events and differential gene expression from different systems, while individual nucleotide resolution crosslinking and immunoprecipitation (iCLIP) highlighted changes in RNA-binding patterns of the TDP-43 mutants. Severe molecular dysregulation was identified in both mouse mutants, alongside opposite changes in TDP-43 splicing activity.

The F210I mutation leads to a dose dependent loss of TDP43 function via diminishing RNA binding capacity. Homozygous animals ($Tardbp^{F210I/F210I}$) are not viable, but depending on genetic background can survive up to E18.5. Heterozygous ($Tardbp^{F210/+}$) mice do not develop neurodegeneration but display extensive genome-wide molecular changes at the splicing and expression levels. Although the total TDP-43 protein levels are not changed by the F210I mutation, we detected changes in the processing of the *Tardbp* transcript potentially impacting on TDP-43 autoregulation mechanism.

The M323K mutation leads to opposite effects to the F210I mutation in TDP-43 mediated splicing activity. The mutation does not affect RNA binding or TDP-43 protein levels. Homozygous mice (*Tardbp*^{M323K/M323K}) survive to adulthood on a mixed genetic background, and we are currently finishing their behavioural and histopathological analysis. Overall, here we show that point mutations within the *Tardbp* gene can differentially affect TDP43 known functions. The array of RNA binding and processing features disrupted by each mutation

provides novel insights into how TDP-43 regulates RNA processing *in vivo*, revealing potential early molecular mechanisms in the development of ALS.

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Poster

301. Motor Neuron Disease: Animal Models I

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Topic: C.05. Neuromuscular Diseases

Support: NIH NINDS 5T32NS041218

Title: Muscle fiber atrophy in tibialis posterior of a TDP-43 over-expressing mouse

Authors: *L. HEYBURN¹, M. HEBRON², C. E. MOUSSA², B. T. HARRIS² ²Neurol., ¹Georgetown Univ., Washington, DC

Abstract: TAR DNA binding protein (TDP-43) is a DNA/RNA binding protein whose expression is altered in diseases of TDP-43 pathology including amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease affecting both upper and lower motor neurons in the brain and spinal cord, respectively. ALS affects neurons that innervate skeletal muscles and control voluntary movement. Some forms of FTLD also involve motor neuron degeneration (FTLD-MND). Loss of motor neuron input into the skeletal muscles leads to muscle weakness and atrophy, eventually leading to respiratory failure. Roughly 70% of ALS patients present with limb onset weakness, with symptoms eventually spreading to other areas. Patients with mostly lower motor neuron forms of ALS have a slower disease progression and have single muscle fibers with a larger cross-sectional area than patients with a quicker disease progression. There is currently no cure for TDP-43 pathologies, however, our group has been investigating the use of tyrosine kinase inhibitors (TKIs) for the treatment of neurodegenerative diseases. Nilotinib is a TKI that is FDA-approved for the treatment of chronic myelogenous leukemia. We have previously shown that it can induce autophagy and reduce levels of the ALS-associated protein TDP-43 in vitro and in vivo. In this study, we use a hemizygous transgenic mouse model that overexpresses human wild-type TDP-43 in neurons to examine spinal cord and skeletal muscles for signs of changes like those seen in TDP-43 pathology. This model uses a Thy1 promoter, which restricts transgene expression to neurons, allowing us to understand how changes to TDP-43 in motor neurons may affect muscles. Homozygous TDP-43 overexpressors do show signs of an ALS-like phenotype, but the hemizygous mice used in this study do not have an overt

phenotype of muscle weakness or motor deficits. We treated these hemizygous transgenic mice (n=4 per group) with nilotinib for 4 weeks and examined spinal cord motor neurons and muscle fibers in the tibialis posterior and compared with untreated transgenic and wild-type mice. We found that overexpression of TDP-43 in this mouse does not lead to a significant reduction in spinal cord motor neuron count, but does lead to a significant reduction in average muscle fiber area, indicating that these hemizygous mice exhibit low level muscle atrophy. In addition, treatment with nilotinib did not significantly increases muscle fiber size or spinal cord motor neuron count compared to control-treated transgenic mice.

Disclosures: L. Heyburn: None. M. Hebron: None. C.E. Moussa: None. B.T. Harris: None.

Poster

301. Motor Neuron Disease: Animal Models I

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 301.15/V15

Topic: C.05. Neuromuscular Diseases

Support: VA CDA2 # I01BX007080

Title: Regulation of CDC7 kinase activity controls pathological TDP-43 phosphorylation

Authors: *N. LIACHKO, H. CURREY, B. C. KRAEMER GRECC, VA Puget Sound Hlth. Care Syst., Seattle, WA

Abstract: Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are two devastating fatal neurodegenerative diseases. Over 90% of ALS cases and approximately 50% of FTLD cases are characterized by pathological inclusions containing the protein TDP-43. Mutations in TARDBP, the gene coding for TDP-43, cause some cases of familial ALS and have been identified in patients with sporadic ALS and FTLD, indicating that TDP-43 functions are critical for neuronal health. While numerous post-translational modifications of TDP-43 have been identified in disease, phosphorylation events at TDP-43 serines 409 and 410 are consistently present and used diagnostically to identify pathological TDP-43-positive inclusions. Phosphorylation of TDP-43 (pTDP) reduces its turnover, increases its aggregation, and promotes neurotoxicity and neurodegeneration. We found that the kinase CDC7 is a key regulator of pTDP accumulation in C. elegans and cell culture models of ALS and FTLD [1]. By phosphorylating TDP-43, CDC7 increases levels of neurotoxic pTDP and worsens disease phenotypes, including neurodegeneration. We have identified post-translational modifications of CDC7 that coincide with a shift in its cellular localization. This shift precedes TDP-43 phosphorylation, and may represent a cellular response to genotoxic stress. Understanding the regulation of TDP-43 phosphorylation will provide insight into the causes of TDP-43 proteinopathies, and may lead to additional targets for therapeutic intervention.

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1. Liachko NF, McMillan PJ, Guthrie CR, Bird TD, Leverenz JB, Kraemer BC. CDC7 inhibition blocks pathological TDP-43 phosphorylation and neurodegeneration. Ann Neurol. 2013;74(1):39-52. doi: 10.1002/ana.23870.

Disclosures: N. Liachko: None. H. Currey: None. B.C. Kraemer: None.

Poster

301. Motor Neuron Disease: Animal Models I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: C.05. Neuromuscular Diseases

Support: KAKENHI (16K07045)

Daiichi Sankyo Foundation of Life Science

Takeda Science Foundation and KAKENHI

The Nakabayashi Trust for ALS Research

Title: Activation of PI3K/mTOR pathway alleviates TDP-43-induced axonopathy in the spinal motor neuron in a zebrafish ALS model

Authors: *K. ASAKAWA^{1,2}, K. KAWAKAMI^{1,2}

¹Natl. Inst. of Genet., Mishima / Shizuoka, Japan; ²SOKENDAI, MIshima / Shizuoka, Japan

Abstract: TAR DNA-binding protein 43 (TDP-43, *TARDBP*) is a major component of the cytoplasmic inclusions that are a pathological hallmark of amyotrophic lateral sclerosis (ALS). While the spinal motor neuron is a primary target for degeneration in ALS, how TDP-43 leads to dysfunction and degeneration of the spinal motor neuron has not been fully understood. In order to understand early pathological changes caused by TDP-43 toxicity, an animal model that allows for monitoring an identified single spinal motor neuron *in vivo* should prove effective. Exploiting the optical and genetic accessibility of zebrafish, we focus on the spinal motor neuron CaP, which is uniquely present in each spinal hemisegment and innervates a defined territory within the ventral myotome. We find that both systemic knockout and targeted overexpression of TDP-43 result in axonal outgrowth defects in CaPs, suggesting that dysregulation of TDP-43 at the protein level causes a reduction in motor unit size. Furthermore, *in vivo* two-photon calcium imaging reveals that the CaPs overexpressing TDP-43 display attenuated calcium transients in both the cell body and axon terminals during fictive swimming, indicating a reduced neuronal excitability. Consistent with this observation, pan-neuronal overexpression of TDP-43 results in

an impaired swimming activity. By using this transgenic zebrafish ALS model, we explore biological pathways that rescue the toxicity associated with an elevated TDP-43 level. We discover that the targeted activation of PI3K α effectively restores the axonal outgrowth of CaPs overexpressing TDP-43. The defects in neuromuscular synapse formation and neuronal excitability are also rescued, albeit partially, by the PI3K α activation. The bath application of rapamycin, an inhibitor of the mTOR protein kinase, blocks the PI3K α -mediated restoration of the axonal outgrowth, suggesting that mTOR is a downstream effector the rescue effect. Intriguingly, while rapamycin treatment inhibits the axonal outgrowth of CaPs in the wild type fish, it does not exacerbate the axonal outgrowth defect caused by TDP-43 overexpression, implying that TDP-43 causes the axonal outgrowth defect through inhibiting the mTOR pathway. These observations suggest that an elevated TDP-43 level hampers axonal outgrowth through attenuating the PI3K α /mTOR pathway that promotes axonal outgrowth in the spinal motor neuron. Therefore, activation of the PI3K α /mTOR pathway might serve as a potential strategy to maintain or restore motor unit size in ALS.

Disclosures: K. Asakawa: None. K. Kawakami: None.

Poster

301. Motor Neuron Disease: Animal Models I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: C.05. Neuromuscular Diseases

Support: MNDRIA and Cure For MND Foundation (GIA1638)

Title: *In vivo* characterisation of neurodegeneration and TDP-43 redistribution following UV induced stress in the Zebrafish spinal cord

Authors: *A. J. SVAHN, R. RADFORD, E. K. DON, N. J. COLE, R. CHUNG, M. MORSCH MND Res. Centre, Dept. of Biomed. Sci., Macquarie Univ., Sydney, Australia

Abstract: The clinical progression of motor neuron disease (MND) suggests a pathological spread of neurodegeneration through the nervous system. The mechanisms that are responsible for the spread of neurodegeneration are not known. *In vitro* evidence has emerged for a 'prion-like' spread of aggregates from cell to cell.

To follow the time-course of neurodegeneration and examine the potential for transfer of proteins following cell stress *in vivo*, we took a live imaging approach in the Zebrafish spinal cord. We tracked fluorescently labelled cell membranes and the RNA binding protein TDP-43, which has been identified as the main protein of pathological inclusions that characterise MND and frontotemporal lobar dementia (FTLD).

Live imaging following selective UV pulse targeting of a single motor neuron in the zebrafish
spinal cord induced cell stress and an apoptotic or necrotic process. Ablated and dying neurons showed characteristic morphological changes, including the shrinkage of the cell soma, and progressive anterograde degeneration (blebbing) commencing at the target site and continuing along the axon over time. In the healthy, developing spinal cord this resulted in rapid attraction of local microglia and take-up of the cell body containing TDP-43. In the absence of microglia following PU.1 (spi1b) knockdown, stressed motoneurons underwent a stereotyped process of neurodegeneration including redistribution of TDP-43 from the nucleus, exosome shedding and eventual cell breakdown.

This real-time representation of TDP-43 redistribution and microglial phagocytosis in the living zebrafish spinal cord provides novel opportunities to study the mechanisms of the spread of neurodegeneration.

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Poster

301. Motor Neuron Disease: Animal Models I

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Title: Impaired mitophagy in ALS mice results in degeneration of neuromuscular junctions

Authors: *R. S. ROGERS¹, S. TUNGTUR¹, T. TANAKA¹, Y. BADAWI¹, L. L. NADEAU¹, H. WANG², H.-M. NI², W.-X. DING², H. NISHIMUNE¹ ¹Anat. & Cell Biol., ²Pharmacology, Toxicology and Therapeut., Univ. of Kansas Med. Ctr., Kansas City, KS

Abstract: Amyotrophic lateral sclerosis (ALS) is a motor neuron disease starting from degeneration of pre-synaptic nerve terminals, known as a dying back neuropathy. Defects in autophagy play an important role in the progression of nerve terminal degradation in ALS.

Importantly, some familial ALS patients have a mutation in an important autophagic gene, SQSTM1. Using an animal model of ALS, SOD1^{G93A} mice, we examined whether defects in mitophagy, the autophagic process of degrading depolarized and degenerating mitochondria, play a role in motor neuron degeneration. Neuromuscular junctions (NMJs) of SOD1^{G93A} mice have a greater number of autophagosomes and degenerated mitochondria compared to wild-type mice. These changes precede similar changes that occur in motor neuron cell bodies. Importantly, we detected mitophagosomes at NMJs of SOD1^{G93A} and wild-type mice, but the number of mitophagosomes in NMJs did not increase in parallel with the accumulation of degenerated mitochondria – indicating a potential defect in mitophagy in SOD1^{G93A} mice. In addition, content of mitophagic proteins were lower in motor neuron cell bodies of SOD1^{G93A} mice compared to wild-type mice. To establish a causal role for defective mitophagy in the progression of motor neuron degeneration, we examined double knockout mice of PTENinduced putative kinase 1 (Pink1) and Parkin. Pink1 and Parkin are essential for sensing depolarized, degenerating mitochondria and recruiting autophagosomes to these mitochondria. Pink1/Parkin double knockout mice at 4 months of age exhibited degeneration and denervation of NMJs and accumulation of mitochondria in NMJ presynaptic terminals. Together these data support that defective mitophagy plays a role in motor neuron degeneration in ALS.

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Poster

301. Motor Neuron Disease: Animal Models I

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Topic: C.05. Neuromuscular Diseases

Support: NIH Grant NINDS NS098523

Title: A resource for research on peripheral neuropathy using mouse models at The Jackson Laboratory

Authors: *K. L. SEBURN, R. W. BURGESS, C. M. LUTZ Jackson Lab., Bar Harbor, ME

Abstract: Inherited peripheral neuropathy, or Charcot-Marie-Tooth disease (CMT), constitutes an important area of research given its cumulative frequency of greater than 1 in 2500 people and the lack of any curative treatments. Mouse models of inherited peripheral neuropathy contribute to this research effort, but also present challenges in terms of accessibility, varying degrees of face validity, and poorly defined genetic backgrounds. To improve the usefulness of mouse

models of inherited peripheral neuropathy, and to facilitate rigor and reproducibility between investigators, we have created the Resource for Research on Peripheral Neuropathy at The Jackson Laboratory (www.jax.org/rrpn, email: peripheral.neuropathy@jax.org), funded by NINDS (R24 NS098523). The goal of this resource is to import existing models of CMT, and to create new models using genetic engineering when suitable models do not already exist. We anticipate adding five to ten models per year to this resource, with advice on prioritization coming from our external steering committee. These mice are then placed on a defined genetic background and made available to the research community through The Jackson Laboratory's Mouse Repository under standard terms of use. In addition to these models being available through the highly visible and accessible JAX Catalog, the resource will also perform baseline phenotyping using a battery of clinically relevant tests. This data, and the phenotyping protocols, will also be publicly available. In this way, the models are validated for their disease relevance, and interested investigators can use public data concerning sex differences, age of disease onset, and severity of disease for planning experiments, performing power analyses, etc. Thus, the Resource for Research on Peripheral Neuropathy serves as an example of how animal models can be generated and made available for research with optimal rigor and reproducibility of both the mice and the data.

Disclosures: K.L. Seburn: None. R.W. Burgess: None. C.M. Lutz: None.

Poster

301. Motor Neuron Disease: Animal Models I

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Topic: C.05. Neuromuscular Diseases

Support: NSF DDRIG

COSMOS Club Foundation

GWU Luther Rice Fellowship Award

Title: Animal model indicating an interaction between MS and exercise's impact on adult hippocampal neurogenesis

Authors: *J. KLEINER¹, B. M. SCHILDER¹, C. SHERWOOD², K. A. PHILLIPS³ ²Anthrop., ¹George Washington Univ., Washington, DC; ³Psychology, Trinity Univ., San Antonio, TX

Abstract: While multiple sclerosis (MS) is typically understood as an autoimmune disease that degrades motor functioning, it can also have significant impacts on cognition. Previous studies suggest that MS affects the hippocampus, damaging patients' cognitive spatial and memory

abilities.Dysregulation of adult hippocampal neurogenesis (AHN), the birth of new granule cells (neuroblasts) which are necessary for proper memory formation, may be a mechanism by which MS disrupts hippocampal functioning. Some research suggests that exercise may lessen some neuropathological effects of MS. The present study utilized marmoset monkeys (Callithrix *jacchus*) to model the neurobiological impacts of MS and exercise therapy on AHN. Eight adult male marmosets were sensitized to myelin oligodendrocyte (MOG) glycoprotein (MOG 34-56) in incomplete Freund adjuvant. This was injected into eight adult male marmosets, inducing autoimmune encephalomyelitis (EAE) which models the neurobiological effects of MS in humans with relapse-remitting MS. An additional four control subjects were injected with incomplete Freund adjuvant alonesaline. Half of the EAE and control subjects engaged in aerobic exercised for 30 minutes, three days/week for 10 weeks. Sections across the anteroposterior length of the hippocampus were immunohistochemically stained for doublecortin (DCX) as a marker for neuroblasts. Stereologic counts of the absolute number, proportion (relative to granule cells) and density of DCX cells were quantified in the dentate gyrus of the hippocampus, divided into the Granule Cell Layer (GCL) and the Subgranular Zone (SGZ), where neuroblasts proliferate. Our findings showed that although neither EAE nor exercise statuses had a significant main effect on any of the DCX cell measures alone (p><0.05), there was a significant interaction between these two variables (ANOVA, Fdf=7.103, p=0.029). Exercise increased absolute DCX cell numbers in the EAE group but decreased it in the control group. Similarly, non-significant trends were observed for %DCX cells and DCX cell density. These results suggest a relationship between exercise and EAE's impact on AHN. This supports the hypothesis that exercise mitigates the impacts of MS on AHN. Thus, this interaction may be a physiological explanation of the benefit human patients see with exercise therapy, regarding spatial and memory cognition.

Disclosures: J. Kleiner: None. **B.M. Schilder:** None. **C. Sherwood:** None. **K.A. Phillips:** None.

Poster

301. Motor Neuron Disease: Animal Models I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 301.21/V21

Topic: C.05. Neuromuscular Diseases

Title: Environmental and genetic contributions in an ALS rat model: Failed recovery and enhanced ventral horn inflammation after peripheral nerve injury

Authors: *S. SCHRAM¹, D. CHUANG², H. PIPONOV², C. HELDER², G. SCHMIDT², R. MICHAEL², F. SONG¹, J. KERNS², M. GONZALEZ², J. LOEB¹ ¹Neurosci., Univ. of Illinois - Chicago, Chicago, IL; ²Univ. of Illinois at Chicago, Chicago, IL

Abstract: Background: ALS is poorly understood and no effective therapeutics exist to stop its insidious progression. For some it starts in a hand or a foot, and in others with trouble swallowing. Once it starts, the disease progresses along the spinal cord, ultimately resulting in respiratory failure. Clinicopathologic human studies have shown a clear relationship between disease onset and lower motor neuron loss, with the most severe lower motor neuron loss at the site of disease onset. One long entertained observation is that ALS may be precipitated by nerve or brain injury. In fact, many patients with ALS are veterans or athletes (Lou Gehrig) and may have suffered minor nerve injuries in the limb where ALS first presents. However, it is unclear whether and how nerve injury plays a role in ALS development. A growing number animal models for ALS are based on human genetic forms of the disorder, but a large number of therapeutic interventions that showed efficacy in these models have failed in human clinical trials. Our lab and others have previously shown an increase in spinal cord microglial activation in post-mortem ALS patient tissue, as well as at early stage in animal models of ALS, suggesting microglial activation contributes to early stages of neurodegeneration. Objectives: Our current study seeks to link an environmental factor (nerve injury) with a genetic factor (SOD1 mutation) to induce symptom onset and disease progression. Methods: We performed sciatic nerve crush injuries in SOD1 G93A rats and littermate wild type controls in early disease stage, prior to any known symptoms. Functional recovery using the Extensor Postural Thrust (EPT) test was tracked weekly. Spinal cord tissue was collected at different stages of disease for staining and quantitative analysis. Results: Significantly enhanced and sustained microglial activation was seen in the ventral horns of SOD1 rats at 1 and 2 weeks after injury that spread to nearby, uninjured motor neuron pools. This microglial activation in the SOD1 animals subsided by 7 weeks. While wild type animals showed full functional recovery by 4 weeks, the SOD1 animals never fully recover. Long term effects of this early injury on survival and the underlying mechanism by which enhanced inflammatory mechanisms lead to failed recovery are currently being investigated. Discussion: These studies take a unique approach to understand the effects of early environmental contributions (nerve injury) in a genetic model of ALS. The system developed could be an important new model for drug development that focuses on disease onset and progression rather than traditional models of survival and may therefore translate better to the human condition.

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Poster

301. Motor Neuron Disease: Animal Models I

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Program#/Poster#: 301.22/V22

Topic: C.05. Neuromuscular Diseases

Support: DC Intellectual and Developmental Disabilities Research Center (U54HD090257)

GW Program for Pediatric Dysphagia (P01HD083157)

Title: Neurodegenerative changes in the hypoglossal nucelus of the LgDel mouse model of DiGeorge/22q11 Deletion Syndrome

Authors: *A. S. POPRATILOFF¹, X. WANG², P. MISTRY³, L. MATSIYEVSKIY³, C. CLARKSON-PAREDES³, C. A. BRANTNER³, T. M. MAYNARD², D. W. MEECHAN², S. A. MOODY⁴, D. S. MENDELOWITZ², A. S. LAMANTIA² ²Pharmacol. and Physiol., ³Nanofabrication and Imaging Ctr., ⁴Anat. and Regen Biol, ¹George Washington Univ., Washington, DC

Abstract: DiGeorge/22q11 Deletion Syndrome (22q11DS), a micro-deletion of 30 to 50 genes on human chr.22, is associated with neurodevelopmental disorders. Cardiovascular and craniofacial abnormalities as well as intellectual disability, schizophrenia, autistic spectrum or attention deficit/hyperactivity disorders define the 22q11DS clinical phenotypic spectrum. 22q11DS craniofacial anomalies are also linked to common perinatal feeding and swallowing disorders (dysphagia) whose origins remain poorly understood. We have shown that the LgDel mouse model of 22q11DS recapitulates the human phenotypic spectrum including perinatal dysphagia, prefigured by disruption of hindbrain patterning and cranial nerve differentiation in LgDel embryos. We also found that changes in neuronal excitability in hypoglossal nucleus (nXII) motoneurons recorded in brainstem slices from one week old LgDel mouse pups accompany LgDel perinatal dysphagia. We therefore asked whether nXII cytological abnormalities are also associated with LgDel feeding/swallowing difficulties. We used a ChAT:GFP reporter to identify and visualize nXII motorneurons. Motorneuron morphology and perikaryal volumes are altered in LgDel nXII. Confocal 3D reconstructions of single biocytininjected nXII motorneurons demonstrate dendritic and axonal abnormalities, including degenerating dendrites. Finally, block-face scanning electron microscopy allowed integrated analysis of nXII location, neuronal identity and ultrastructural characteristics in individual mouse pups. We confidently identified dilated mitochondrial cristae, pale mitochondrial matrix, and swollen Golgi and endoplasmic reticulum in nXII motoneurons of one week old LgDel but not wild type pups. At later ages, we found that LgDel nXII motorneurons and interneurons have cytoskeletally sparse, organelle depleted, pale cytoplasm, as well as reduced synaptic frequency. In addition, there is pronounced hypertrophy of perineuronal as well as perivascular astrocytic processes, especially around capillaries. In these *LgDel* mice there is limited evidence of dark degeneration; however we find no evidence of condensed chromatin and nuclear envelope changes associated with neuronal apoptosis. These results suggest that non-apoptotic neuronal degeneration in LgDel nXII may contribute to perinatal dysphagia. The developmental and degenerative changes in cranial nerve circuits for feeding and swallowing may reflect diminished dosage of several 22q11 genes that regulate neuronal mitochondrial metabolism.

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301. Motor Neuron Disease: Animal Models I

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Program#/Poster#: 301.23/V23

Topic: C.05. Neuromuscular Diseases

Support: CIHR MOP-111070

ALS Society of Canada

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Muscular Dystrophy Association

Canadian Foundation of Innovation

Fonds Recherche Québec-Santé Leader Opportunity Fund

Title: Branch-specific assembly and disassembly precedes global motor-unit degeneration in a model of amyotrophic lateral sclerosis

Authors: *E. MARTINEAU¹, A. DI POLO^{1,2}, C. VANDE VELDE^{1,2}, R. ROBITAILLE¹ ¹Dept. de neurosciences, Univ. de Montréal, Montreal, QC, Canada; ²Ctr. de recherche du Ctr. Hospitalier de l'Université de Montréal, Montréal, QC, Canada

Abstract: Amyotrophic lateral sclerosis (ALS) is a late-onset neurodegenerative disease affecting motor neurons. Although loss of neuromuscular junction (NMJ) is an early event, it remains unclear whether it is a consequence of local pathological signals or the expression of a degenerating neuron. Single time point observations show that a subgroup of degenerating motor neurons retract from their NMJs while a distinct population compensates via collateral sprouting. This strongly suggests that NMJ denervation is mainly dependent on the state of the neuron which innervates it. However, the rescue of motor neuron death does not necessarily rescues NMJ loss, suggesting that the local neuromuscular environment may play a key role. If that is the case, the initial loss of NMJs should be branch-specific and asynchronous within a single axonal arborization (motor-unit). Thus, we sought to directly assess the time course of structural changes within single motor-units using repeated in vivo imaging over a 3 month period in a slow progressing model of inherited ALS (SOD1^{G37R} mice). Here we show in symptomatic mice that individual terminal axon branches and synapses are dismantled asynchronously for weeks before the whole motor axon degenerates. Denervation events tend to propagate from the first lost NMJ in an axonal arbor, with distal branches being more susceptible. This precise spatiotemporal pattern implies that local micro-environment factors are involved. Surprisingly, we observed that dismantling of individual axonal branches is accompanied by simultaneous axonal

sprouting and synapse formation onto nearby NMJs. Axonal arborizations sprouted almost exclusively onto NMJs that they did not initially innervate, thus increasing motor-unit size. Paradoxically, motor-units failed to re-innervate their dismantled NMJs which further suggest that local signals dictate the pattern of neuromuscular remodeling in ALS. This motor-unit dynamism was phenotypically silent as indicated by grip strength and body weight measurements. Our results support a model in which NMJ denervation in ALS is a dynamic, initially slow, process of continuous denervation and new innervation. This raises stabilization of NMJs or enhancement of re-innervation as attractive therapeutic targets for ALS.

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Poster

301. Motor Neuron Disease: Animal Models I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 301.24/V24

Topic: C.05. Neuromuscular Diseases

Support: MNDRIA Grant-in-aid

Title: Novel mouse model of an amyotrophic lateral sclerosis-associated profilin 1 mutation

Authors: *M. E. BRETTLE, H. STEFEN, A. DJORDJEVIC, J. W. CHAN, S. FOK, F. DELERUE, Y. D. KE, L. M. ITTNER, T. FATH Sch. of Med. Sci., UNSW Australia, Randwick, Australia

Abstract: Amyotrophic lateral sclerosis (ALS) is the most common form of motor neuron disease. Sporadic and familial forms of disease present with similar clinical symptoms and histopathology. Understanding the underlying pathogenesis of the disease is essential for the development of treatments. Mutations in the actin-associated protein, profilin 1, have been identified as a rare cause of familial ALS. We have developed a novel mouse model to elucidate the role that PFN1^{C71G} plays in ALS. Expression of V5-tagged PFN1^{C71G} is targeted to α -motor neurons in the spinal cord. Initial data shows V5-PFN1^{C71G} expression in the anterior horn of the neural tube starting from embryonic stages in transgenic mice. Motor testing shows that transgenic mice have motor deficits on RotaRod commencing at 1 months of age. This novel mouse model of PFN1^{C71G} will provide a potential tool to understand the role that PFN1 plays in the pathogenesis of ALS and could be used for testing future ALS therapeutics.

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302. Mechanisms of Neuroprotection

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 302.01/V25

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: This work was supported by the Intramural Research Programs of the National Eye Institute.

Title: Exosome-derived from bone marrow mesenchymal stem cells promote retinal ganglion cell survival

Authors: B. MEAD¹, *S. I. TOMAREV²

¹Section of Retinal Ganglion Cell Biol., Natl. Eye Institute, NIH, Bethesda, MD; ²SRGCB, LRCMB, NEI, NIH, Bethesda, MD

Abstract: The loss of retinal ganglion cells (RGC) and their axons is a leading cause of blindness and includes traumatic (optic neuropathy) and degenerative (glaucoma) eye diseases. Mesenchymal stem cells (MSC) have demonstrated significant neuroprotective and axogenic effects on RGC in both of the aforementioned models. The present study aimed to test neuroprotective properties of exosomes isolated from bone marrow-derived MSC (BMSC) in a rat optic nerve crush (ONC) and microbeads or laser photocoagulation glaucoma models. Exosomes were isolated from human BMSC and characterized by NanoSight, flow cytometry and CD63 ExoELISA. Using an *in vitro* axotomized rat RGC model and *in vivo* rat models of ONC and glaucoma, $3x10^9$ exosomes were treated/injected into the cell culture well/vitreous. To measure their neuroprotective and axogenic capacity immunochemistry, optical computerized tomography (OCT) and electroretinography (ERG) were used. The composition of miRNA in exosomes from human BMSC and control human fibroblasts was investigated by RNA sequencing and used to identify candidate target mRNA in RGC.

Both BMSC and fibroblasts secrete similar numbers of exosome as detected and analysed by ExoELISA, electron microscopy, flow cytometry and NanoSight. Treatment of RGC cultures with exosomes led to significant RGC survival (299 ± 24.1 RGC/well) compared to both fibroblast exosome treated (72.3 ± 6.4 RGC/well) and untreated (121.3 ± 6.2) cultures. Following intravitreal transplantation, exosomes successfully integrated into the inner retinal layers, including RGC layer. After ONC (21d), BMSC exosomes provided significant therapeutic effects as compared with fibroblast exosomes or uninjected eyes. For the three measured outputs, the thickness of the retinal neve fibre layer was $33.8 \pm 4.8 \mu m$, $21.6 \pm 1.5 \mu m$, and $18.0 \pm 2.1 \mu m$, respectively; RGC density was $73.3 \pm 7.8/mm$ of retina, $20 \pm 2.2/mm$ of retina, and $23.6 \pm 7.7/mm$ of retina, respectively; and positive scotopic threshold response was $28.6 \pm 8.1 \mu v$, $13.2 \pm 3.4 \mu v$, and $13.7 \pm 1.1 \mu v$, respectively. The significant therapeutic benefits

were also seen in the treatment of glaucoma models. The therapeutic benefit of BMSC exosomes was reduced significantly if isolated from BMSC following knockdown of Argonaute 2, a protein that complexes with miRNA and is integral to their function. RNAseq analysis of miRNA from BMSC and fibroblasts exosomes identified over 20 candidate miRNA that were expressed exclusively or significantly higher in BMSC exosomes.

We demonstrate for the first time that BMSC-derived exosomes offer significant therapeutic benefit to the protection of RGC, an effect mediated at least partially by their miRNA.

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Poster

302. Mechanisms of Neuroprotection

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 302.02/V26

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Biomedical Advanced Research and Development Authority Interagency Agreement

Title: Natural history model of soman-induced epileptogenesis and brain pathology in rats: A long-term study

Authors: *L. A. LUMLEY¹, M. .. DE ARAUJO FURTADO^{2,3}, C. R. SCHULTZ¹, M. Q. PHAM¹, M. BELLIN¹, M. F. STONE¹, B. M. MARRERO-ROSADO¹, F. ROSSETTI⁴ ¹USAMRICD, Gunpowder, MD; ²Anatomy, Physiol. and Genet., Uniformed Services Univ. of the Hlth. Services, Bethesda, MD; ³BioSEaD, LLC, Rockville, MD; ⁴Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: The exposure to organophosphorous (OP) compounds such as the chemical warfare nerve agent soman (GD) can induce *status epilepticus* (*SE*) and brain damage through inhibition of acetylcholinesterase followed by glutamate excitotoxicity. Studies were conducted to investigate the long-term neurological and cardiovascular changes that occur after subcutaneous exposure to 1.0 LD₅₀ GD. Adult male Sprague-Dawley rats implanted with telemetry devices to continuously record EEG, ECG, EMG, body temperature and activity received 1.0 LD₅₀ GD (s.c.) followed by an admix (i.m.) of atropine sulfate (ATS; 2 mg/kg) and the oxime HI-6 (93.6 mg/kg) at 1 min after exposure to increase survival, and diazepam (DZP; 10 mg/kg; s.c.) at 30 min after onset of *SE*. Rats were continuously monitored for up to 10 months after GD exposure for physiological changes. Rats exposed to GD that developed *SE* displayed an increase in the EEG power in the delta and theta band. The power in delta, theta and alpha band remained chronically increased following GD exposure until the end of the study, while the power in the beta band decreased. GD-exposed rats that had *SE* developed spontaneous recurrent seizures (SRS; latency 5-120 days) with increased incidence over time. By 10 days post-SE, ~30% of rats

developed SRS; by 60 days post-SE, ~70% of rats developed SRS, and by 120 days post-SE, 100% of rats had developed SRS. Rats also presented hyperactivity, prolonged QTc intervals and transient increase of heart rate and ECG-derived respiration (EDR). The EMG power spectra increased during behavioral seizure activity and decreased after treatment with DZP. Transient drop in body temperature occurred in all GD-exposed rats but was faster in rats that did not seize. GD-exposed rats that developed *SE* and SRS had significant neuronal loss 10 months after exposure. This natural history study demonstrates that with time, epileptogenesis occurs in the majority of GD-exposed rats that display *SE*.

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Poster

302. Mechanisms of Neuroprotection

Location: Halls A-C

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Program#/Poster#: 302.03/W1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant U01-NS074926

DSI supplied HD-S02 telemetry implants

Title: Evaluation of an improved telemetry transmitter (HD-S02) in experimental pharmacology studies to assess seizure activity in rats

Authors: *M. D. FURTADO^{1,2}, C. SCHULTZ³, T. HAAS⁴, F. ROSSETTI⁵, M. STONE³, B. MARRERO-ROSADO³, L. A. LUMLEY³

¹Anatomy, Physiol. and Genet., USUHS, Bethesda, MD; ²BioSEaD, LLC, Rockville, MD; ³US Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD; ⁴Data Sci. Intl., St. Paul, MN; ⁵Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: The use of telemetry for EEG recording is the most reliable option in experimental pharmacology and physiology because it reduces animal stress, which leads to an increase in data quality. Despite some disadvantages, such as a reduced number of channels when compared to tethered EEG systems, it better identifies oscillatory brain patterns that are pathological as a result of neurological injury. The development of small wireless telemetry systems with good

signal to noise ratio and spectral resolution enables an in-depth inspection of EEG activity in small animals during events such prolonged seizures (status epilepticus - SE). Also, EEG recordings allow us to detect more epileptic abnormalities than does observation of behavioral seizures alone. We present here a telemetry transmitter (HD-S02) capable of signal acquisition at a higher sampling rate (375 Hz) when compared to our previous iterations (240 Hz). Additionally, an automatic calibration of each channel is implemented with no need to manually input the specific frequencies for each device. The battery can last for up to 5 months of continuous use, which is crucial in experimental epilepsy studies when spontaneous recurrent seizures (SRS) can be delayed in development and brief compared to the initial status epilepticus. Also, the longer battery life of the HD-SO2 reduces need for multiple surgeries to replace battery packs as is needed with some other transmitter types. The battery run-time is conveniently monitored and cross-talk is detected when multiple animals are placed closer than the recommended distance. Each transmitter broadcasts its own signal identification, and as a result, the recording stops when an animal is placed on the wrong receiver or too close to the receiver, thus avoiding the recording of invalid data. To test the efficacy of the new transmitters, male rats implanted with HD-S02 to continuously record the EEG, body temperature and activity received 1.2 LD₅₀ GD (s.c.) followed by an admix (i.m.) of atropine sulfate (ATS; 2 mg/kg) and the oxime HI-6 (93.6 mg/kg) at 1 min after exposure to increase survival, and midazolam (MDZ; 3 mg/kg; i.p.) with or without an experimental antiepileptic drug (lacosamide, rufinamide) at 40 min after onset of SE. Rats were continuously monitored for up to 14 days after GD exposure for physiological changes. EEG seizures were successfully recorded after exposure, and SRS were detected during the sub-chronic period. These findings validate the use of HD-S02 in experimental pharmacology studies. In addition, the higher sampling rate can potentially permit the detection of more features in the EEG signal, ranging from 0.5-100 Hz.

Disclosures: M.D. Furtado: A. Employment/Salary (full or part-time):; BioSEaD, LLC. C. Schultz: None. T. Haas: A. Employment/Salary (full or part-time):; Data Sciences International. F. Rossetti: None. M. Stone: None. B. Marrero-Rosado: None. L.A. Lumley: None.

Poster

302. Mechanisms of Neuroprotection

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 302.04/W2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Evaluation of cannabinoids for anticonvulsant and neuroprotective efficacy in a rat model of soman-induced status epilepticus

Authors: *B. MARRERO-ROSADO¹, C. R. SCHULTZ¹, E. KUNDRICK¹, M. F. STONE¹, S. O'BRIEN¹, K. WALKER¹, F. ROSSETTI², L. A. LUMLEY¹ ¹US Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD; ²Blast Induced Neurotrauma Br., Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: Cannabidiol (CBD), the non-psychoactive component of cannabis, has received increased attention as a potential therapy for treatment-resistant epilepsy. Cannabinoids have beneficial effects in multiple animal models of seizure, ischemia, and traumatic brain injury. Exposure to soman (GD), a chemical warfare nerve agent, induces status epilepticus (SE) and spontaneous recurrent seizures (SRS), leading to extensive neuronal damage and cognitive impairment. When treatment of SE is delayed, the standard anticonvulsant midazolam is ineffective at preventing the development of epilepsy. In the present study, we evaluated the potential benefits of CBD and of JZL195, a drug that increases endogenous cannabinoids through inhibition of degradative enzymes, against GD-induced seizure activity and neuropathology. Adult male Sprague-Dawley rats were surgically implanted with transmitters to continuously record electroencephalographic (EEG) activity. Rats received a lethal dose of GD (sc) followed by an admix (im) of atropine sulfate (ATS; 2 mg/kg) and the oxime HI-6 (93.6 mg/kg) at 1 minute after GD exposure to increase survival. Rats received JZL195 (5, 10 or 20 mg/kg, ip) 1 min after exposure or 40 minutes after SE onset. JZL195 (20 mg/kg) significantly decreased cell death in the basolateral amygdala and reduced the loss of inhibitory interneurons (GAD67+) in the piriform cortex at 72 hours after GD exposure. At 2 weeks after exposure, JZL195 significantly reduced the loss of mature neurons (NeuN+) in the basolateral amygdala and medial thalamus. In a second experiment, rats received 150 mg/kg (ip) of CBD either at 60 or 30 minutes before GD exposure or at 1 minute after GD exposure and MDZ (3 mg/kg; sc) at 30 minutes after SE onset. CBD administered as pretreatment reduced the risk of developing SRS and the number of SRS events in a time-dependent manner with the 60-minute pretreatment being the most beneficial. Preliminary data suggest a decrease in mature neuronal cell loss in CBD pretreatment groups at 2 weeks following GD exposure. Altogether, data from these studies suggest that cannabinoids reduce the toxic effects of GD poisoning. This research was supported by an NIH Interagency Agreement with the USAMRICD.

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Poster

302. Mechanisms of Neuroprotection

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Program#/Poster#: 302.05/W3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Shota Rustaveli NSF grant FR/617/7-270/13

Title: Neuroprotective effects of active fraction of flavonoids from Saperavi on kainic acidinduced epilepsy in rats

Authors: M. QURASBEDIANI¹, N. DOREULEE¹, *M. ALANIA¹, B. CHKHARTISHVILI¹, B. PARTSVANIA², M. CHIKOVANI¹, R. BUKIA¹

¹I.Javakhishvili Tbilisi State Univ., Tbilisi, Georgia; ²Georgian Tech. University, Inst. of Cybernetics, Tbilisi, Georgia

Abstract: Temporal lobe epilepsy (TLE) is one of the common human seizure disorders characterized by seizures poorly controlled with anticonvulsant medications. Earlier reduction of the total antioxidant status and increase of production of nitric oxide (NO) in epileptogenesis were demonstrated. In new approaches of TLE treatment plant flavonoids are very important, because of their abilities to scavenge reactive oxide species and to inhibit pathological NO. In our previous experiments antioxidant potency of flavonoids from saperavi (SF) was revealed. The aim of the first series of current experiments was to define a role of supplementation of rats during the early stages of epileptogenesis with SF (8 days, 25mg/kg per day, after single i.p. injection of kainic acid (15mg/kg)) on a number and duration of the behavioral seizure attacks. The effects of SF were compared with the effects of flavonoid quercetin and L-NAME (a nonselective inhibitor of NO synthase) (8 days of administration, 25mg/kg and 40mg/kg per days, respectively). Behavioral seizures were monitored during open field and T-maze laboratory tests. It was revealed that behavioral alterations in Kainic Acid-Status Epilepticus (KA-SE) animals were abolished by administration of SF. The frequency and duration of behavioral seizures in KA-SE rats statistically decreased and correction in learning/memory ability were detected. The efficiency of quercetin on epilepsy induced cognitive impairment compared to the SF was less pronounced. L-NAME effectively blocked the KA-SE-induced seizure frequency and duration, but exacerbate memory deficit induced by epilepsy. The aim of the next series of experiments was to determine the influence of early postnatal feeding of rats with SF on electrophysiological characteristics of neurons in the CA1 field of the hippocampus. The specific objectives were to investigate the effects of SF on background spiking activity and frequency/amplitude characteristics of epileptiform discharges induced by high frequency electrical stimulation. Early postnatal feeding of rats with SF raised the network desynchronization: in the background activity the number of discharges was increased and the amplitude was decreased. High frequency electrical stimulation induced epileptiform discharges with lower amplitude and frequency in SF fed animals compared to the control group. It was suggested that SF has protective effects on the early stages of epileptogenesis.

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Poster

302. Mechanisms of Neuroprotection

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Program#/Poster#: 302.06/W4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Coordinación de la Investigación Científica. Universidad Michoacana. CIC-1821649

Title: Neuroprotective effect of raloxifene under chronic cerebral hypoperfusion, in ovariectomized rats

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Abstract: Selective estrogen receptor modulators have been used as a part of therapeutic schedules to prevent cognitive decline associated with aging, in which estrogenic deficiency and possible chronic cerebral hypoperfusion (CCH) may occur in pre- and post-menopausal women. The present study was aimed to evaluate the neuroprotective effect of Raloxifene in an experimental model of CCH (occlusion of both common carotid arteries, one-week apart), under absence of estrogenic cerebral influence mainly dependent on ovarian secretion. Twenty-five Sprague-Dawley female rats (250-300 g bw) were ovariectomized and randomly allotted, two weeks later, to the following groups: Sham (surgical procedures for CCH, without interrupting cerebral blood flow, n=10); CCH+Veh (CCH+vehicle, 10% dimethylsulfoxide in water, 0.3 ml/day, sc, n=7); CCH+Raloxifene (CCH+Raloxifene, 3.0 mg/Kg/day, sc, n=8). After 60 days of vehicle or Raloxifene treatment, spatial learning and memory were evaluated in the Morris water maze, and brains were processed to evaluate hippocampal CA1 pyramidal neuron population. Parameters of spatial learning and memory (escape latencies, swimming path lengths) did not significantly differ along the seven successive testing days in the three experimental groups. However, during the probe trial (day 8) swimming through the target quadrant, as well as number of crossings over the precise former escape platform location, were similar in the CCH+Raloxifene and the Sham groups, and significantly higher (p<0.05) than in the CCH+Veh group. Hippocampal CA1 pyramidal neuron population was significantly reduced (p<0.05) to a 75%, as compared to the Sham group (100%). Raloxifene treatment prevented this neuronal loss, and resulted in a slight non-significant increase in the pyramidal neuron population (115%). These results support the neuroprotective effects of Raloxifene on the brain structures being a

part of the neural substrate for cognitive functions, affected by estrogenic deprivation and chronic cerebral hypoperfusion.

Disclosures: L. Salcido-Cadena: None. G. Letechipia-Vallejo: None. M. Olvera-Cortes: None. G. Morali: None. M. Cervantes: None.

Poster

302. Mechanisms of Neuroprotection

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 302.07/W5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Neuroprotective effects of Aristotelia chilensis (Maqui berry) extract and dapsone on neonatal status epilepticus model induced by kainic acid

Authors: *L. A. BAUTISTA OROZCO¹, S. A. OROZCO-SUAREZ² ¹Inst. Mexicano Del Seguro Social, Ciudad DE Mexico, Mexico; ²Inst. Mexicano del Seguro Social, México, Mexico

Abstract: The neuroprotection after an initial insult as seizure activity is considered essential in order to avoid the establishment of epilepsy. The aim of this study was to evaluate the neuroprotective effects of Aristotelia chilensis (Maqui berry, MB) extract and Dapsone (D) on the epileptogenesis associated to the damage produced by status epilepticus induced with kainic acid (KA) in neonate rats. Male Sprague Dawley rats (96, 12 days old) were divided into 5 groups: Control group (C), AK group, AKMB group, AKD group and AKMBD group with 3 evaluation time (24 h, 5 and 20 days). The kainic acid was administered once with a 3 mg/kg dose to induce status epilepticus. Seizure activity was assessed daily, The brains were obtained by craniotomy which were processed for immunofluorescence techniques to identify proinflammatory proteins (NFkB, IL-1 ß and COX2 and immunoperoxidase for GFAP) and assess the damage by Fluorojade-stain, and other brains were processed for western blot to quantify NFkB, IL-1 β and COX2. The *Maqui berry* extract reduced the number of seizures at 17 and 32 PN days, as well as the number of cells on degeneration process and the expression of pro-inflammatory proteins at 24 h after kainic acid injection, as compared with AK (P<0.05), however Dapsone had a greater effect in reducing the number of seizures at 17 and 32 PN days, the number of cells on degeneration process and also reduced the relative expression of proinflammatory proteins at 24 h after seizures. Maqui berry extract has neuroprotective and antiinflamatory effects, these effects were greater with Dapsone. This study suggests that early intervention on the inflammation and antioxidant pathways reduces the risk of developing epilepsy.

Disclosures: L.A. Bautista Orozco: None. S.A. Orozco-Suarez: None.

Poster

302. Mechanisms of Neuroprotection

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Program#/Poster#: 302.08/W6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Ferulic acid enhances the neuroprotective properties of calorie restriction against acrylamide model in *Drosophila melanogaster*: Neurobehavioral and biochemical evidences

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Abstract: Protein malnutrition is associated with neurochemical implications, and behavioural deficits in experimental animals. Oxidative stress is a pivotal denominator underlying the protein malnutrition physiology. However, limited dietary restriction is implicated with reduced redox markers among different neuron types in vivo. Accordingly it was inquisitive to assess possible therapeutic property for dietary protein in a neurodegenerative model. Casein (the complete protein) was selected as a dietary modulant against acrylamide induced neurotoxicity in Drosophila melanogaster, which is an extensively used in vivo system to assess the relation between dietary intervention and neuronal oxidative stress. In our study, adult (8-10d) males were maintained on varying concentrations of dietary casein (0.1-2%; n=20 flies/vial, 3vials/group) for 2 weeks were coexposed to acrylamide (Acr 2mM). Motor function and mortality were noted at regular intervals. Terminally, the fly homogenates were subjected to biochemical analysis for redox markers, mitochondrial function and neuronal function markers. Acr induced significant mortality in a time dependent manner (25%) and locomotor phenotype among survivors (35%) which was markedly reduced among casein groups. Casein also reduced the oxidative markers (hydroperoxides, protein carbonyls, GSH, SOD) and neurotoxicity parameters (AChE, BChE) among fly homogenates. Interestingly, coexposure with ferulic acid (FA, 100µM) among casein flies further protected the flies against Acr-neurotoxicity. In addition, modifications in the dietary carbohydrate resulted in obvious fatigue among the flies and therefore was not pursued in this study. From our study, dietary casein proved to be a significant modulator of neuronal health in vivo along with FA. Our data suggests a therapeutic role for dietary protein and ferulic acid-like polyphenols for neurodegenerative diseases.

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Poster

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Research foundation Flanders (FWO-Vlaanderen)

Agency for innovation and entrepreneurship Flanders (Vlaio)

Title: Shining a light on glaucoma: Optogenetic brain stimulation confers retinal neuroprotection

Authors: *E. GEERAERTS, M. CLAES, E. DEKEYSTER, C. VAN DEN HAUTE, M. SALINAS-NAVARRO, L. H. ARCKENS, L. MOONS KU Leuven, Leuven, Belgium

Abstract: Glaucoma is a neurodegenerative disease characterized by a progressive loss of the retinal ganglion cells (RGCs). The central brain targets of the RGCs are well-known to be important for the survival and activity of their retinal connections. To investigate this two-way connection between the brain and the RGCs as a neuroprotective strategy in glaucoma, a series of experiments was performed in which the major retinal projection in the mouse, the superior colliculus (SC), was optogenetically activated in an established experimental mouse glaucoma model.

Optogenetic activation of the SC was performed via injection of an adeno-associated viral vector coding for the stable step-function opsin (SSFO). The SSFO is a channelrhodopsin 2 mutant with slow closing kinetics, thus facilitating prolonged neuronal activation. Transgene expression was shown to cover a large area of the SC. After optic fiber implantation, animals were subjected to the previously reported laser-induced ocular hypertension model (OHT), which induces glaucoma-like RGC loss. Starting one day before OHT induction, the experimental group received light stimulation twice daily until the animals were sacrificed at 14 dpi. Neuronal activation of the SC was validated via immunostaining for the immediate-early gene c-Fos and behavioral analysis. Activation of the SC is known to induce specific behavioral changes, such as turning, freezing and running, which were all observed upon repeated optogenetic stimulation. Behavioral changes lasted for 20 - 30 minutes, coinciding with the SSFO kinetics. Together, these data show that repeated optogenetic stimulation of the SC is possible. Furthermore, following semi-automated quantification of RGCs on Brn3a-stained retinal flatmounts, the optogentically stimulated group showed an significant increase in RGC survival, with 91% (± 4% SEM, N=21) survival versus 75% (± 6% SEM, N=16) in the non-stimulated group, compared to untreated eyes. Our data clearly reveal that repeated stimulation of central brain targets can contribute to neuroprotection of RGCs. Ongoing work includes elucidation of a

possible mechanism, with a focus on involvement of neurotrophins. In conclusion, these results unveil exciting new possible treatment paradigms for glaucoma and other optic neuropathies.

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Poster

302. Mechanisms of Neuroprotection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Phenytoin promotes remyelination of the corpus callosum in the adult mouse brain

Authors: *J. M. VEGA-RIQUER^{1,2}, N. IBARRA-CASTAÑEDA¹, D. ZARATE-LOPEZ¹, G. MENDEZ-VICTORIANO¹, N. MOY-LOPEZ¹, J. GUZMAN-MUNIZ¹, O. GONZALEZ-PEREZ¹

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Abstract: Introduction: Oligodendrocytes loss and myelin sheet destruction are one of the main events that characterize Multiple Sclerosis, a chronic neurodegenerative disease. Endogenous neural precursors in the adult brain may be good candidates to help restore the oligodendroglial population. Phenytoin promotes cell proliferation of neural precursor cells (NPCs) in the postnatal brain. Hence, the pharmacological stimulation of NPCs with phenytoin may represent a viable alternative to promote white matter regeneration. **Purpose:** To evaluate whether phenytoin promotes remyelination in the corpus callosum of adult mice intoxicated with cuprizone. **Methods:** 24 CD1 male mice were exposed to 0.2% cuprizone mixed with food chow for 8 weeks; then we assembled two groups: phenytoin-treated group and the untreated control group. The treated group received oral phenytoin (10 mg/kg) for 4 weeks upon cuprizone removal. The control group only received vehicle solution (0.1ml of 25% ethanol v/v solution). To evaluate remyelination, we quantified the number of oligodendrocyte precursor cells (**OPC**) and mature oligodendrocytes that expressed Olig2/Brdu, NG2/Brdu, RIP/Brdu cell markers, and the expression level of myelin basic protein (**MBP**), as well as and the muscle strength and

motor coordination. **Results:** The number of OPC and oligodendrocytes significantly increases after the phenytoin administration as compared to untreated group. Densitometric analysis also show an increase in the expression of MBP in the corpus callosum. Functional test show a significant improvement in the score rates obtained with horizontal bars test in the phenytoin group. **Conclusions:** Phenytoin stimulates OPC proliferation that contributes to reestablish the oligodendroglial population and promotes remyelination of the corpus callosum. These cellular effects are associated with functional recovery in motor coordination and strength.

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Poster

302. Mechanisms of Neuroprotection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH U54NS083924

8612MD007583

Title: Alpha7 nAChR activation or inhibition can mediate neuroprotective activity in hippocampal slices and in isolated mitochondria

Authors: V. A. ETEROVIC¹, H. R. ARIAS³, M. SKOK⁴, D. PEREZ², *P. A. FERCHMIN² ¹Neurosci., ²Biochem., Univ. Central del Caribe; Sch. of Med., Bayamon, PR; ³Dept. of Basic Sci., California Northstate Univ. Col. of Med., Elk Grove, CA; ⁴Dept. of Mol. Immunol., Palladin Inst. of Biochem., Kyiv, Ukraine

Abstract: 4R-cembranoid (4R), methyllycaconitine (MLA) and 3-furan-2-yl-N-p-tolylacrylamide (PAM-2) are neuroprotective drugs that target the alpha-7 nicotinic acetylcholine receptor (α 7). However, while 4R and MLA neuroprotective activities are mediated by inhibition of α 7, PAM-2 is a positive allosteric modulator of α 7. The objective of this work is to compare the neuroprotective activity of PAM-2 with those of 4R and MLA, in rat hippocampal slice and in isolated mice mitochondria expressing α 7 receptors. Recording of population spikes (PSs) in rat area CA1 of acute hippocampal slices was used to assess the injury caused by NMDA excitotoxicity and the neuroprotection by 4R, MLA, and PAM-2. The PS is the sum of axon potentials of functionally active pyramidal neurons synaptically elicited by stimulation of the Schaffer collaterals. Excitotoxicity was produced by a 10 min application of 0.5 mM NMDA which decreased the PS recovery to 40% of ACSF controls. The neuroprotective drugs were

applied for one hour 30 min after NMDA. In each experiment, the initial PS was measured before NMDA and the final PS, one and a half hour after NMDA. Data were analyzed by Kruskal-Wallis ANOVA, followed by Dunn's test. The recovery of PS was significantly increased when NMDA was followed by the application of 10 µM PAM-2 (68%), 10 µM 4R (82%), or 10 nM MLA (54%). Upon simultaneous application of 10 µM PAM-2 and 10 µM 4R, the PS recovery was 60%, while the application of 10 µM PAM-2 plus 10 nM MLA caused a recovery to 61%. All differences shown were significant (p<0.05). In conclusion, significant PS recovery after NMDA was observed when applying PAM-2, a positive α 7 modulator, and two selective inhibitors of a7 nAChR, MLA and 4R. This finding could be explained by the involvement of different synaptic circuits. On the other hand, the fact that the positive effects of PAM-2 and 4R were not additive could be explained by direct competition of a positive modulator and an inhibitor at the a7 receptor. The activity of PAM-2 and 4R was determined in liver mitochondria obtained from WT and mutant mice. The results indicated that PAM-2 efficiently attenuated Cyt c release in WT, but not α 7-/- or α 7/ β 2-/- mice mitochondria exposed to high Ca^{2+} , but not to H_2O_2 or wortmannin, whereas 4R activity was effective against Ca^{2+} and H₂O₂, but less against wortmannin. These results suggest that PAM-2 and 4R inhibit Cyt c release by distinct intracellular pathways, where PAM-2 activity is mediated by α 7-containing AChRs triggering CaKMII-dependent, but not Src- or PI3K-dependent, intracellular pathways.

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Poster

302. Mechanisms of Neuroprotection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01AG0145264

Title: Palmitate-enriched diet mitigates BDNF expression in the brain

Authors: *G. A. MARWARHA¹, J. LILEK², J. SCHOMMER², O. GHRIBI¹ ¹Biomed. Sci., Univ. of North Dakota Sch. of Med., Grand Forks, ND; ²Biomed. Sci., Univ. of North Dakota, Grand Forks, ND

Abstract: A multitude of epidemiological, clinical, and laboratory studies have suggested that diets rich in saturated fat precipitate cognitive impairment and exacerbate cognitive decline in the elderly leading to an increase in the risk for dementia and neurodegenerative disorders. Palmitic acid (palmitate) is the most abundant saturated free fatty acid (sFFA) present in the saturated fat higher palmitate levels in the plasma inversely correlate with cognitive function.

However, the molecular mechanisms underlying the effects of a palmitate-enriched diet in the derangement of cognitive function and evoking learning and memory deficits has not been characterized. Herein, we determined the effects of a palmitate-enriched diet and exogenous treatment on Brain-derived Neurotrophic Factor (BDNF) expression, in the mouse brain and SH-SY5Y human neuroblastoma cells, respectively. We fed a cohort of nine-month old wild-type C57BL/6J mice (n=15), a palmitate-enriched diet or a control-chow diet for three months and determined the effects on BDNF expression in cortex and hippocampus and elucidated the role of changes in cAMP/PKA/CREB signaling pathways that results in changes in CREB-binding to the BDNF promoter by ChIP-qPCR. We further determined the extent to which exogenous palmitate treatment of human neuroblastoma SH-SY5Y cells evokes changes in cAMP/PKA signaling pathway that results in altered CREB activation and CREB-mediated BDNF expression and delineated the signaling cascades by selective knock-down of kinases and ectopic expression of transcriptionally-inert mutant forms CREB. We demonstrate, in palmitate-treated human neuroblastoma cells and in the brains of palmitate-enriched diet-fed mice, that palmitate evokes a reduction in cAMP levels leading to a mitigation in PKA-mediated CREB activation that culminates in attenuated BDNF expression. We further show that the mitigation of CREB transcriptional activity is essential for the palmitate-induced decrease in BDNF expression, as constitutively active CREB precludes the deleterious effects of palmitate on BDNF expression. Our study highlights the derangements in cAMP/PKA/CREB signaling pathway as the conduit for palmitate-induced down-regulation of BDNF, the most abundant neurotrophin and growth factor in the brain that is indispensable for neuronal survival and synaptogenesis, and other facets of neuronal physiology integral in learning and memory.

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Poster

302. Mechanisms of Neuroprotection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NSERC Grant RGPIN-2015

Title: Knockdown of heat shock proteins HSPA6 (Hsp70B[`]) and HSPA1A (Hsp70-1) affects the viability of differentiated human neuronal cells following thermal stress

Authors: *C. A. DEANE, I. R. BROWN

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Abstract: Heat shock proteins (Hsps) are highly conserved proteins that play important roles in cellular repair and protective mechanisms that counteract protein misfolding and aggregation that are features of neurodegenerative diseases. HSPA6 (Hsp70B') is an inducible member of the HSPA (Hsp70) multigene family that has received little attention compared to the more widely studied HSPA1A (Hsp70-1). Interestingly, the HSPA6 gene is found in the human genome but is not present in mouse and rat. Hence it is absent in current animals models of neurodegenerative diseases which have been characterized as protein misfolding disorders. To advance knowledge of this little studied HSPA member, the effect of selective knockdown of HSPA6 and HSPA1A was examined in relation to the ability of differentiated human SH-SY5Y neuronal cells to survive thermal stress. Induction of Hsps by low dosage co-application of celastrol and arimoclomol was observed to enhance the ability of differentiated neuronal cells to tolerate heat shock. Small interfering RNA (siRNA) knockdown of HSPA6, or HSPA1A, resulted in loss of the protective effects of co-application of celastrol/arimoclomol. More pronounced effects were observed at 44°C heat shock compared to 43°C. These results suggest that induction of both HSPA6 and HSPA1A is required for the protection of differentiated human neuronal cells from cellular stress.

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Poster

302. Mechanisms of Neuroprotection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: GGET ARISTEIAII (KA3948)

University of Crete (ELKE, KA4371)

Title: Neuroprotective and anti-inflammatory effects of the microneurotrophin BNN27 in the STZ-model of diabetic retinopathy

Authors: *R. IBÁN-ARIAS¹, S. LISA², N. MASTRODIMOU¹, I. CHARALAMPOPOULOS¹, A. GRAVANIS³, K. THERMOS¹

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Abstract: Diabetic retinopathy (DR) is the most common ocular disease of people under diabetes, which gradually leads to visual impairment and blindness. Three major pathogenic

components define DR, namely neovascularization, neurodegeneration and inflammation. Available therapeutics target only the formation of new blood vessels. Recent findings propose that the imbalance between prosurvival neurotrophic factors and inflammatory components lead to apoptosis and pro-inflammatory responses. We have shown that BNN27, a novel synthetic DHEA derivative that interacts with neurotrophin receptors -thus called *microneurotrophin*-, reverses the diabetes-induced retinal damage (on amacrine retinal cells and ganglion cell axons). More specific, it activates the NGF TrkA receptor and its downstream prosurvival signaling in the Streptozotocin (STZ)-model of DR. The main aim of the present study was to examine the effect of BNN27 on the diabetes-induced changes a) in the immature isoform of NGF, proNGF, which binds with high affinity to the p75 ^{NTR} neurotrophin receptor, b) on gliosis, and c) on the pro- and anti-inflammatory mediators in the STZ-model of DR. BNN27 attenuated the diabetesinduced increase in proNGF, p75^{NTR}, glial fibrillary acidic protein (GFAP) and Iba-1 (microglia marker) expression. The levels of the transcription factor NFkB, known to be implicated in the production of pro- inflammatory cytokines, were increased in the diabetic retina. BNN27 attenuated the diabetes-induced increase in the pro-inflammatory cytokines TNF α and IL-1 β . In addition, it up-regulated the levels of the anti-inflammatory cytokines IL-10 and IL-4. The PI3K/Akt signaling pathway is also implicated in survival by regulating the production of proinflammatory cytokines. In the diabetic retina, Akt kinase phosphorylation was amplified, while BNN27 attenuated the phosphorylation of Akt kinase in a dose-dependent manner. In conclusion, these results provide important findings regarding the effects of the microneurotrophin BNN27 on the expression of proNGF and p75^{NTR}, gliosis and inflammation in the diabetic retina. However, further studies are necessary in order to support its beneficial use as therapeutic for the treatment of diabetic retinopathy.

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Poster

302. Mechanisms of Neuroprotection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH RO1

Title: Genetic deletion of Sarm1 prevents axon degeneration in chemotherapy induced peripheral neuropathy

Authors: *A. CETINKAYA-FISGIN¹, X. LUAN¹, N. REED¹, A. HOKE² ²Neurol., ¹Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Although increasing numbers of cancer patients are experiencing excellent outcomes with prolonged survival with chemotherapy, many chemotherapeutic agents often cause Chemotherapy Induced Peripheral Neuropathy (CIPN), a serious, painful, and dose-limiting complication. There are currently no effective therapies that prevent CIPN. In order to examine the mechanisms that lead to axon degeneration in CIPN and identify new therapeutic pathways for the treatment, we asked if genetic deletion of Sarm1 (sterile α-motif-containing and armadillo-motif containing protein) which has a pivotal role in activating axonal degeneration following injury, is protective against axonal degeneration in mouse CIPN models. Paclitaxel (25 mg/kg three times for one week), Cisplatin (4 mg/kg once a week for 4 weeks) and Bortezomib (0.8 mg/kg twice a week for 4 weeks) are administered by tailvein injections to wild-type mice and Sarm1 knockout mice. The key manifestations of mouse peripheral neuropathy models, including loss of intra epidermal nerve fibers (IENF), thermal hyperalgesia, and reduction in sensory evoked responses were observed in wild-type mice, but Sarm1 knockout mice did not show loss of IENF, thermal hyperalgesia or reduction in sensory evoked responses. As genetic deletion of Sarm1 blocks the development of peripheral neuropathies induced by these three different chemotherapy drugs that cause peripheral neuropathy via different mechanisms of actions, our findings suggest that axonal degeneration in CIPN has a final common pathway mediated by Sarm1. Therapeutic strategies aimed at reducing or inhibiting SARM1 activity may offer a new therapeutic target for CIPN.

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Poster

302. Mechanisms of Neuroprotection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NS094507

Title: Intestinal dysbiosis affects motorsensory function after intracerebral hemorrhage

Authors: *G. KONE, C. IADECOLA, J. ANRATHER Feil Family Brain and Mind Res. Inst., Weill Cornell Med., New York, NY

Abstract: While intracerebral hemorrhage (ICH) accounts for only 15-20% of all strokes, it is the most deadly of stroke subtypes with a 40-50% mortality within 1 month. The brain damage that occurs following ICH is in part determined by inflammation, mediated by both innate and

adaptive immune cells. In particular, regulatory T-cells and $\gamma\delta T$ cells have both been shown to infiltrate the brain following ICH (Mracsko et al., Stroke, 2014). Furthermore, regulatory T-cell implants have been shown to limit neuroinflammation and improve functional outcomes following ICH (Yang et al., International Immunopharmacology, 2014). We have previously shown that intestinal dysbiosis, induced by antibiotic-produced alterations of the intestinal microbiota, can alter T-cell trafficking to the brain and reduce brain injury following ischemic stroke (Benakis, et al., Nature Medicine, 2016). In the present study we explored whether intestinal dysbiosis can similarly provide neuroprotection in ICH. C57BL/6 mice treated with amoxicillin and clavulanic (AC) acid in tap water were housed alone (AC sensitive) or co-housed with seeder mice that had AC resistant gut flora which were then transferred to their cage mates (AC resistant). ICH was induced via stereotaxic injection of 0.075U bacterial collagenase into the striatum. Sensorimotor function was assessed using the tape test 1 day prior and on days 1, 3, and 7 following ICH and measuring the time to contact and removal of tape on the forepaws. On day 7, brains were sectioned to measure swelling by comparing the volumes of the hemisphere ipsilateral and contralateral to the lesion. AC sensitive mice made earlier contact with tape on their contralesional forepaw on day 1 ($42\pm 29s$) and day 3 ($13\pm 7s$) than AC resistant mice made on day 1 (91 \pm 36s, p = 0.0049; Mann-Whitney U test) and day 3 (55 \pm 33s, p=0.0007, n=15). Similarly, AC sensitive mice more quickly removed tape from their contralesional forepaw on day 1 ($86\pm 37s$) and day 3($65\pm 30s$) than AC resistant mice did on day 1($150\pm 24s$, p=0.0120) and day 3 (147 ± 21 s, p= 0.0001, n=13). Brains from AC sensitive mice showed a trend for less swelling of the injured hemisphere, but this change did not reach statistical significance in this small group (p>0.05; Mann-Whitney U test). These results suggest that the gut-brain axis may play a role in the evolution of functional decline following ICH, and suggest a novel therapeutic avenue for attenuating the inflammatory response following ICH.

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Poster

302. Mechanisms of Neuroprotection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Photoregulin3 prevents retinal degeneration in mice

Authors: *P. A. NAKAMURA¹, A. SHIMCHUK¹, S. TANG², S. DING², T. REH¹ ¹Biol. Structure, Univ. of Washington, Seattle, WA; ²Gladstone Inst., San Francisco, CA

Abstract: Nr2e3 is a retina-specific nuclear receptor and a master regulator of photoreceptor gene expression. Chemical modulation of this receptor has great potential as a therapeutic strategy to treat retinal degenerative diseases like Retinitis Pigmentosa (RP) and macular

degeneration. Toward this end, we have identified several series of small molecule modulators of Nr2e3 that regulate the expression of photoreceptor-specific genes. Thus far, we have focused on two series of chemically distinct compounds, the Photoregulin1 (PR1) series and the Photoregulin2 (PR2) series. Manipulation of photoreceptor expression with either PR1 or PR2 slows the progression of retinal degeneration in vitro in the Rho^{P23H} and $Pde6b^{Rd1}$ models of RP (Nakamura et al., 2016). However, in vivo analyses with these series, especially the PR1 series, were limited by the compounds' potency, solubility, and stability in vivo. We have also identified a third series, Photoregulin3 (PR3), which is more potent than the first two series. After systemic delivery, PR3 has large effects on rod photoreceptor gene expression (e.g. Nrl, *Rhodopsin*, *Gnat1*) by RNAseq analysis. In *Rho^{P23H}* mice, a model of autosomal dominant RP, most rod photoreceptors undergo cell death by the end of the third postnatal week, and we hypothesized that decreasing Rhodopsin expression with PR3 treatment would prevent retinal degeneration in this model. We treated Rho^{P23H} mice with PR3 or vehicle from postnatal day 12-14 (P12-14) until P20 and assessed retinal structure and function at P21. PR3-treated Rho^{P23H} mice had larger scotopic and photopic ERG responses than littermate controls, in addition to significantly decreased degeneration of photoreceptors. Together, our data suggest that pharmacological disruption of Nr2e3 signaling may be a therapeutically advantageous strategy for the treatment of RP and other degenerative diseases of the retina.

Disclosures: P.A. Nakamura: None. A. Shimchuk: None. S. Tang: None. S. Ding: None. T. Reh: None.

Poster

302. Mechanisms of Neuroprotection

Location: Halls A-C

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: DE17794

DE22743

NS87988

Title: Intrathecally injected bone marrow stromal cells produce sustained neuropathic pain reduction via neuro-immune regulation

Authors: *Y. D. HUH, G. CHEN, R.-R. JI Dept. of Anesthesiol., Duke Univ. Med. Ctr., Durham, NC

Abstract: Chemotherapy-induced peripheral neuropathy is the *de facto* toxic effect that limits the administration of anti-neoplastic agents such as paclitaxel in the treatment of cancer. This

toxic effect limits the utilization of aggressive chemotherapeutic regimens associated with an increase in patient survival from cancer. Understanding the mechanisms of chemotherapyinduced peripheral neuropathy may enable the development of treatments to curtail the neurotoxicity of chemotherapeutic agents and allow for increased usage of aggressive treatment regimens in oncology. Chen et al. discovered that intrathecal injection of bone marrow stromal cells (BMSCs) reduced peripheral neuropathy for more than two months in murine chronic constriction injury and spared nerve injury models. The study found that TGF- β 1 was the factor secreted by BMSCs responsible for the reduction in neuropathic pain, and furthermore the injected BMSCs migrated specifically to injured dorsal root ganglia through an interaction between CXCR4 and CXCL12. In this study, we examined the effects of BMSC intrathecal injections on peripheral neuropathy in the murine paclitaxel model (four intraperitoneal injections on alternate days at a concentration of 2.0mg/kg). We discovered that at a single injection of a similar dosage (3.0 x 10⁵ cells) of BMSCs into the intrathecal space of paclitaxeltreated mice produced a significant reduction in mechanical allodynia that lasted for more than six weeks. The analgesic effect of intrathecally injected bone marrow stromal cells in paclitaxelinduced peripheral neuropathic mice was reversed by an intrathecal injection of anti-TGF-B1 antibody, suggesting TGF-B1 as the specific factor secreted by BMSCs that inhibits neuropathic pain. We also confirmed that TGF-β1 injected intrathecally at a dosage of 100ng produced an immediate but transient reduction in mechanical allodynia in paclitaxel-treated mice. These findings suggest that bone marrow stromal cells injected into the intrathecal space secreting factors including TGF-B1 can produce significant and sustained analgesia for pain associated with chemotherapy-induced peripheral neuropathy.

Disclosures: Y.D. Huh: None. G. Chen: None. R. Ji: None.

Poster

302. Mechanisms of Neuroprotection

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Program#/Poster#: 302.19/W17

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Efficacy of novel acetylcholinesterase reactivators against organophosphate poisoning: In silico and *In vitro* studies

Authors: *M. ARSHAD¹, M. Q. FATMI¹, K. MUSILEK², K. KUCA³, S. M. NURULAIN¹ ¹Biosci., Comsats Inst. Of Information Technol. Islama, Islamabad Gpo, Pakistan; ²Biomed. Res. Ctr., Univ. Hosp. Hradec Kralove, Hradec Kralove,, Czech Republic., Czech Republic; ³Univ. of Defense, Hradec Kralove, Czech Republic, Czech Republic

Abstract: Organophosphate (OP) poisoning is a global issue. The organophosphorus compounds manifest toxicity by inhibiting neurochemical, acetylcholinesterase (AChE).Oxime is used to

reactivate the inhibited AChE. First oxime was developed in 1956, named pralidoxime. However, its efficacy is controversial. Hence, there is pressing need to develop a new efficacious oxime. Present study was aimed and designed to introduce a novel efficacious oxime and compared with another experimental oxime, K27. *In silico* and *in vitro* methods were employed to assess the potency and compare the efficacy of novel oximes against paraoxon (POX) inhibited AChE.

Molecular docking of the oximes revealed that novel oximes K378 and K727 binds at peripheral anionic site (PAS) with low binding affinity and high binding energy. Other tested oximes were having high binding affinity and low binding energy for the active catalytic site at the bottom of the gorge. LogP determination showed that novel oxime K727 possess low permeability to blood brain barrier. Intrinsic toxicity of K727 and K378 (in term of IC₅₀) were 0.99 μ M and 0.94 μ M respectively. Percent reactivation (R₅₀) for PAM-Cl, K27, K727 and K378 were found to be 135.45 μ M, 2.68 μ M, 1.7109X10¹⁴ μ M and 2.67X10¹⁷ μ M respectively. Apparent rate constant of the oximes revealed slow apparent rate constant for K727 (≈0.002 moles/minute) and K378 (≈0.005 moles/minute) in comparison K27 (≈0.06 moles/minute), and PAM-Cl (≈0.02 moles/minute). The delayed time point application of oximes showed that K27 is effective even, when applied after 20 minutes of intoxication by POX. Other oximes decreased the efficacy with increase in time point of oxime application

It is concluded that K378 and K727 are not efficacious than pralidoxime. However, pralidoxime is inferior in efficacy than K27. Further work with structurally different organophosphates is suggested.

Disclosures: M. Arshad: None. M.Q. Fatmi: None. K. Musilek: None. K. Kuca: None. S.M. Nurulain: None.

Poster

302. Mechanisms of Neuroprotection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Pennsylvania State CURE funding mechanism

Title: Mechanism of action of retinal pigment epithelial (RPE) grafts reducing drug seeking in high-dose cocaine taking rats after a period of abstinence

Authors: *K. VENKITESWARAN¹, T. CAYTON¹, S. SINGH¹, A. PATEL¹, T. SUBRAMANIAN¹, P. SUE GRIGSON² ¹Neurol & Neural & Behav Sci., Penn State Milton S Hershey Med. Ctr., Hershey, PA; ²Neural & Behavioral Sci., Penn State Col. of Med., Hershey, PA

Abstract: Prolonged exposure to cocaine affects VTA-NAc dopaminergic neurons resulting in imbalance to mesolimbic pathway in vivo, contributing to relapse seen in chronic cocaine addiction. Our previous study in rat model of cocaine addiction demonstrated that retinal pigment epithelial (RPE) cells grafted into NAc, helped to reduce drug seeking in high-dose cocaine-self-administering animals after a period of abstinence (Venkiteswaran et al. 2016). To verify this and to understand the underlying mechanisms, we investigated if cocaine induced neuronal death and RPEC-mediated rescue could occur in primary cultures of mouse fetal ventral mesencephalic (mFVM) neurons. mFVM tissue dissected from E13.5 mouse embryos was made to single cell preparation and was cultured in vitro. These cultures were exposed to increasing doses of cocaine (3 µM to 1300 µM) in vitro for 24 hours. After this, the cultures were fixed and stained for tyrosine hydroxylase (TH) and quantified the TH positive neurons by stereology. The study showed in vitro dose responsive neuronal degradation and also upregulation of glial cell proliferation in the mFVM cultures when exposed to increasing doses of cocaine. Cocaine treated mFVM cultures were treated with RPEC conditioned medium (RPE-CM) 1) 24 hours prior to cocaine administration (pretreatment), 2) with cocaine administration (simultaneous treatment), and 3) 24 hours after cocaine administration (post treatment) in vitro. As controls, Glial Derived Neurotropic Factor (GDNF, 1µg/ml) and Pigment epithelial derived factor (100ng/ml) were used to treat separately on the mFVM, cultured in vitro. The cultures were fixed on day 6 and the dopaminergic neurons were analyzed by TH immunocytochemistry, stereology and neuron tracing techniques. TH positive neurons were quantified by petrimetrics and neuronal characteristics were measured using Neurolucida. GDNF and PEDF treatments showed better survival of dopaminergic neurons in the cocaine treated mFVM cultures. However the neuronal characteristics were different for the two growth factors. Treatment with RPE-CM showed better survival of TH positive neurons with healthier neuronal characteristics.

Disclosures: K. Venkiteswaran: None. **T. Cayton:** None. **S. Singh:** None. **A. Patel:** None. **T. Subramanian:** None. **P. Sue Grigson:** None.

Poster

302. Mechanisms of Neuroprotection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R15NS090384

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Title: Using Trichostatin A to rescue Trka+ neurons in a mouse model of familial dysautonomia

Authors: *R. G. BUKSCH, III, J. WALTERS, J. GOFFENA, L. GEORGE Biol. and Physical Sci., Montana State Univ. Billings, Billings, MT

Abstract: Familial Dysautonomia (FD) is a severe neurodevelopmental and neurodegenerative disease that devastates the peripheral nervous system, culminating in death of most patients by age 40. FD results from a single base mutation in the gene Ikbkap/Elp1 that encodes the scaffolding unit for the Elongator complex. A hallmark feature of FD, present at birth, is a severely reduced number of TrkA+ pain and temperature sensing nociceptors in the dorsal root ganglia. Previous studies in a mouse model for FD have shown that impaired neurogenesis during embryonic development contributes to this decreased number of nociceptors. Multiple studies in both familial dysautonomia patients and mouse models of FD have also documented reduced levels of acetylated histone H3. To examine whether depleted histone acetylation may play a causal role in the failed production of TrkA+ neurons during embryogenesis, we evaluated the ability of the histone deacetylase inhibitor, Trichostatin A (TSA), to rescue TrkA+ neuron number in Wnt1-Cre; Ikbkap^{LoxP/LoxP} conditional knockout (CKO) mice where Ikbkap/Elp1 is selectively ablated in the peripheral nervous system. Although CKO pups die within 24 hours of birth, their survival through development allowed us to ask whether hyper-acetylation of histones during embryogenesis could rescue TrkA+ neuron number. Pregnant dams were treated with either 1mg/kg of Trichostatin A (experimental) or vehicle alone (control), at E8.5, E10.5, and E12.5, a time frame corresponding to neurogenesis in the mouse dorsal root ganglia. Quantification of TrkA+ neuron number at E17.5 shows a significant increase in TSA-treated embryos over vehicle controls (132.9% increase; p<.00001). We are currently exploring the molecular and cellular mechanisms via which histone deacetylase inhibition rescues TrkA+ neuron number during embryogenesis.

The post-natal death of *Wnt1-Cre; Ikbkap^{LoxP/LoxP}* pups has been attributed to complications of a cleft palate. Interestingly, TSA treated CKO embryos show significant changes in head morphology at E17.5. We are currently investigating whether this altered head development includes correction of cleft palate such that CKO pups survive beyond their first 24 hours. Further studies will also evaluate the ability of Trichostatin A to protect cultured mature neurons *in vitro*, as well as to prevent neurodegeneration *in vivo*, in adult mice.

Disclosures: R.G. Buksch: None. J. Walters: None. J. Goffena: None. L. George: None.

Poster

302. Mechanisms of Neuroprotection

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The Henry Smith Charity

Title: Rescuing synaptic activity in prion-disease mice

Authors: *J.-M. BOURGOGNON, J. R. STEINERT

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Abstract: It has been suggested that nitric oxide and S-nitrosylation are involved in the pathogenesis of various neurodegenerative disorders including Parkinson's disease (PD), and Alzheimer's disease (AD). Elevated neuroinflammation levels that characterize these pathologies are largely associated with an increased production of nitric oxide (NO) leading to abnormal protein S-nitrosylation. We use a mouse model of neurodegeneration to investigate the role of nitric oxide mediated pathways at the synapse and its contribution to neuronal decline. We show that in prion-infected mice synaptic activity is dramatically decreased. Indeed the amplitude and frequency of miniature EPSCs are lowered together with potassium currents. However chronic injection of a NOS inhibitor L-NAME totally rescues presynaptic release probability / the number of functional synaptic sites and the density/conductance of postsynaptic receptors at individual synapses. We evaluate the impact of blocking nitric oxide synthase on memory and survival of the prion mice. We further examine the expression of synaptic proteins like synapsin and complexin1/2 in prion-infected mice as the disease progresses using Western blotting and immunohistochemistry. Future work will study the effect of chronic L-NAME treatment on synaptic protein expression and nitrosylation status of synaptic proteins.

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Poster

302. Mechanisms of Neuroprotection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: A.S. is funded by a grant from the German Research Foundation (DFG; SCHU 3039/1-1).

Title: GDPGP1 is a novel and conserved stress-responsive gene in neurons

Authors: *A. SCHULZ¹, M. HAMMARLUND²

¹Program in Cell. Neuroscience, Neurodegeneration and Repair, ²Yale Univ. Sch. of Med., New Haven, CT

Abstract: Our mechanistic understanding of how neurons respond to stress and injury is incomplete, and this lack of fundamental knowledge impedes the development of urgently needed therapies for neurological diseases. We used an RNA-Seq screen against multiple stress conditions to unravel the common transcriptional signature of acutely stressed cortical mouse

neurons. Our expression screen and subsequent retests identified GDPGP1 as a novel stressresponsive gene. GDPGP1 expression is down-regulated in primary cortical mouse neurons following hypoxic and oxidative stress. Further, knockdown of GDPGP1 in primary cortical mouse neurons leads to widespread neuronal cell death. To characterize the *in vivo* function of GDPGP1 in the neuronal response to stress and injury, we used a genetic approach in *C. elegans*. GDPGP1 has a single homologue in *C. elegans*, *mcp-1*. *mcp-1* shows neuron-specific expression and, consistent with our results in cultured mouse cortical neurons, is transcriptionally downregulated in response to a range of stresses. Loss-of-function mutations of *mcp-1* improve axon regeneration after axotomy, but lead to increased degeneration of GABA neurons as well as reduced survival of worms following environmental stress. Taken together, our data identifies GDPGP1/*mcp-1* as a novel stress-responsive gene in nematode and mammalian neurons, and suggest that GDPGP1 has an important *in vivo* function in mediating neuronal responses to multiple stresses. The downregulation of GDPGP1 in injured or stressed neurons might contribute to loss of cell survival.

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Poster

302. Mechanisms of Neuroprotection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: FAPESP 2014/06892-3

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CNPq 300552/2013-9

Title: Neuroprotection and neurodegenerative response by CB1 and CB2 cannabinoid receptor inactivation after neonatal peripheral nerve lesion

Authors: *M. PEREZ¹, L. P. CARTAROZZI¹, G. B. CHIAROTTO¹, F. S. GUIMARÃES², A. L. R. OLIVEIRA¹ ¹Unicamp, Campinas, Brazil; ²Univ. of São Paulo, Ribeirão Preto, Brazil

Abstract: Neonatal peripheral nerve lesion results in degeneration of spinal motoneurons and dorsal root ganglia (DRG) already at the acute phase post-axotomy. Neurotrophic substances may prevent such cell loss, allowing axonal regeneration and rehabilitation. We have previously shown that cannabidiol (CBD), a non-psychotropic component of cannabis leaves, is neuroprotective for both motoneurons and DRG neurons, when administrated during the five subsequent days post nerve lesion. CBD can activate the endocannabinoid system, via

cannabinoid receptors, namely CB1 and CB2. However, the relative importance of each receptor is elusive regarding neuronal response to axonal transection. Therefore, the aim of present work was to investigate the neuroprotective effects of CBD by selectively inactivating CB1 and CB2 receptors following neonatal sciatic nerve lesion. For this purpose, Wistar neonatal rats (P2) were subjected to unilateral sciatic nerve injury and treated with CBD (15mg/Kg) or vehicle solution for five consecutive days. CB1 and CB2 were blocked alone or simultaneously by the use of AM251 (CB1 antagonist) and AM630 (CB2 antagonist). Neuronal survival was assessed by direct counting in Nissl stained sections. RT-qPCR was used to measure gene expression of CB1, CB2, BDNF, and GDNF both in the lumbar intumescence and DRGs. Similarly, immunohistochemistry was used to evaluate protein expression in situ. Unexpectedly, the spinal motoneuron survival was 40% greater than vehicle when CBD alone or the combination of AM251 and AM630 were used. Also, qRT-PCR analysis demonstrated that AM251 and AM630 alone or together generated 40% decrease of CB1 receptor and 20% of BDNF gene expression. Importantly, the use of CB1 and CB2 antagonists result in a significant increase of CB2 and GDNF gene expression in the DRGs (60% upregulation), suggesting that cannabinoid receptors blockade also impacts the primary afferents response to injury. Taking together, the results herein show that the cannabinoid receptors can play a role in the neuroprotective and regenerative response after peripheral nerve lesion during the neonatal phase.

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Poster

302. Mechanisms of Neuroprotection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 NS40433

Title: Interleukin-10 expression in the facial motor nucleus: Roles for motoneuron survival after axotomy

Authors: ***E. M. RUNGE**^{1,2}, D. O. SETTER^{1,2}, F. M. KENNEDY^{1,2}, V. M. SANDERS³, K. J. JONES^{1,2}

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Abstract: Maintaining motoneuron (MN) survival is critical for successful neuroregeneration after peripheral nerve injury. Elucidating the cellular mechanisms that determine whether a MN

survives or perishes after a peripheral nerve injury will be necessary for the development of therapies for the treatment of nerve injury and may yield important clues about the pathogenesis of amyotrophic lateral sclerosis (ALS), a disease which is also thought to be initiated by a distal insult to the MN axon. Of particular interest to our laboratory is interleukin-10 (IL-10), an antiinflammatory cytokine. We have previously found that a central source of IL-10 is required for CD4+ T cells to mediate MN survival after facial nerve axotomy (FNA) and that axotomy induces astrocytes to upregulate IL-10 receptors. The objective of this study is to investigate the cellular source and role of IL-10 in mediating neuroprotection after FNA. Immunohistochemistry and utilization of an IL-10/GFP reporter mouse revealed that astrocytes were positive for IL-10 after FNA, whereas microglial production of IL-10 was undetectable. Unexpectedly, MN themselves appeared to be constitutive producers of IL-10. To determine whether a glial source of IL-10 is critical for MN survival, we generated Cre/lox mouse strains to selectively knock down IL-10 in microglia and astrocytes. In accordance with our previous findings, knockdown of IL-10 in microglia had no effect on MN survival after axotomy. To our surprise, knocking down IL-10 in astrocytes also did not affect MN survival. These results have led to the hypothesis that a neuronal source of IL-10 is necessary for MN survival after FNA, potentially by modulating glial reactivity. Experiments to address this neuronal source of IL-10 are underway. The pattern of IL-10 expression in the mSOD1^{G93A} mouse model of ALS after FNA is also under investigation. These studies pave the way for future application of immune-modulating therapies after nerve injury.

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Poster

302. Mechanisms of Neuroprotection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Activating transcription factor 3 protects retinal ganglion cells and promotes regeneration after optic nerve crush

Authors: C. KOLE¹, T. ZHAO², L. BONET-PONCE¹, *N. NAKAYA¹, B. MEAD¹, S. I. TOMAREV¹ ¹SRGCB/LRCMB, ²RNPS, Natl. Eye Inst., Bethesda, MD

Abstract: Injured mature CNS axons do not regenerate in mammals. Activating transcription factor 3 (ATF3) is a member of a family of ATF/cyclic AMP responsive element binding

transcription factors and was identified as a protein whose activity is regulated by stressful stimuli. In the peripheral nervous system, upregulation of ATF3 expression is closely linked to the survival of neurons and the regeneration of their axons following axotomy. Here, we sought to determine whether ATF3 promotes the survival of CNS neurons and the regeneration of their axons using an optic nerve crush model. In zebrafish which are able to regenerate the optic nerve after transection, a 19.2 fold upregulation of atf3 expression was demonstrated in the retina 48h after optic nerve crush compared with uninjured retina. In order to elucidate whether overexpression of ATF3 may stimulate regeneration of the optic nerve in mammals, we performed intravitreal injections of recombinant AAV2.9/2yF vector encoding mouse ATF3 2.3E+10 viral particles into adult mouse eyes one week before the optic nerve crush, AAV2.9/2yF encoding for mCherry was used as negative control. Survival of retinal ganglion cells (RGCs) and regeneration of their axons were analyzed one week after crush. RGC were identified by positive RBPMS staining whereas axons were labeled with the anterograde fluorescent tracer cholera toxin beta. Intravitreal injection of AAV encoding ATF3 increased survival of RGCs by 23% relative to eyes injected with control AAV encoding cherry protein or non-injected eyes. ATF3 overexpression induced significant regeneration of RGC axons with an approximate 3-fold higher number of axons 1mm distal to the laminin⁺ crush site compared with controls. At 40d post-crush, some axons were identified within the optic chiasm after ATF3 overexpression. . The optomotor test was done using the OptoMotry system and was used to record head movements (optomotor reflex; OMR) in an animal in response to a rotating visual stimulus. ATF3 overexpression led to a partial restoration of OMR compared to control animals 35 days after the crush. Altogether, these results indicate that ATF3 has a neuroprotective and axogenic effect on injured RGCs, promoting the restoration of some visual-dependant behaviours.

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Poster

302. Mechanisms of Neuroprotection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CONAyT grant 220365

CONACyT scholarship 380140

Title: Cromolyn sodium administration after pilocarpine-induced Status epilepticus reduces the subsequent neuronal damage in hippocampus of rats
Authors: *M. G. VALLE DORADO¹, S. A. OROZCO², L. ROCHA¹

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Abstract: Pretreatment with Cromolyn sodium (CGS) produces neuroprotector effects in experimental hypoxia-ischemia and Status epilepticus (SE). However, at present it is unknown if its administration after SE is able to prevent the subsequent neuronal damage. This study was focused to investigate if a single administration of CGS applied 1 h after pilocarpine-induced SE can reduce the neuronal damage in hippocampus. Rats (n=20) received one saline solution administration (1 ml/kg, i.p.) per day for 5 consecutive days for habituation to manipulation. Twenty-four hours after the last saline injection, animals received the administration of lithium chloride (3 mEq/kg i.p.), and 18 h later, methylscopolamine (1 mg/kg, s.c.) was applied. Pilocarpine (30 mg/kg, i.p.) was injected 30 min after methylscopolamine to induces SE. Two hours after the establishment of SE, rats were injected with diazepam (2.5 and 1.25 mg/kg, i.m, 1 h and 8 h after SE, respectively) to reduce the convulsive seizures. A group of animals (CG-SE n=6) was injected with CGS (50 mg/kg s.c.) 1 h after the first administration of diazepam. The SE control group (SS-SE n=8) was manipulated as described above except that vehicle was applied instead of CGS. A control group (SS, n=6) received vehicle instead of pilocarpine. Animals were sacrificed 24 h after SE and the brain was used to evaluate neurons in process of death using Fluoro-Jade B. We found that the control group presented scarce cell in process of death. In contrast, SS-SE group showed neuronal damage 24 h after SE in dorsal hippocampus (dentate gyrus, 363.4±128 mm³; hilus, 186.7±28 mm³; CA1, 233.3±155 mm³ and CA3, 260.3±48 mm³), lateral-dorsal thalamic nucleus (260.3±24 mm³) and layers V and VI of cortex (213.3±49 mm³). When compared with SS-SE group, CGS-SE group presented less neuronal damage induced in the dorsal hippocampus (dentate gyrus, 86% p=0.0158; hilus, 42% p=0.0122; CA1, 80% p=0.0377 and CA3, 68% p=0.0093). This situation was not evident in the lateraldorsal nucleus of thalamus and neocortex. In conclusion, our data indicate that CGS applied after SE can be used as a therapeutic strategy to reduce the subsequent neuronal damage.

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Poster

302. Mechanisms of Neuroprotection

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 2UO1NS058162

Title: Analysis of Recurrent Seizures after soman-induced status epilepticus: Comparison between Tezampanel versus Diazepam when treatment is delayed

Authors: *T. H. FIGUEIREDO¹, J. P. APLAND², M. D. FURTADO³, V. ARONIADOU-ANDERJASKA⁴, M. M. F. BRAGA⁴

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Abstract: The use of nerve agents (NA) in recent years, even against civilians, bring again to the forefront the question of readiness and whether the existing medical countermeasures can save lives and protect against the long-term health consequences of exposure. The primary action of NA is the inhibition of acetylcholinesterase, which, in addition to the peripheral cholinergic crisis, induces status epilepticus (SE); if SE is not adequately controlled, death may ensue, or brain damage, with long-lasting neurological and behavioral consequences. The total duration of SE after exposure determines to a significant degree the extent of brain damage. In previous research from our group, antagonists of GluK1-containing kainate receptors (GluK1Rs) were found to be effective against soman-induced seizures even when administration is delayed 1 hour or later. On the other hand, diazepam (DZP), the only FDA-approved anticonvulsant for the treatment of NA-induced seizures, has been demonstrated in animal models to lose efficacy with delayed administration; furthermore, we have observed significant recurrence of convulsive seizures after the initial SE is terminated by DZP, making the total duration of SE with 24 hours post-exposure no different from that in animals who do not receive anticonvulsant treatment. In the present study, we compared the occurrence of recurrent seizures after treatment of somanexposed rats with tezampanel (LY293558) or DZP. We have analyzed video-EEG of animals exposed to 1.4X LD50 soman (pretreated 30 min earlier with 125 mg/kg HI-6 and administered 2 mg/kg Atropine Sulfate at 1 min after exposure) and treated with DZP (10 mg/kg) or LY293558 (50 mg/kg) at 1 to 2 hours after exposure. The latency to the appearance of recurrent convulsive seizures was significantly longer in animals treated with LY293558 (1020 ± 78 min) versus DZP $(330 \pm 28 \text{ min})$. We also used power spectrum analysis to study the progression of recurrent electrographic seizures during the 24-hour period post-exposure. Although seizures recurred after either LY293558 or DZP treatment, LY293558 was significantly more efficacious in suppressing them, thus reducing the total duration of SE in the 24-hour post-exposure period significantly more than DZP. The data suggest that targeting the glutamatergic system to suppress seizures after NA exposure will not only terminate the initial SE but also reduce significantly the total duration of post-exposure seizures, which predicts significant protection against brain damage; suppression of the total duration of seizures in the 24-hour post-exposure period cannot be achieved by drugs targeting the GABAergic system, such as DZP.

Disclosures: T.H. Figueiredo: None. J.P. Apland: None. M.D. Furtado: None. V. Aroniadou-Anderjaska: None. M.M.F. Braga: None.

Poster

302. Mechanisms of Neuroprotection

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 302.29/DP04/W27 (Dynamic Poster)

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Felix and Carmen Sabates Missouri Endowed Chair in Vision Research

Title: Steroid hormone receptors control neuronal inositol 1,4,5-trisphosphate receptor activity in the nucleus

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Abstract: Controlling the nucleoplasmic concentration of free calcium ions (Ca²⁺) is essential for the physiological activity of neurons, such as gene expression and neuronal survival under disease conditions. At the same time, the steroid hormones progesterone and the estrogen 17βestradiol have been identified as neuroprotective and critical for proper neuronal viability as transcription factors directly controlled by extracellular hormone signaling. 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 in the nuclear envelope control the Ca^{2+} concentration of the nucleoplasm in neurons. Specifically, it was identified how direct binding of estrogen receptors to inositol 1,4,5-trisphosphate receptors on the nucleoplasmic face of the nuclear envelope affects release of Ca^{2+} from the nuclear envelope by controlling the activity of this major type of ligand-gated intracellular Ca²⁺ release channels in the nuclear envelope of 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 in the nucleus after binding of steroid hormone receptors were determined. Binding of steroid hormone receptors to the intracellular Ca²⁺ 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 Ca²⁺ from the nuclear envelope and in the nucleoplasm as well as altered susceptibility of neurons to stimuli selectively elevating nucleoplasmic Ca^{2+} levels. The work indicates that Ca^{2+} signaling in the nucleus of neurons mediated by inositol 1,4,5-trisphosphate receptors as Ca^{2+} dependent intracellular Ca^{2+} release channels is critically controlled by steroid hormone receptor binding. Such nucleoplasmic Ca^{2+} signaling controlled by protein-protein interactions in neurons of the central nervous system potentially provides a novel mechanism for both genomic as well as non-genomic actions of steroid hormone receptors as a new target for drug development in the area of neurodegeneration.

Disclosures: P. Koulen: None.

Poster

302. Mechanisms of Neuroprotection

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 302.30/W28

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: EphB2 controls depression & cognitive impairment by regulating neurogenesis in hippocampus

Authors: *Y. XIAOKAITI¹, V. LURIA², H. ZHANG³, J. O'DONNELL¹, Y. XU¹ ¹Dept. of Pharmaceut. Sci., State Univ. of New York at Buffalo, Buffalo, NY; ²Dept. of Systems Biol., Harvard Med. Sch., Boston, MA; ³Depts Behav Med. & Psych, Pharm, West Virginia Univ. Hlth. Sci. Ctr., Morgantown, WV

Abstract: EphB2 activates signaling transduction in central nervous system by interacting with membrane-anchored ligands ephrins. EphB2 (receptor tyrosine kinase) is considered to mediate development of the neurogenic niche of excitatory neurons, implicating its role in neuropsychiatric disorders. However, how EphB2 controls depression and memory and cognitive processes remain partially understood. The present study examined depression-like behaviors and cognitive impairment induced by EphB2 knockout (KO) in series behavioral tests and the correlation with hippocampal neurogenesis. The EphB2 KO mice produced depression-like behaviors and deficits in spatial memory and cognition in comparison with matched wild-type littermates in tail suspension, forced swimming, Morris water maze, novel object recognition and novel object location tests. Further study suggested that depletion of EphB2 significantly reduced expression of neural stem cells (NSCs)/progenitor cells (NPCs), and also affected differentiation of neural progenitors in the hippocampus. These alterations were accompanied by the findings that increased N-methyl-D-aspartate (NMDA) receptor 2B, decreased phosphorylation of cAMPresponse element binding protein (pCREB) and brain derived neurotrophic factor (BDNF) levels were significant in EphB2 KO mice. These alterations exhibited by knockout of EphB2 highlight that EphB2 receptor controls the progression of depression and cognitive impairment by increased hippocampal neurogenesis, which implicate that EphB2 is one of candidate molecules

for modulating the production and integration of new neurons for treatment of emotional and cognitive disorders.

Key Words: EphB2; depression; cognition; neurogenesis; NMDA-2B receptor

Disclosures: Y. Xiaokaiti: None. V. Luria: None. H. Zhang: None. J. O'Donnell: None. Y. Xu: None.

Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 303.01/W29

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Ministry of Education, Science and Technological Development of the Republic of Serbia III41014

Ministry of Education, Science and Technological Development of the Republic of Serbia OI173056

Title: Time-dependent changes in cholesterol 24-hydroxylase expression in the spinal cords during experimental autoimmune encephalomyelitis

Authors: *I. LAVRNJA, K. SMILJANIC, D. SAVIC, A. MLADENOVIC-DJORDJEVIC, K. TESOVIC, S. KANAZIR, S. PEKOVIC Inst. For Biol. Res. Sinisa Stankovic, Belgrade, Serbia

Abstract: The dysfunction of cholesterol metabolism may be a crucial event that leads to the progression of multiple sclerosis (MS). Using a rat experimental autoimmune encephalomyelitis (EAE) model of MS, the specific changes of main molecule involved in the retaining of cholesterol homeostasis in the spinal cord: cholesterol 24-hydroxylase (CYP46A1) expression was monitored on gene and protein level during the course of disease. The present study is aimed to explore the molecular pattern of CYP46A1 expression over the course of EAE and cell type(s) accountable for its expression. EAE was induced in Dark Agouti rats by immunization with the spinal cord tissue homogenate and Complete Freund's adjuvant. Animals were sacrificed 9, 13, and 22 days after immunization. First signs of the disease appeared at 9 days after immunization and the disease reached a peak at day 13. Clinical signs of EAE decreased afterwards during the period of recovery, and all rats completely recovered by day 22 (end of the disease). Development of EAE signs was accompanied with body weight loss; maximal loss coincided with paralysis at the peak of disease. Myelin lipid debris was seen only at the peak of EAE and was efficiently removed until the end of disease. Since CYP46A1 is a crucial molecule for elimination of cholesterol excess, a detailed cellular profiling of CYP46A1 expression was

undertaken, and revealed regional and temporal specificities in its distribution. Double immunofluorescence staining showed presence of CYP46A1 protein in neurons, infiltrated macrophages, microglia and astrocytes in the areas of demyelination, implying that these cells have a role in cholesterol turnover during EAE. Summarizing, the observed changes in the regulation of cholesterol metabolism EAE may enhance symptoms at the onset and peak of the disease, but during the recovery period could contribute to the regeneration of myelin sheath. The fact that cholesterol is a crucial, rate-limiting factor for myelin growth and that its metabolites may have a role in promoting autoimmunity as inflammatory mediators, warrants further investigation of cholesterol metabolism in autoimmune diseases like MS.

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Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

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Program#/Poster#: 303.02/W30

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Baicalein neutralizes inflammation and nerve growth factor in cerebral cortex in diabetic rat model

Authors: S. S. AL-REJAIE¹, *M. N. ASHRAF²

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Abstract: Diabetes-induced encephalopathy is a well-reported consequence among diabetic subjects. Alteration includes cellular, molecular and functional complications. Nevertheless, the mechanisms responsible for the pathogenesis and the therapeutic strategies are not fully reported. In the present investigation, baicalein as a flavonoid was examined as a protective agent against the experimental diabetic neurodegenerative diseases based on its potent free radical-scavenger activity. Baicalein was orally administered in two different doses (25 and 50 mg kg¹⁻ day¹⁻) to diabetic rats for five consecutive weeks. Following the end of the treatment, cerebral cortex tissues were harvested and directly frozen in liquid nitrogen till analysis. Baicalein markedly lowered the expressions of inflammatory cytokines including interleukin (IL)-1 β , tumor necrosis factor (TNF)- α and IL-6 and corrected the altered levels of neurotrophic factors such as brain derived neurotrophic factor (BDNF), nerve growth factor (NGF) and insulin growth factor (IGF)-1 in the diabetic cerebral cortex tissues compared to untreated diabetic group. Taken together, these finding indicate the beneficial role of baicalein in alleviating the provoked inflammation and enhancing the neurotrophic support in the diabetic cerebral cortex.

Disclosures: S.S. Al-Rejaie: None. M.N. Ashraf: None.

Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: PAEP grant, UNAM

Title: Role of neuroinflammation in the regulation of the cytochromes P450 2C11 and 2J3 in the brain

Authors: *M. M. LOAIZA ZULUAGA, C. NAVARRO-MABARAK, S. L. HERNÁNDEZ-OJEDA, R. CAMACHO-CARRANZA, J. J. ESPINOSA-AGUIRRE Inst. de Investigaciones Biomédicas, Univ. Nacional Autónoma De México, CDMX, Mexico

Abstract: The cytochromes P450 (CYP) are membrane proteins that are expressed in the endoplasmic reticulum of plants, fungi and animal cells. They contribute to the degradation of endogenous and exogenous compounds including drugs and carcinogens. CYP presents a variety of isoforms, 2C and 2J isoforms have epoxygenase activity. CYP epoxygenases have been considered the major producers of short chain fatty acids, such as epoxyeicosatrienoic acids (EETs) from the oxidation of arachidonic acid (AA). EETs have diverse biological properties including anti-inflammatory activities, inhibiting NF- κ B as a transcriptional factor, thus avoiding its participation in the production of pro-inflammatory cytokines.

It has been seen that phenomena such as inflammation and infection can affect the regulation of epoxygenases during the metabolism of AA to EETs, inhibiting their transcriptional expression in tissues like heart, liver and kidney. It has also been shown that these enzymes are expressed in brain, but its regulation by inflammation has not been studied yet in this important organ. Based on previous studies and in the relevance that the EETs have acquired, we seek to determine if there are changes in the expression and protein levels of CYP2J3 and CYP2C11 in the cortex and hippocampus of animals exposed to LPS as a result of the presence of pro-inflammatory cytokines such as IL-6, IL-1 β and TNF- α in the brain. We performed an in vivo model of neuroinflammation by the intraperitoneal administration of LPS (1mg/kg) to male Wistar rats, and as a vehicle, commercial saline solution. The sacrifice was by decapitation at 6, 12, and 24 hours, obtaining the hippocampus and the cerebral cortex of the control and treated rats. We evaluated the transcriptional expression of CY P2J3 and 2C11 in these tissues by RT-PCR, considering GAPDH as the housekeeping gene. Our results show for the first time that LPS treatment in the periphery (i.p. administration) is able to downregulate CYP 2J3 and 2C11 expression in the brain. Pro-inflammatory cytokine levels in the cerebral cortex will be

determined by specific ELISA kits and CYP protein levels will be determined by Western Blot in the hippocampus and cerebral cortex to further confirm our hypothesis.

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Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: 5P20GM109025

Title: Neuromodulators of inflammation in mouse administered with exogenous soluble gp130

Authors: *A. M. SALAZAR, A. S. MURTISHAW, M. M. BOLTON, J. W. KINNEY PSYCHOLOGY, Univ. of Nevada Las Vegas, Las Vegas, NV

Abstract: Neuroinflammation is a multifaceted biological response of the nervous tissue to harmful stimuli, cell damages, and some metabolic diseases. It is a vital component of the immune response to promote healing and restoration. However, prolonged inflammation can be harmful. Emerging evidences suggest that it plays a significant role in the pathogenesis and exacerbation of neurodegenerative diseases such as Alzheimer's Disease, Parkinson's Disease and Multiple Sclerosis. Neuroinflammation is commonly characterized by activated microglia and abnormal levels of cytokines, such as IL-6 that has both pro- and anti-inflammatory effects. IL-6 is needed for the activation of the immune system. IL-6 binds IL-6 receptor and the complex associates with gp130, a signal transducer, to initiate signaling via JAK/STAT pathway. This process contributes to the regenerative and protective effect of IL-6 signaling during inflammation. IL-6 is also found to bind to the soluble form of IL-6 receptor (sIL-6R) and this complex instead induces IL-6 trans-signaling in cells with gp130 only in their membrane. This condition is reported to stimulate inflammation. However, a soluble form of gp130 (sgp130) is described to bind the IL-6/sIL-6R complex that suppresses the IL-6 trans-signaling and regulates inflammation. This effect is characterized by several studies on animal models of human diseases such as intestinal and acute inflammation, rheumatoid arthritis, sepsis, arteriosclerosis, asthma, and various cancers. In a high fat diet-induced obesity model, the increased level of sgp130 in the plasma suggests an antagonistic response to systemic inflammation. While the position of sgp130 in IL-6 signaling is well-established and proofs of its role in regulating inflammation are growing, less information on its effect in neuroinflammation is available. In addition, investigations on the effects of sgp130 in the expression of other inflammation modulators are limited. Thus, we investigated the influence of sgp130 in neuroinflammation. We examined the

effects of exogenous sgp130 in TLR3-mediated inflammation. This study is aimed at describing the effects of a three-consecutive day intraperitoneal administrations of exogenous sgp130 in healthy and immunocompromised wildtype mice to specifically evaluate the expression profiles of cytokines with or without poly I:C. Hippocampal tissues were analyzed for cytokine levels. The results showed that sgp130 alone evoked an immune response and demonstrated significant effects on the expression of several cytokines. We also found that sgp130 altered several cytokines in the poly I:C inflammation model.

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Poster

303. Neuroinflammation: Animal Models

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIAAA R01AA021775

Title: Changes in neuroinflammation markers across brain regions of interest in alcohol-related brain damage

Authors: *P. TOLEDO NUNES¹, L. C. VEDDER², T. DEAK³, M. M. DEAK², L. M. SAVAGE, Ph.D.⁴

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Abstract: Neuroinflamation has been suggested to play a significant role alcohol-related brain dysfunction. Thiamine deficiency (TD) is a key contributor to alcohol-related brain damage. The goal of this study was to determine the long-term impact of Chronic Ethanol Treatment (CET; 20% ethanol in drinking water for 6 months) and thiamine deficiency (induced by pyrithiamine-induced thiamine deficiency and thiamine free diet; PTD) on the expression of several key neuroimmune genes (IL-1 β , IL-6, TNF α , IkB α) in brain regions with established vulnerabilities to alcohol-related brain damage (thalamus, hippocampus, frontal cortex). Using real time RT-PCR assessments, we observed only minor fluctuations in these neuroimmune genes as a result of CET, regardless of the structure being examined. In contrast, PTD treatment led to a profound increase in all four neuroimmune genes within the thalamus, a structure that demonstrates severe pathology as a result of TD. Cytokine changes in the thalamus ranged in magnitude from moderate (3-4 fold increase for IL-1 β and IkB α) to severe (10-40 fold increase in TNF α and IL-

6, respectively). Though a similar pattern was observed in the hippocampus and frontal cortex, overall fold-increases were comparatively small relative to the thalamus. Importantly, neuroimmune gene induction varied significantly as a function of severity of TD, and displayed a gradual decline across recovery (24-hr, and 3 weeks post-treatment). Overall, these data suggest that rapid induction of neuroimmune genes may contribute to the severity of thalamic lesions induced by TD. Furthermore, TD, rather than a life-long history of alcohol consumption per se, appears to contribute greater vulnerability toward neuroinflammation. Finally, the temporal aspects of neuroimmune gene induction suggests the degree of TD may be a key driver of neuroimmune gene expression and subsequent neuroinflammation.

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Poster

303. Neuroinflammation: Animal Models

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NIBIB Grant R01EV018306

Title: Dynamics of blood-brain barrier repair in a neonatal rabbit model of cerebral palsy and its implications in nanoparticle-based drug delivery

Authors: *J. E. PORTERFIELD¹, E. S. SMITH², K. LIAW¹, Z. ZHANG², R. M. KANNAN³, S. KANNAN²

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Abstract: Two of the key physiological hallmarks of cerebral palsy (CP) are an impaired bloodbrain barrier (BBB) and neuroinflammation. Recent nanotechnology efforts have taken advantage of the impaired BBB, for targeted attenuation of neuroinflammation. It has previously been shown in a neonatal model of CP induced by maternal-fetal inflammation that motor function, BBB integrity, and survival can all be increased dramatically by targeted delivery of Nacetyl cysteine, a potent anti-oxidant/anti-inflammatory agent, to activated microglia and astrocytes via dendrimers. We have recently discovered that the restoration of the BBB could be time-dependent, which may have major implications on the development of a dosing regimen for dendrimer-based therapies in preclinical models, due to the fact that neuroinflammation persists, but becomes less accessible over time.

To study BBB repair dynamics after neonatal exposure to maternal inflammation, pregnant New Zealand white rabbits are given an intrauterine dose of LPS endotoxin on gestation day 27, then

labor is induced on day 29 and kits are obtained. To investigate whether the BBB was becoming less penetrable over time, kits were injected i.v. with Evan's Blue Dye (EBD) at varying time points after birth. After sacrifice the brain was perfused with saline, flash frozen, microdissected, and homogenized in 50% trichloroacetic acid for spectroscopic quantification. The quantity of EBD in the brain tissue decreased incrementally over a 24-hour period, indicating a decrease in permeability of the BBB. Additionally, occludin expression is currently undergoing analysis through western blot, which is expected to further confirm the time-dependent repair of the BBB in the absence of therapeutics.

For analysis of the impact on dendrimer therapy, dendrimer-cyanide 5 dye conjugate (D-Cy5) was administered to the kits i.v. at varying time points after birth. 24 hours after D-Cy5 administration, kits were sacrificed and perfused, and D-Cy5 quantification was performed similarly to EBD. The results show significantly decreased uptake of D-Cy5 at later time points, suggesting that the ability of the dendrimer to cross the BBB is diminished over time. This data indicates that BBB repair may function independently of neuroinflammation within this model of CP, and that for effective treatment of CP with dendrimer nanotechnologies, administration may have to occur in the early stages of the disease, or may require higher dosing to achieve the same effective concentration of drug in the brain. These results are integral to understanding mechanisms of CP as well as other neonatal conditions marked by maternal inflammation.

Disclosures: J.E. Porterfield: None. **E.S. Smith:** None. **K. Liaw:** None. **Z. Zhang:** None. **R.M. Kannan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ashvattha Therapeutics, Orpheris Inc. **S. Kannan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ashvattha Therapeutics, Therapeutics, Orpheris Inc.

Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Intranasal IGF-1 protects against neonatal lipopolysaccharide-induced neuroal inflammation in the brain of juvenile rats

Authors: *L.-T. TIEN¹, Y.-J. LEE¹, C.-C. CHIEN^{1,2}, S. LU³, L.-W. FAN³ ¹Fu Jen Catholic Univ., Taipei, Taiwan; ²Anesthesiol., Cathay Gen. Hosp., Taipei, Taiwan; ³Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: Neonatal lipopolysaccharide (LPS) exposure-induced brain inflammation resulted in motor dysfunction and brain dopaminergic neuronal injury, and increased the risks of neurodegenerative disorders in adult rats. Our previous studies showed that intranasal administration of insulin-like growth factor-1 (IGF-1) protects against LPS-induced white matter injury in the developing rat brain. To further examine whether IGF-1 protects against LPSinduced brain neuronal injury and neurobehavioral dysfunction, recombinant human IGF-1 (rhIGF-1) at a dose of 50 µg/pup was administered intranasally 1 hour following intracerebral injection of LPS (1 mg/kg) in postnatal day 5 (P5) Sprague-Dawley rat pups. Neurobehavioral tests were carried out from P7 to P21, and brain neuronal injury was examined at P21. Our results showed that LPS exposure resulted in disturbances of motor behaviors in juvenile rats. Moreover, LPS exposure caused injury to central catecholaminergic neurons, as indicated by reduction of tyrosine hydroxylase (TH) immunoreactivity in the substantia nigra (SN) and ventral tegmental area (VTA), and brain noradrenergic neurons, as indicated by reduction of TH immunoreactivity in the locus coeruleus (LC) of the P21 rat brain. The LPS-induced reduction of TH+ cells were observed at a greater degree in the SN and LC of the P21 rat brain. Intranasal rhIGF-1 treatment attenuated LPS-induced central catecholaminergic neuronal injury and motor behavioral disturbances, including locomotion, beam walking test and gait analysis. Intranasal rhIGF-1 administration also attenuated LPS-induced elevation of IL-1ß levels and numbers of activated microglia, and cyclooxygenase-2+ cells, which were double labeled with TH+ cells in the SN, VTA, and LC of the P21 rat brain. These results suggest that IGF-1 may provide protection against neonatal LPS exposure-induced central catecholaminergic neuronal injury and motor behavioral disturbances, and that the protective effects are associated with the inhibition of microglia activation and the reduction of neuronal oxidative stress by the suppression of the neuronal cyclooxygenase-2 expression.

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Poster

303. Neuroinflammation: Animal Models

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NCI Grant R01CA194024

NINDS Grant P30NS045758

Title: Mammary tumors induce neuroinflammation, but not behavioral deficits in Balb/C mice

Authors: *W. H. WALKER, J. C. BORNIGER, A. A. ZALENSKI, S. GAHOLT, N. ZHANG, A. C. DEVRIES

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Abstract: Women diagnosed with breast cancer are considerably more likely to develop a mood disorder and to report cognitive deficits than women in the general population. Previous studies suggest that peripheral tumors, such as mammary tumors, may create an immune response that can drive neuroinflammation, in turn leading to depression and cognitive deficits. In the present study, two cohorts of female Balb/C mice received bilateral orthotopic injections of syngeneic 67NR, 4T07, or 4T1cells (1x10⁵ cells per injection) to induce mammary tumors. Approximately three weeks later, cognitive function (via fear conditioning) or depressive-like behavior (via tail suspension and forced swim test) was assessed. Tissues were collected following the completion of behavioral testing. Proinflammatory cytokine levels were increased in the serum (IL-1 β , TNF α , IFN γ) and livers (IL-1 β , IL-6, TNF α) of mice injected with 4T07 or 4T1 cell lines compared to the 67NR cell line and vehicle control. IL-1ß was increased in both the hippocampus and cortex of mice injected with 4T07 or 4T1 cell lines relative to the vehicle and 67NR treatment groups. However, mammary tumors had no effect on hippocampal neurogenesis (DCX+ cells) and did not alter depressive-like behavior or induce cognitive deficits. Collectively, these data demonstrate that similarly sized tumors can produce differential immune responses both peripherally and centrally and that tumor-induced neuroinflammation can exist without resulting in the development of depressive-like behavior or cognitive deficits.

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Poster

303. Neuroinflammation: Animal Models

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Application of retinoic acid after optic nerve injury affects optic nerve glia and macrophages in adult frog Rana pipiens

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Abstract: Retinoic acid (RA) is a vitamin A-derived lipophilic molecule that plays major roles during development of the nervous system. The RA signaling pathway is also present in parts of the adult nervous system, and components of it are upregulated after injury. Recently we have shown that the components of RA signaling are present at low to moderate levels in frog retinas and tecta of control, unoperated animals. After optic nerve injury, there is a large increase in the synthetic enzyme RALDH and the retinoic acid receptors (RARs) in the frog retina. It is not known whether similar changes take place in the glia of the injured optic nerve. The objective of the present study is to characterize the RA signaling system in the frog optic nerve and to determine the changes that occur after axotomy and application of RA. We performed optic nerve crush and applied into the nerve either saline solution or retinoic acid. We examined the optic nerves at 48h, one week, and two weeks after axotomy with immunocytochemistry and electron microscopy. Our results indicate that RARs are present in the optic nerve glia at very low levels, and that one week after axotomy there is a significant increase in staining intensity in those glial cells. Electron microscopy studies of the proximal, injury, and distal sites of the optic nerves show macrophages filled with secondary lysosomes and residual bodies. Application of RA to the optic nerve causes a significant increase in the number of macrophages present one week after optic nerve injury. Immunocytochemistry of various macrophage subtypes was carried out followed by confocal microscopy. The vast majority of cells were F4/80-positive in saline- and RA-treated nerves. We also identified a sub-population of anti-inflammatory M2 macrophages (arginase-positive) at the injury and distal sites. We are currently studying the changes in time and location of these populations. In conclusion, axotomy increases RARs in optic nerve cells. The application of RA affects the number and the distribution of macrophages after optic nerve injury and it may play a role in the success of optic nerve regeneration.

Disclosures: V. De La Rosa-Reyes: None. **M.V. Duprey-Diaz:** None. **J.M. Blagburn:** None. **R.E. Blanco:** None.

Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 303.10/X2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Effects of Humanin on cognitive deficit and neuroinflammation by short-term exposure to cuprizone in mice

Authors: M. MURAKAMI¹, *Y. ABE², T. NIIKURA¹

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Abstract: Humanin (HN) is a 24-residue neuroprotective peptide. HN with a substitution of glycine for serine 14 (HNG) is a highly potent HN derivative and shows neuroprotective activity at nano M levels in vitro. HNG reduced amyloid burden and suppressed cognitive impairment in a mouse model of Alzheimer's disease. HNG also ameliorated amnesia induced by a muscarinic receptor antagonist and a GABA_A receptor agonist in mice. In addition, HN is noted to suppress LPS-induced cytokine production and activation of primary astrocytes in vitro. This indicates that HN has anti-inflammatory function to glial cells. However, the effects of HN on neuroinflammation in vivo remain unclear. Cuprizone (CPZ), a cupper chelator, induces demyelination in the central nervous system when mice are exposed to it for four to eight weeks (long-term exposure). A short-term (one week) exposure to CPZ triggers schizophrenia-like behavior in mice. In these mice, neuroinflammatory responses, up-regulation of a proinflammatory cytokine and glial activation, but not demyelination were observed, indicating that inflammatory responses occur prior to demyelinateion. Here, we tested the effect of HNG on these responses induced by short-term CPZ exposure in mice. Intraperitoneal injection of HNG for one week reversed the CPZ-induced deficit in object recognition memory but not in working memory. We detected activation of microglia and astrocytes in these mice by immunohistchemistry. Quantitative analysis showed no significant changes by HNG in CPZinduced glial activation. We then analyzed levels of transcripts for glial markers in hippocampi by quantitative PCR. Though the effect was limited, HNG significantly suppressed CPZ-induced activation of microglia and astrocytes. On the other hand, HNG did not alter the reduced level of myelin-specific transcript as a result of CPZ treatment. These results imply cell-type-specific action of HN. These findings suggest that HN can suppress neuroinflammation and associated cognitive deficit in a wide range of neurological disorders.

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Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 303.11/X3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Mast cells and their involvement in neurological damage in a Huntington's disease model

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Abstract: Huntington's disease (HD) is a hereditary neurodegenerative disorder caused by a mutation in exon 1 of the huntingtin gene. HD courses with neuronal loss and neuroinflammatory processes, among other pathological processes. Mast cells (MCs) participate in inflammatory processes, where histamine (Hist) is the principal mediator. In different neuroinflammation models, His potentiates NMDA receptor-mediated cytotoxic effects. Intrastriatal (i.e.) administration of quinolinic acid (QUIN; NMDA agonist) in rodents mimics the most representative neurochemical characteristics of HD. In this study, we set to investigate the participation of MCs in the neurological and oxidant damage observed in a neurochemical model of HD. For this purpose, were used MCs-deficient Kit w-sh / w-sh mice (Wsh) and C57BL6 / J mice administered with QUIN (30nmol/µL, i.e.) as a reference for damage. To verify the participation of MCs in the QUIN-induced damage, Wsh mice were reconstituted with MCs precursors derived from mouse bone marrow (BMMCs). Reconstitution with BMMCs was performed by two routes: intracerebroventricular (ICV) (5x105 cells) and intraperitoneal (IP) (2x106 cells). In parallel, a group of animals was administered with Hist ICV (5 µg). Ipsilateral turns, striatal GABA content, lipid peroxidation (LP) and reactive oxygen species (ROS) were measured. Wsh mice presented a lower number of ipsilateral turns, GABA, LP and ROS levels in response to QUIN, in comparison to the control. I.p. reconstituted mice showed no significant difference from un-reconstituted Wsh mice. In contrast, Wsh mice ICV reconstituted with BMMCs or administered ICV with Hist increased the number of ipsilateral turns and GABA levels similar to control. Our results demonstrate that, at central level, His secreted by MCs potentiates the neurological and oxidative damage in this neurochemical model of Huntington disease.

Disclosures: P.E. Martínez-Gopar: None. F. Perez: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Conacyt Grant 241911. C. Rios: None. L.A. Tristan-lopez: None. C. González-Espinosa: 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 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.; Grant Conacyt 188565.

Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

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Program#/Poster#: 303.12/X4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: National Multiple Sclerosis Society PP-1510-06517

NINDS R01NS081040

Title: Fibroblasts infiltrate the spinal cord in experimental autoimmune encephalomyelitis

Authors: *S. L. YAHN, J. LI, R. BRAMBILLA, J. K. LEE Neurolog. Surgery, Univ. of Miami, Miami, FL

Abstract: Remyelination failure is a crucial component of disease progression in the autoimmune demyelinating disease Multiple Sclerosis (MS). The regenerative capacity of oligodendrocyte progenitor cells to differentiate into myelinating oligodendrocytes is likely influenced by many aspects of the lesion environment including inflammatory signaling and extracellular matrix deposition. These features of MS lesions are typically ascribed to infiltrating leukocytes and reactive astrocytes. Here we demonstrate that fibroblasts also infiltrate the spinal cord parenchyma in the animal model of MS, experimental autoimmune encephalomyelitis (EAE). Using Col1 α 1-GFP transgenic mice, we show that perivascular/meningeal fibroblasts are activated in the spinal cord at EAE onset and infiltrate the parenchyma by the peak of behavioral deficits. Furthermore, infiltrating fibroblasts are associated with areas of macrophage accumulation, demyelination, and fibronectin deposition. These results implicate fibroblasts in EAE pathology and suggest that fibrotic mechanisms may serve as novel therapeutic targets.

Disclosures: S.L. Yahn: None. J. Li: None. R. Brambilla: None. J.K. Lee: None.

Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

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Program#/Poster#: 303.13/X5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: TSGH-C106-019

Title: Low dose of Diphenyleneiodonium (DPI), specific NADPH oxidase 2 (NOX2) inhibitor, targets NOX2 & ameliorates experimental autoimmune encephalomyelitis (EAE) severity in mouse model

Authors: *C.-F. HU¹, S.-J. CHEN¹, J.-S. HONG² ¹Pediatric Dept., Tri-Service Gen. Hosp., Taipei, Taiwan; ²NIEHS, Rtp, NC

Abstract: Nicotinamide adenine dinucleotide phosphate oxidase, type 2, (NADPH oxidase 2, NOX2), a key superoxide-producing enzyme which forms reactive oxygen species (ROS), plays a critical role in microglia-mediated chronic neuroinflammation & subsequent progressive

dopaminergic neurodegeneration in Parkinson's disease in mouse model. However, seldom articles discuss about the values & effects on different diseases, like experimental autoimmune encephalomyelitis (EAE) in mouse model. The aim of this study was to evaluate the characteristics & effects of low dose of NOX2 inhibitor, diphenyleneiodonium (DPI) in moderate severity of EAE mouse model. Furthermore, we want to ask whether NOX2 knockout mice show disease protection through reducing superoxide production & subsequent inflammatory process.

Our preliminary data reveal that pre-treatment strategy with DPI (both intravenous injection & drinking water formula) significantly attenuates progressive encephalomyelitis & reduces the severity of limbs paralysis of mice after disease induction. Meanwhile, NOX2 knockout mice show EAE protection through the inhibition of microglia activation, less peripheral macrophages infiltration in the central nervous system (CNS), & also a reduction in the expression of proinflammatory factors.

Disclosures: C. Hu: None. S. Chen: None. J. Hong: None.

Poster

303. Neuroinflammation: Animal Models

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIMH Grant R21 MH10815

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Title: Intestinal microbiota modulates depressive-like behavior in a mouse model of multiple sclerosis

Authors: *J. E. GOERTZ, I. A. MARIN, A. GAULTIER Univ. of Virginia, Charlottesville, VA

Abstract: In addition to the traditional motor impairments that are hallmark symptoms, depression has been found to be one of the most common symptoms in multiple sclerosis (MS) patients. While the immune system was previously thought to be the main link between this autoimmune disease and depression, new evidence points to the microbiome, the collection of organisms that live in and on the body, as an active player in depression etiology. The microbiome has recently been implicated in autoimmunity, as data has shown that autoimmune disease cannot be induced in germ-free mice and also that the microbiota composition may be responsible for the gender bias observed in the prevalence of autoimmune diseases. Previous

studies from our lab have shown that the microbiome, and in particular *Lactobacillus*, are involved the development of stress-induced depression in mice. Therefore, we hypothesize that changes in the gut microbiota in response to autoimmune disease induce depressive behavior. In order to tackle this problem, we induced experimental autoimmune encephalomyelitis (EAE), the mouse model of MS to examine the effect of disease on behavior and microbiota composition. We utilized the sucrose preference test and the social preference test to measure depressive behavior as the disease progressed. 16S sequencing showed robust changes in the composition of intestinal microbiota. These data suggest the microbiota in the gut, specifically *Lactobacillus*, is altered in mice affected by CNS autoimmune disease in correlation with their depressive behavior. Further research in this area may suggest new leads in developing novel therapies that could be used to treat depression in MS patients.

Disclosures: J.E. Goertz: None. I.A. Marin: None. A. Gaultier: None.

Poster

303. Neuroinflammation: Animal Models

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Program#/Poster#: 303.15/X7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: GM109086

Title: Dose-dependent effects of systemic inflammation on murine cortical activity

Authors: *E. R. JAECKEL¹, M. I. BANKS¹, C. N. MURPHY², S. M. GRADY¹, P. A. BARNARD³, S. KAUR³, E. A. TOWNSEND⁴, R. D. SANDERS¹ ¹Anesthesiol., ²Physiol. Grad. Training Program, ³Col. of Letters and Sci., ⁴Sch. of Med. and Publ. Hlth., Univ. of Wisconsin, Madison, WI

Abstract: Introduction: Neuroinflammation is associated with cognitive and behavioral anomalies, but the mechanisms are unclear. At its extreme, during delirium, profound changes in cognitive function are accompanied by increased slow wave activity, and decreased 8-14Hz phase lag index (PLI) connectivity, in the EEG. We sought to model the changes in the behavioral and EEG responses to systemic inflammation by administering Lipopolysaccharide (LPS). Methods: All procedures were approved by the UW-Madison Animal Care and Use Committee. 14 mice (male; BL6 background; 12-20 wks old) were instrumented with bilateral parietal and frontal skull screw EEG electrodes. After 5-7 days recovery, mice were given intraperitoneal (IP) saline injections, and 1-2 days later IP injections of LPS (12.5, 25 or 125 ug/kg). Animals were tethered to a lightweight headstage during the experiment allowing movement to signify wakefulness. Data were collected for 1 hr prior and at 2-3 hrs post-injection. Average power was computed in the EEG δ (1-4 Hz), θ (4-10Hz), α (10-20 Hz), β (20-

30 Hz) and γ (30-100Hz) bands during movement defined via video recording. Comparisons were between the post-injection:control ratios of normalized band power (to total power) for saline versus LPS. Additionally, 8-14Hz PLI was calculated between frontal and parietal electrodes. Results: LPS increased average delta power across frontal and parietal electrodes at 12.5 ug/kg (p=0.0079), 25 ug/kg (p=0.0016) and 125 ug/kg (p=0.0022) during wakefulness. At 125 ug/kg (p=0.06) or 25 ug/kg (p=0.11). Multi-way ANOVA showed a trend to effect for LPS dose (p=0.06) but not electrode (p=0.39). LPS also decreased alpha and gamma power at each dose tested (p<0.05) but with no effect of dose in multi-way ANOVA (alpha LPS dose effect p=0.15; gamma LPS dose effect p=0.24). 125 ug/kg LPS also decreased frontoparietal 8-14Hz band PLI (p=0.0057) with no effect at the other doses (p>0.05). Conclusions: Ongoing studies will resolve the dose-dependence, and electrode site-dependence of inflammation. LPS injection is a promising model for neuroinflammatory effects on brain activity in wakefulness confirmed by spontaneous movement.

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Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: The Michael J. Fox Foundation

Title: Correlation analysis of [¹⁸F]ROStrace *Ex vivo* autoradiography and dihydroethidium fluorescent images in lipopolysaccharides treated animals

Authors: *C.-C. WENG, C. HOU, C. ZENG, C.-J. HSIEH, S. LI, H. LEE, R. H. MACH Radiology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Reactive oxygen species (ROS) play a critical role in the "neurotoxic" form of inflammation occurring in several neurodegenerative disorders. However, there is little data regarding the relationship between the in vivo ROS and neuroinflammation. For a better understanding, herein we performed the distribution correlation analysis between [¹⁸F]ROStrace and some common inflammation markers in a lipopolysaccharides (LPS) treated animal model. The LPS-treated mice (female Balb/c, body weight 20-25 g) received a 5 mg/kg i.p. injection of LPS 24 hr before study. Firstly, a 60-min dynamic PET scan was performed after an i.v. injection of [¹⁸F]ROStrace (~11MBq in 0.1 mL saline solution). To verify the ROS distribution, a dose of 15 mg/kg dihydroethidium (DHE) was also i.v. injected to the same animal after imaging, and it

was sacrificed 30 min after DHE injection. The brain was sectioned and acquired for ex vivo [¹⁸F]ROStrace autoradiography and DHE fluorescence. For further information on the inflammation, the sections were also stained for GFAP and Iba-1 and fused with the DHE images.

From the in vivo PET images of LPS-treated animals, we found a high uptake of the [¹⁸F]ROStrace in the brain, and a steady-state was reached roughly 30 min after tracer injection. Examination of the ex vivo autoradiography showed that the uptake ratio of the whole brain is about 3-fold higher in the LPS-treated group compared to the control one, and the tracer uptake was mainly distributed in the subregions of cortex, thalamus, hippocampus, and cerebellum. This showed a good correlation with the DHE fluorescent images and the antibody staining results. The [¹⁸F]ROStrace PET images can clearly delineate the ROS generation in the LPS-treated animal, and the ex vivo autoradiography further shows the tracer uptake regions were colocalized with the ROS markers, DHE and inflammation antibodies. This study provides evidence that [¹⁸F]ROStrace is useful for further in vivo ROS research.

Disclosures: C. Weng: None. C. Hou: None. C. Zeng: None. C. Hsieh: None. S. Li: None. H. Lee: None. R.H. Mach: None.

Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 303.17/X9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Persistence of cognitive deficits in the absence of systemic inflammation following acute LPS administration in immune-competent and immunodeficient mice

Authors: *S. REGE, H. HACKBART, A. TEICHERT, J. MASUMI, S. P. BRAITHWAITE, S. MINAMI

Alkahest, Inc., San Carlos, CA

Abstract: Peripheral inflammation has been linked to cognitive dysfunction in both animal models and in humans. Elevated levels of peripheral cytokines, classical hallmarks of inflammation, have been associated with lower cognitive performance, and there exists a well-established relationship between peripheral inflammation and cognitive dysfunction in patients experiencing acute infection and recent surgical procedures. However, there is little known about the long-lasting effects of transient systemic inflammation on cognitive function. Here we demonstrate that an acute dose of LPS peripherally can result in persistent cognitive dysfunction in the absence of elevated systemic inflammation. We administered a high (5mg/kg) and low (1mg/kg) dose of LPS to 2-month-old C57Bl/6 mice and assessed cognitive performance one week and one month later. The high dose group demonstrated diminished cognitive performance

in hippocampus dependent tests, like novel object recognition and y-maze, both one week and one month after administration of LPS. The low dose group was less affected after one month, with the only deficiency observed in the novel object recognition test. There were no effects on locomotor function or general physical health of the mice at either one week or one month after treatment. Immunocompromised mice are often used as a model to test human protein-containing therapies; in order to test the utility of these mice in an acute peripheral inflammation-induced cognitive dysfunction model, we treated NOD.Cg-*Prkdc^{scid}Il2rg^{tm1Wjl}*/SzJ (NSG) mice and found that their performance in hippocampus-dependent tests was slightly impaired. Additionally, we measured levels of circulating inflammatory cytokines immediately after LPS induction and terminally, at which point we assessed brain tissue by histological methods and performed protein analysis of neuronal and inflammatory cell markers. These results indicate that LPS can be utilized to establish a model of cognitive dysfunction induced by acute systemic inflammation in both immune-competent and immunodeficient mice, thus enabling future studies to better understand the relationship between inflammation and cognition.

Disclosures: S. Rege: A. Employment/Salary (full or part-time):; Alkahest, Inc. H. Hackbart: A. Employment/Salary (full or part-time):; Alkahest, Inc. A. Teichert: A. Employment/Salary (full or part-time):; Alkahest, Inc. J. Masumi: A. Employment/Salary (full or part-time):; Alkahest, Inc. S.P. Braithwaite: A. Employment/Salary (full or part-time):; Alkahest, Inc. S. Minami: A. Employment/Salary (full or part-time):; Alkahest, Inc..

Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 303.18/X10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Baicalein neutralizes brain oxidative stress in diabetic rat model

Authors: *S. ALSHARARI¹, S. AL-REJAIE²

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Abstract: Diabetic-induced encephalopathy is a well-documented complication in diabetic patients, which is characterized by cellular, molecular and functional alterations. Nevertheless, the mechanisms responsible for the pathogenesis and the therapeutic strategies are not fully reported. In the present investigation, a flavonoid baicalein was examined as a protective agent against the experimental diabetic neurodegenerative diseases based on its potent free radical-scavenger activity. Baicalein was orally administered in two different doses (25 and 50 mg kg1-day1-) to diabetic rats for five consecutive weeks. At the end of the treatment period, the cerebral cortex tissues were harvested and directly frozen in liquid nitrogen till analysis. Baicalein,

especially the higher dose, improved insulin and blood glucose levels. In addition, diabetic associated oxidative injury in the diabetic cerebral cortex tissues was ameliorated by baicalein, which was manifested by enhanced glutathione, superoxide dismutase and catalase activities and decreased lipid peroxidation products compared to untreated diabetic rats. Taken together, these finding indicate the beneficial role of baicalein in alleviating the provoked oxidative stress in the diabetic cerebral cortex.

Disclosures: S. Alsharari: None. S. Al-Rejaie: None.

Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Ministry of Science and Technology of China (2016YFA0501000)

Title: CCL2 increases neuronal excitability during the early stage of systemic inflammation

Authors: *L.-H. DUAN^{1,2}, X.-D. ZHANG^{1,2}, W.-Y. MIAO¹, H.-M.-Z. LI¹, X. YU^{1,2} ¹Inst. of Neurosci., ShangHai, China; ²Univ. of Chinese Acad. of Sci., Beijing, China

Abstract: Acute infection by bacteria and viruses, if not kept in check, can quickly become systemic, leading to inflammatory responses in the brain. Young children are particularly vulnerable, with inflammation-induced cephalomeningitis being a leading cause of death among newborns, especially premature babies. The long-term effects of neuroinflammation on the brain function and behavior have been studied extensively, while its acute effects (within the first few hours) are relatively unknown. Here, using different systemic inflammation models, including Lipopolysaccharides (LPS), Poly(I:C) and ODN 1668, to respectively mimic infections mediated by bacteria, RNA virus and DNA viruses, we demonstrated that during the early stage of infection (within 2 hours), mice exhibited increased neuronal excitability and increased susceptibility to kainic acid induced seizure. High level expression of the chemokine (C-C motif) ligand 2 (CCL2) was observed in all three models, with the effects of LPS being highest. Overproduction of CCL2 may interfere with normal brain development and neuronal function, which may in turn, result in inflammation-induced neuronal hyperexcitability. Consist with this hypothesis, bath application of CCL2 in acute brain slices increased the frequency of miniature excitatory postsynaptic currents (mEPSCs) in hippocampal and cortical pyramidal neurons, as well as their neuronal firing. Further investigations demonstrated that the effects of LPS induced increase in mEPSC frequency and neuronal excitability were blocked in CCL2 knockout mice. Together, these results demonstrate that CCL2 plays a key role in regulating excitatory synaptic transmission and neuronal excitability, during the early stage of systemic inflammation.

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Poster

303. Neuroinflammation: Animal Models

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: This work is supported by the National Research Foundation of Korea Grant funded by the Korean Government (NRF-2015R1C1A1A02037060)

Title: Establishment of minimal positive conditions to ensure brain safety for rapid development of emergency vaccines

Authors: ***H. BAEK**¹, G. KIM¹, M. PARK¹, B. KO¹, K. KIM¹, H. SEO², S. YI¹ ¹Soonchunhyang Univ., Asan, Korea, Republic of; ²Konyang Univ., Nonsan, Korea, Republic of

Abstract: With the increase in international human and material exchanges, contagious and infectious epidemics are being discovered. One of the effective methods of inhibition is the rapid development and supply of vaccines. Considering the safety of the brain during vaccine development is very important. However, manuals for brain safety assays for new vaccines are not uniform and efficient globally. Therefore, the aim of this study is to establish positive control protocol for effective brain safety test to help rapid vaccine development. Using the characteristics of tight junction of blood-brain barrier with the selective defense of the brain, it is possible to destroy this important micro-structure by administering lipopolysaccharides (LPSs), thereby artificially increasing the permeability of the parenchyma. The conditions are established so that the degree of brain penetration or brain destruction of a newly developed vaccine can be quantitatively identified. The most effective conditions were suggested by measuring the time-dependent expression of two types of mice (C57BL/6 and ICR), two types of LPSs (*Salmonella* and *Escherichia*), and tight junction biomarkers (ZO-1 and Occludin) after exposure to LPSs. In the future, we hope the positive-protocol will be able to speed up the determination of brain safety of various vaccines through this condition.

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Poster

303. Neuroinflammation: Animal Models

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Program#/Poster#: 303.21/X13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Deficits in touchscreen-based operant tasks and fear conditioning memory in the cuprizone model of multiple sclerosis

Authors: A. J. NURMI¹, M. KOPANITSA¹, K. LEHTIMÄKI¹, M. FORSMAN¹, A. SUHONEN¹, J. KOPONEN¹, *A.-M. KÄRKKÄINEN¹, A. MERENLENDER-WAGNER², J. KAYE², A. ORBACH²

¹Charles River Discovery, Kuopio, Finland; ²Teva Pharmaceut. Industries Ltd., Netanya, Israel

Abstract: Cognitive problems frequently accompany neurological manifestations of multiple sclerosis (MS). Therefore, evaluation of cognitive performance in mouse models of MS is needed to determine learning and memory parameters that change with the emergence of MSrelated symptoms and can be used as benchmark phenotypes for testing of drug candidates. We assessed cognitive behavior of female 9-10-week-old C57Bl/6J mice subjected to continuous cuprizone exposure by oral gavage (400 mg/kg daily) for two or four weeks and compared their performance to that of vehicle-treated mice. Cuprizone causes death of mature oligodendrocytes leading to demyelination, mostly, in the corpus callosum, that resembles pathology seen in MS patients. Cuprizone-treated mice demonstrated multiple deficits in a sequence of touchscreenbased operant tasks of gradually increasing difficulty. In particular, in comparison to vehicletreated animals, cuprizone-treated mice completed fewer trials, responded more slowly to visual stimuli, and were also slower to collect food reward after correct responses. Furthermore, cuprizone-treated mice exhibited significantly lower accuracy of responses during "Punish incorrect" stage of operant training. In contextual/cued fear conditioning experiments, in the 2week cohort, cuprizone-treated mice demonstrated significantly lower levels of both contextual and cued freezing than vehicle-treated mice. In the 4-week treatment cohort, a significant effect of treatment was observed only on contextual freezing level. Mild demyelination was observed in the 2-week cuprizone-treated cohort as reflected by significantly lower fractional anisotropy values in forceps minor of the corpus callosum and external capsule of cuprizone-treated animals. Stronger demyelination was induced by the longer, 4-week treatment, as indicated by larger and more widespread decreases of fractional anisotropy values in the brain of cuprizonetreated mice (forceps minor of the corpus callosum, genu of corpus callosum, body of corpus callosum, splenium of corpus callosum, external capsule, internal capsule) compared to myelination levels in the brain of animals that received vehicle. Our results suggest that touchscreen operant tests and fear conditioning paradigms sensitively detect cognitive consequences of MS-like anatomical brain abnormalities caused by relatively short exposures to cuprizone. Therefore, novel drug candidates for MS-treatment could be screened for their ability to reverse those cognitive deficits and to improve brain structural parameters in longitudinal experiments in the same animals.

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Poster

303. Neuroinflammation: Animal Models

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Immunophenotyping and characterization of PLP and MOG induced relapsing/remitting and chronic mouse models of experimental autoimmune encephalomyelitis

Authors: J. KURKIPURO, *M. VIHMA, L. KOISTINEN, *M. VIHMA, A. OKSMAN, L. TAHTIVAARA, A. J. NURMI Charles River Discovery, Kuopio, Finland

Abstract: The experimental autoimmune encephalomyelitis (EAE) is an inflammatory demyelinating disease of the central nervous system characterized by infiltration of immune cells into the CNS and subsequent demyelination by activated myelin-specific T-cells. We have characterized two models of EAE; the chronic myelin oligodendrocyte glycoprotein (MOG) induced model in C57BL/6J mice and the relapsing/remitting proteolipid protein (PLP) induced model in SJL mice. Altogether 20 female C57BL/6J and SJL/J between 9-10 weeks of age were inoculated with MOG₃₅₋₅₅/CFA or PLP₁₃₉₋₁₅₁/CFA, respectively, without PTX for the induction of the chronic and relapsing/remitting EAE models. Mice were then monitored for clinical signs of disease development and changes in body weight for three weeks before sacrificing the cohorts for harvesting of draining lymph nodes (DLN), brain and spinal cord samples for immunophenotyping and characterization by flow cytometry and immunohistochemistry. The EAE development in both models showed first clinical signs roughly 10 days after the inoculations. The chronic C57BL/6J model reached higher clinical score and showed no relapse of the disease development whereas this was observed in the SJL model. In the relapsing/remitting SJL model, the clinical signs were also less severe in comparison to chronic model. Flow cytometry analysis of the brain tissue showed elevated T-cell populations, significant invasion of CNS-associated monocytes from periphery as well as upregulation of the microglial population in both models. Finally, the analysis of the DLN tissues showed that the levels of NK-cells seem to be more elevated in the MOG model than in the PLP model.

Disclosures: J. Kurkipuro: None. L. Koistinen: None. M. Vihma: None. A. Oksman: None. L. Tahtivaara: None. A.J. Nurmi: None.

Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 303.23/X15

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: University of Otago PhD project funding

Title: Treatment of brain injury due to extreme prematurity: Effect of melatonin on ADHD-like hyperactivity and midbrain dopaminergic neurons

Authors: *O. OKPE, ***O. OKPE**, L. GODDARD, D. E. OORSCHOT Dept. of Anat., Univ. of Otago, Dunedin, New Zealand

Abstract: The birth of an extremely premature baby (≤28 weeks of gestation) is costly to health systems. Children born extremely prematurely can experience repeated hypoxic injury to the brain and can develop attention deficit hyperactivity disorder (ADHD). In a new Sprague-Dawley rat model of repeated hypoxic brain injury during the equivalent of extreme prematurity, ADHD-like hyperactivity and a lower absolute number of midbrain dopaminergic neurons in the ventral tegmental area was observed. Yet, treatment with melatonin, which is safe in clinical neonatology and a known antagonist of the proposed biological mechanisms that generate a lower number of midbrain dopaminergic neurons in the ventral tegmental area, has not been investigated. Nor has the effect of melatonin on ADHD-like hyperactivity been investigated. Hence, the aim of this study was to investigate whether treatment with melatonin rescues ADHD-like hyperactivity and midbrain dopaminergic neurons in the ventral tegmental area. Postnatal day 1-3 Sprague-Dawley male rats were exposed to repeated hypoxia and divided into treatment groups of repeated hypoxia diluent-treated (n = 9 pups) and repeated hypoxia melatonin-treated (n = 10). At 9 months-of-age, each rat was tested for ADHD-like hyperactivity using a well characterised fixed interval test. At 10 months-of-age, each rat was perfuse-fixed, and its right cerebral hemisphere was serially sectioned and then immunohistochemically stained with an antibody to the catecholaminergic enzyme, tyrosine hydroxylase, to label dopaminergic neurons. The absolute number of dopaminergic neurons in the ventral tegemental area was then measured using the Cavalieri and disector methods. There was no significant difference between the repeated hypoxia melatonin-treated and the repeated hypoxia diluent-treated animals for the extent of ADHD-like hyperactivity (repeated measures ANOVA, p = 0.35) and the absolute number of midbrain dopaminergic neurons in the ventral tegmental area (Student's *t*-test, p =0.15, two-tailed test, n = 7/group and 6/group, respectively). The results of this study suggest that melatonin does not rescue ADHD-like hyperactivity and dopaminergic neurons in the ventral tegmental area after repeated hypoxic brain injury during the equivalent of extreme prematurity.

Disclosures: O. Okpe: None. L. Goddard: None. D.E. Oorschot: None.

Poster

303. Neuroinflammation: Animal Models

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EKFS 2015_A113 to K.G. DFG GO2505/1-1 to K.G. DFG ME3283/5-1 to S.G.M. CIM ME 3283/6-1 to S.G.M. SFB TRR 128/2 2016 TP B06, TP B12

Title: NADPH oxidase type 4 inhibits immune cell trafficking into the central nervous system during neuroinflammation

Authors: *A. M. HERRMANN¹, K. GÖBEL¹, S. EICHLER¹, J. BREUER¹, S. GLUMM¹, S. KOVAC¹, T. RUCK¹, M. MERKER¹, E. GEUSS², P. HUNDEHEGE¹, C. KLEINSCHNITZ³, H. H. W. SCHMIDT⁴, S. G. MEUTH¹

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Abstract: Transendothelial trafficking of immune cells into the central nervous system (CNS) and disruption of the blood brain barrier (BBB) are pathophysiological hallmarks of neuroinflammatory disorders like multiple sclerosis (MS). Accumulating evidence suggest that oxidative stress plays a major role in the pathogenesis of MS, whereas a specific influence of oxidative stress on BBB dysfunction in MS was unclear so far. Here, we identify NADPH oxidase type 4 (NOX4) as a specific and direct modulator of BBB integrity. Deficiency of NOX4, but not NOX1 or NOX2, rendered mice more susceptible to experimental autoimmune encephalomyelitis (an animal model of MS) and was accompanied by a remarkable enhancement of BBB disruption and CNS inflammation. Murine and human *in vitro* analysis revealed that lack

of NOX4 amplifies leukocyte trafficking by modified endothelial cells. Further, reduced endothelial NOX4 expression was found in CNS tissue of individuals suffering from MS indicating an important role of NOX4 also in humans. Our study demonstrates, for the first time, that NOX4 is an important and direct regulator of BBB integrity.

NOX4 activation can decrease BBB damage and cell invasion during neuroinflammation and may offer a novel strategy for the treatment of MS.

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Poster

303. Neuroinflammation: Animal Models

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NIH/NINDS R01NS080844

Newborn Medicine Funds from the Department of Pediatrics, University of Mississippi Medical Center

Title: Celecoxib ameliorates neonatal lipopolysaccharide-enhanced adult susceptibility to the rotenone-induced neurodegenerative disorder

Authors: *L.-W. FAN¹, L.-T. TIEN², A. M. KAIZAKI³, S. LU¹, J. W. LEE¹, J. P. SHAFFERY⁴, X. DAI⁵, N. B. OJEDA¹, A. J. BHATT¹, R. D. SAVICH¹, Y. PANG¹ ¹Pediatrics/Newborn Med., Univ. of Mississippi Med. Ctr., Jackson, MS; ²Sch. of Med., Fu Jen Catholic Univ., New Taipei City, Taiwan; ³Dept. of Pharmacology, Toxicology & Therapeutics, Div. of Toxicology, Sch. of Pharm., Showa Univ., Tokyo, Japan; ⁴Dept. of Psychiatry and Human Behavior, Animal Behavior Core, ⁵Dept. of Anesthesiol., Univ. Mississippi Med. Ctr., Jackson, MS

Abstract: Chronic brain neuronal inflammation has been proposed to play an important role in the development of neurodegenerative disorders in adult life. Our previous study showed that perinatal lipopolysaccharide (LPS) exposure induced a chronic neuronal inflammation, as indicated by increases in cytokines and pro-inflammatory molecules-induced cyclooxygenase-2 (COX-2)+ cells, and enhanced adult susceptibility to develop neurodegenerative disorders triggered by rotenone, a commonly used pesticide. The objective of the current study was to examine whether celecoxib, a selective COX-2 inhibitor, has long-lasting protective effects and attenuates LPS-induced motor behavioral dysfunction and LPS-enhanced susceptibility to

rotenone toxicity in later life of these rats. Intraperitoneal (i.p.) injection of LPS (2 mg/kg) or saline was performed on postnatal day 5 (P5) Sprague-Dawley male rat pups, and celecoxib (20 mg/kg) or vehicle was administered (i.p.) 5 min after LPS injection. On P70, rats were challenged with rotenone through subcutaneous mini-pump infusion at a dose of 1.25 mg/kg per day for 14 days. Motor behavioral tests were carried out from P70 to P98. Rats were sacrificed and LPS-induced injury to the dopaminergic system was assessed by losses of tyrosine hydroxylase immunoreactive neurons in the substantia nigra. Our results showed that neonatal administration of celecoxib provided protection against rotenone-induced neurobehavioral impairments including bradykinesia, akinesia, and rigidity in rats with the neonatal exposure to LPS. Celecoxib treatment also provided protection against LPS-enhanced rotenone-induced brain injury in adult rats, including loss of tyrosine hydroxylase positive neurons, a decrease in mitochondrial complex I activity, and an increase in the number of activated microglia in the substantia nigra of P98 rats. Results from the current study suggest that treatment with celecoxib has long-lasting protective effects against perinatal brain inflammation-enhanced adult susceptibility to environmental toxin-induced neurodegenerative disorders. Our results are useful for studying mechanisms involved in the pathogenesis of dopaminergic neuronal injury induced by infection/inflammation and the development of potential therapeutics.

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Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 MH53032

NIH T32 GM007270

Washington Research Foundation - Hall Fellowship

Title: The role of inflammation in healthy and natural neruodegenerative states of a sensorimotor pathway responsible for song production

Authors: *T. LARSON¹, Y. TOKAREVA², M. MERRITT-COLE1², E. A. BRENOWITZ³ ¹Dept. of Biol., Univ. of Virginia, Charlottesville, VA; ²Psychology, ³Psychology and Biol., Univ. of Washington, Seattle, WA

Abstract: Neuroinflammation is typically considered a negative response following neuronal injury and damage. For example, inflammatory microglia become polarized from the "inactive" surveying (or ramified) state following neuronal loss and promote a classic cytokine-mediated inflammatory response. Neuroinflammation can, however, play a beneficial role in the healthy adult brain. Ramified microglia elicit positive effects through neuronal and synapse pruning to maintain proper neuronal number and connections. To identify the role of inflammation in both healthy and natural degenerative states, we employed the natural seasonal regression of the motor pathway responsible for song production in Gambel's white crowned sparrow (Zonotrichia leucophrys gambelii). Neuronal number in the song control nucleus HVC (proper name) changes seasonally such that as male sparrows transition between breeding and nonbreeding conditions, HVC neuronal number increases and decreases by about 68,000 neurons, respectively. As a result of the seasonal apoptosis of HVC neurons, neural stem cell proliferation increases - a process termed natural reactive neurogenesis. With this unique model of natural and rapid neurodegeneration, we show that inflammation is both necessary and sufficient for increases in reactive neurogenesis using pro- and anti-inflammatory agents during health and degenerative states, respectively. Quantification of the number of ramified and activated microglia in HVC using immunohistochemistry further suggests that local inflammatory status is conferred to the neural stem cells via microglia.

Disclosures: T. Larson: None. Y. Tokareva: None. M. Merritt-Cole1: None. E.A. Brenowitz: None.

Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 303.27/X19

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Differential anticonvulsant responses of the basolateral amygdala and area tempestas to muscarinic receptor subtype antagonists following soman-induced seizures

Authors: *K. LAITIPAYA¹, J. K. CHANDLER², E. A. JOHNSON³, J. W. SKOVIRA⁴ ¹USAMRICD, Gunpowder, MD; ²US Army Med. Res. Inst. of Chem. Def, Aberdeen Proving Ground, MD; ³Pharmacol., US Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD; ⁴US Army Med. Res. Inst. of Chem. Def., Aber Prov Grd, MD

Abstract: Excessive cholinergic neurotransmission, particularly that involving the muscarinic receptor (MR), initiates seizure activity following exposure to the organophosphorus nerve agent soman. The MR antagonist scopolamine has been shown to prevent the onset of soman-induced seizures when given as a pretreatment or shortly after soman exposure. Scopolamine, however, is a non-specific MR subtype antagonist. The purpose of this study was to determine if antagonism

of specific MR subtypes is necessary to prevent soman-induced seizures. Using an up-down dosing procedure over successive animals we evaluated the microinjection of MR subtype specific antagonists into the basolateral amygdala or area tempestas (structures implicated as possible foci for soman-induced seizures) for their ability to pharmacologically modulate seizure activity. Rats were surgically prepared one week prior to the experiment with electrodes to record brain electroencephalographic (EEG) activity and with guide cannula bilaterally directed toward a designated brain structure. On the day of the experiment, animals were pretreated by microinjection (1 µl/cannula) with a MR subtype specific antagonist and intraperitoneal HI-6 (150 mg/kg). Thirty minutes later, animals were injected subcutaneously with 180 µg/kg soman (1.6 x LD₅₀) followed one minute later by 2.0 mg/kg atropine methyl nitrate intramuscularly. Animals were then returned to a cage where EEG was monitored for seizure activity. The MR subtype specific antagonists telenzepine (M_1) and 4-DAMP (M_3) provided an anticonvulsant effect when injected into the basolateral amygdala (anticonvulsant $ED_{50} = 30.06$ and 40.79 $\mu g/\mu l/cannula$, respectively). Rats injected with telenzepine into the basolateral amygdala also had the highest survival rate, followed by those injected with 4-DAMP. In the area tempestas only the M_2 antagonist methodramine produced an anticonvulsant effect (anticonvulsant $ED_{50} =$ 107.90 μ g/ μ l/cannula) while also having the highest survival rate. The results show that different MR subtypes within the basolateral amygdala and area tempestas respond to produce an anticonvulsant effect against soman-induced seizures while also increasing survivability of the specimens. More MR subtypes provided an anticonvulsant response in the basolateral amygdala than in the area tempestas. These results suggest that the response of multiple MR receptor subtypes within a brain structure may thus contribute to a greater anticonvulsant effect by nonspecific MR subtype antagonists such as scopolamine.

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Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 303.28/X20

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Ppary Agonism in alzheimer's disease and irradiated mouse models

Authors: *D. CORTEZ¹, L. A. DENNER², K. T. DINELEY, 77550³ ¹UTMB, Galveston, TX; ²Intrnl. Med., Univ. Texas Med. Br., Galveston, TX; ³Neurol., The Univ. of Texas Med. Br., Galveston, TX

Abstract: Converging evidence from animal and human studies suggest that an early target of Alzheimer's disease (AD) amyloid pathology is synaptic activity within the entorhinal cortex

(EC), dentate gyrus, and Cornu Ammonis 3 (EC/DG/CA3) network. A major function of this hippocampal network is to perform pattern separation; the ability to process overlapping environmental cues into unique representations and distinguish similar, yet non-identical contexts. We have previously shown that aged Tg2576 mice (a model for early AD that recapitulates amyloid pathology, hippocampal cognitive deficits and disrupted EC/DG/CA3 circuitry) exhibit improved circuit and cognitive performance with peroxisome proliferator activated receptor gamma (PPAR γ) agonism. PPAR γ is a nuclear receptor and transcription factor that can be activated with FDA-approved drugs. Since we have found that the PPAR γ transcriptome and proteome contains mediators and markers for neurogenesis, we postulated that cognitive tests that require continued adult neurogenesis (e.g., context discrimination) would be altered in Tg2576 AD mice compared to littermate controls and that RSG treatment would normalize this behavior. Consistent with enhanced acute BrdU uptake, a mitotic marker, Tg2576 performed superiorly to littermate controls in context discrimination and cranial irradiation significantly impaired both groups' ability to distinguish between similar contexts. Further, PPARy agonism improved wild type performance. Conversely, Tg2576 groups exhibited delayed context discrimination with rosiglitazone treatment suggesting an effect of PPARy agonism on subgranular zone mitosis as measured with BrdU incorporation. Interestingly, doublecortin quantitation of neuronal precursor cells and immature neurons incorporated into the granule cell layer of the hippocampus revealed no significant difference between untreated and rosiglitazone treated wild type and Tg2576 groups.

Thus, superior context discrimination in Tg2576 appears to be due to enhanced mitosis in the subgranular zone; however, the extent to which subsequent adult-born neuronal precursors and immature neurons contribute to the dentate gyrus circuit is still open. PPAR γ agonism appears to reduce BrdU incorporation with no net effect on doublecortin-positive neuronal precursors and immature neurons leaving it open still as to the mechanism underlying improved context discrimination in wildtype mice with rosiglitazone treatment.

Disclosures: D. Cortez: None. L.A. Denner: None. K.T. Dineley: None.

Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 303.29/X21

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Imaging the 18-kda translocator protein (a marker for neuroinflammation) in alcoholism

Authors: *R. E. TYLER¹, S. KIM¹, Y. JANG¹, C. WIERS¹, L. VENDRUSCOLO², G. KOOB², G.-J. WANG¹, J.-S. LIOW³, M. GUO¹, R. DAMADZIC¹, N. VOLKOW¹ ¹NIAAA, NIH, Bethesda, MD; ²NIDA, NIH, Baltimore, MD; ³NIMH, NIH, Bethesda, MD **Abstract:** Chronic alcohol consumption is associated with neurotoxicity and cognitive impairment. However, the neurobiological mechanism underlying alcohol's neurotoxic effects is poorly understood. We postulate that alcohol-induced neuroinflammation plays a role in alcohol's neurotoxic effects. Here, we determine if chronic alcohol use is associated with neuroinflammation. Towards this aim, we used [¹¹C]PBR28 positron emission tomography (PET) to measure the expression of the 18-kDa Translocator Protein (TSPO) in a rat model of alcoholism *in vivo*. TSPO is predominantly expressed in activated microglia and astrocytes in the brain, and therefore considered a biomarker of neuroinflammation.

10 rats (Wistar, male, mean weight= 547 ± 3) became alcohol dependent (AD) by passively inhaling alcohol sprayed in their cage for 14hrs/day during a 1.5-2.5 month period (blood alcohol levels averaged 196 mg/dL). [¹¹C]PBR28 brain PET scans were performed for each rat along with a control (n=10, mean weight= $568\pm6g$). After scanning, rats were sacrificed and brains harvested for [³H]PK-11195 autoradiography.

In vivo results show consistently lower whole brain uptake of [¹¹C]PBR28 in AD rats compared to controls. Autoradiography confirms these results, showing lower specific binding of [³H]PK-11195 in brains from AD rats. These results are consistent with our findings that human subjects with alcohol use disorder (AUD) express lower levels of TSPO in their brains. This may reflect alterations in the innate immune response of rats and humans exposed to chronic alcohol. Experiments to determine whether this alteration can recover after 1 month of abstinence are underway.

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Poster

303. Neuroinflammation: Animal Models

Location: Halls A-C

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Program#/Poster#: 303.30/X22

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Axonal conduction is a useful measurement to assess demyelination and remyelination in preclinical models and correlates with histology

Authors: *P. PANG, B. GIACOMOZZI, N. PANDE, W. M. SIDERS, C. E. PEDRAZA Sanofi, Framingham, MA

Abstract: The corpus callosum (CC) is the largest fiber bundle in the CNS connecting left and right cerebral hemispheres and mediating interhemispheric communication. In multiple sclerosis (MS) patients, the CC exhibits focal demyelinating lesions and axonal damage from early disease stages. CC damage may contribute to cognitive dysfunction in MS. Although several

immunomodulating therapies have been approved for MS patients, no therapies exist that target remyelination. **Objective:** Using systemic cuprizone and focal lysolecithin induced damage models of demyelination/remyelination, we assessed neuronal functional decline and recovery using axonal conductance and histological analysis of myelin. Methods: For the cuprizone model, mice were fed with cuprizone for 5 weeks to induce demyelination and then returned to normal diet for 8 weeks to allow remyelination to occur. The CC was dissected at several time points over the 13 week period for electrophysiological and histological analyses. One cohort of cuprizone fed animals was treated with Triiodothyronine (T3), a pro-myelinating agent, to determine whether enhancing remyelination affects axonal conductance. For the focal demyelination model, lysolecithin was injected directly into the CC, tissue was collected at various time points and myelin was analyzed. Results: In the cuprizone model, CC axonal conductance was correlated with histological analysis of demyelination during the 5 weeks of the cuprizone diet. Upon removal of cuprizone, CC axonal conductance slowly recovered as did axon remyelination. However, axonal conductance did not reach control values within the 13 week period examined. As expected, T3 treatment enhanced myelination and preserved neuronal function as reflected by axonal conductance. In the lysolecithin model, demyelination peaked at one week post-injection and was followed by spontaneous remyelination over 2-3 weeks. Axonal conductance is being assessed in this model following treatment with compounds known to enhance remyelination. Conclusions: Axonal conductance provides a direct measurement of neuronal function in vivo, reflecting changes in myelin integrity during demyelination and remyelination. This functional readout can be used in combination with histology to assess remyelination enhancing therapeutics for MS.

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Poster

304. Neuroinflammation in Neurodegenerative Diseases

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 304.01/X23

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Cognitive impairment induced by systemic inflammation in mice

Authors: *E. ANDRIAMBELOSON, B. HUYARD, E. POIRAUD, F. LAUGA, C. NEVEU, S. WAGNER NEUROFIT, ILLKIRCH, France

Abstract: Systemic inflammation is believed to play a role in ageing as well as in various dementia-related neurological disorders. Indeed, several lines of evidence indicate positive association between systemic inflammation and cognition and other aspects of behaviour in the

elderly and demented.

The present study was conducted to investigate the impact of systemic inflammation on cognitive function in mice. To this end, a single intraperitoneal administration of a non-septic dose of lipopolysaccharide (LPS) (0.25 mg/kg) was undertaken and the time course of cognitive decline was assessed. Reduced spontaneous alternation of mice in a T-maze was used as a measure of cognitive deficit.

Results showed that 1 week after the injection of LPS, a dramatic reduction in the alternation of mice in the T-maze was observed which suggests a cognitive deficit in LPS-treated mice. Although the cognitive deficit persisted for up to 3 weeks following LPS injection there was no change of the general health of mice. Furthermore, the locomotor behavior of LPS-mice as assessed by spontaneous free exploration in the open-field was comparable to that of naive mice. The treatment of LPS- treated mice with Memantine, a drug approved for cognitive enhancement in the elderly, fully restored their cognitive performance. Similar pattern of result was obtained with donepezil, another approved cognitive enhancer drug. Moreover, LPS-induced cognitive deficit was fully prevented by treatment of mice with anti-inflammatory drugs such as dexamethasone or ibuprofen, which confirmed the inflammatory-driven mechanism of LPS-induced cognitive relapse was observed after discontinuation of the anti-inflammatory therapy, which indicates the presence of an underlying chronic and persistent inflammation process.

Taken together, these above results suggest that low grade systemic inflammation is capable of mediating sustained cognitive impairment in mice.

Disclosures: E. Andriambeloson: None. B. Huyard: None. E. Poiraud: None. F. Lauga: None. C. Neveu: None. S. Wagner: None.

Poster

304. Neuroinflammation in Neurodegenerative Diseases

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Program#/Poster#: 304.02/X24

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Prior exposure to repeated LPS injections prevents further accumulation of hippocampal beta-amyloid

Authors: *J. L. PETERMAN, J. D. WHITE, A. HARDY, M. J. EIMBERBRINK, K. PAULHUS, M. THOMPSON, G. W. BOEHM, M. J. CHUMLEY Psychology, Texas Christian Univ., Fort Worth, TX

Abstract: Alzheimer's disease (AD) is characterized by the accumulation of beta-amyloid plaques and neurofibrillary tangles. Our laboratory has previously demonstrated that repeated
bouts of inflammation increase beta-amyloid within the hippocampus of C57BL6/J mice. Given the relationship between inflammation and AD pathology onset and progression, we sought to explore how a second series of lipopolysaccharide (LPS) injections will impact beta-amyloid levels. Male C57BL6/J mice were given daily injections of LPS (250µg/kg) or sterile saline for seven days. Fourteen days following the last injection, animals were administered another series of daily injections of LPS or sterile saline. Hippocampal tissue was collected and assayed to quantify total beta-amyloid levels. Results demonstrated that animals given one round of LPS injections had significantly more beta-amyloid than saline injected controls, whereas animals administered two rounds of LPS had intermediary levels of beta-amyloid. This effect appears to be driven by the reduced inflammatory response to the secondary exposure to LPS, following a fourteen-day recovery. Specifically, four hours following the first LPS injection of the second series, animals previously exposed to LPS had significantly less central and peripheral inflammation than animals exposed to LPS for the first time. The mechanism behind this diminished inflammatory response may not be due to endotoxin tolerance but, rather, result from antibody production against LPS or beta-amyloid, as IgM levels were increased in animals exposed to LPS in the first injection series. The specificity of these antibodies is currently being explored.

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Poster

304. Neuroinflammation in Neurodegenerative Diseases

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Support: NIH Grant 5R01NS092122

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Bluefield Project to Cure FTD

Title: Progranulin loss dysregulates splenic and peripheral blood immune cells populations and may contribute to neuroinflammation and neurodegeneration in early-onset frontotemporal dementia

Authors: *T. L. KUKAR¹, G. T. KANNARKAT², K. P. MACPHERSON², E. M. KLINE³, C. J. HOLLER¹, M. A. JOHNSON¹, M. G. TANSEY⁴ ¹Pharmacol., ²Physiol., ³Emory Univ., Atlanta, GA; ⁴Physiol., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Autosomal dominant mutations in the progranulin gene (GRN) cause a drastic reduction in progranulin (PGRN) and granulin levels contributing to the pathogenesis of familial frontotemporal degeneration (FTD), the most common form of early-onset dementia. Rare homozygous GRN mutations in humans cause neuronal ceroid lipofuscinosis (NCL), which shares neuropathological features with FTD-GRN patients, suggesting shared disruption of lysosomal pathways. Multiple studies have implicated PGRN as a key regulator of neuroinflammation and neurodegeneration. Genetic ablation of the GRN gene is associated with aberrant increases in phagocytosis and pro-inflammatory cytokine production in microglia. Further, global Grn KO and microglia-specific Grn KO mice have increased sensitivity to the neurotoxin MPTP. Most recently Grn deficiency was shown to promote circuit-specific synaptic pruning by microglia via complement activation. While intense focus has been placed on investigating the effect of GRN deficiency in microglia, we have focused our efforts on investigating the effects of GRN loss in all major subsets of peripheral immune cells based on the rationale that peripheral-central immune cross-talk is key for brain health and disruptions in these pathways may promote degeneration. We performed deep-immunophenotyping using flow cytometry of brain, spleen, and peripheral blood of *Grn* KO and WT mice ages 18-22 months. We found a generalized increase in cell numbers in the blood with an increased frequency of NK cells and Ly6C+ monocytes in blood as well as increased MHC-II expression on B cells and monocytes in blood; and a decreased proportion of non-CD4/CD8 T-cells made up CD3+ T cell populations. In addition, Grn KO mice displayed decreased NK cells, B cells, and macrophages in the spleen with decreased expression of MHC-II; and non-CD4/CD8 T-cells make up a larger proportion of CD3+ T cells in their spleen. These novel findings support a model for PGRN in regulation of peripheral immune cell populations and raise the interesting possibility that analysis of peripheral immune cells could shed light on the role of central-peripheral immune cross-talk in the pathogenesis of FTD patients with GRN mutations. Immunophenotyping analyses of immune populations in the brain of *Grn* KO vs WT mice is underway and will be compared with changes observed in the periphery [Funding provided by NIH/NINDS 5R01NS092122 and 5R01NS093362, and The Bluefield Project to Cure FTD].

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Poster

304. Neuroinflammation in Neurodegenerative Diseases

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 304.04/X26

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: The TRAIL DR5 receptor substantially contributes to amyloid beta-related neurotoxicity in the mouse

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Abstract: TRAIL, a proapoptotic cytokine belonging to the TNF superfamily, is a potent inducer of neuronal death in animal models of Alzheimer's disease (AD). TRAIL effects appear mediated by its death receptor 5 (DR5). Immunoneutralization of TRAIL results in restored cognition, as well as in blunted expression of inflammatory molecules in the AD 3x transgenic mouse brain. To better understand the role of TRAIL in neurodegeneration, in-vitro experiments were run to analyse the impact of treatment with amyloid-beta (AB), as compared to TRAIL, on survival of dispersed Trail- r-deficient mouse embryonal hippocampal cells. Next, the expression of TRAIL, TRAIL-R, as well as microglia and astrocytes markers were studied in the hippocampi of animals undergone treatment with fragment 1-42 of AB. Hippocampal cells from Trail-rdeficient mice showed significant resistance to death induction by both AB 1-42 or TRAIL, as compared to cells Trail-r-proficient mice. Immunofluorescence showed decreased activation of microglia (Iba-1) and astrocytes (GFAP) after stereotactical treatment of Trail-r-deficient mice with AB 1-42. Finally, western blot analysis of hippocampi from Trail-r-deficient mice treated with AB 1-42, showed decreased expression of either IL-1, iNOS and phosphorylated tau protein. The bulk of these results demonstrate that lack of TRAIL-R is associated with substantial decline of noxious effects of AB 1-42, providing genetic evidence that, during ABrelated neuroinflammation, TRAIL supplies a pivotal contribute to subsequent neurodegenerative processes. In conclusion, according to these results, it is plausible to hypothesize that the TRAIL system may be regarded as a potential target for innovative AD therapy.

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Poster

304. Neuroinflammation in Neurodegenerative Diseases

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CAPES (Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior) PhD Scholarship

FINEP (Financiadora de Estudos e Projetos)

FAPERJ (Fundação de Apoio a Pesquisa do Estado do Rio de Janeiro)

Title: A model of neonatal Zika virus infection for the study of neurodevelopmental disorders

Authors: *I. NEM DE OLIVEIRA SOUZA¹, P. DA SILVA FROST², R. LEÃO SILVA NERIS³, S. TEIXEIRA FERREIRA⁴, F. GUARINO DE FELICE⁴, A. THOMPSON DA POIAN⁴, I. ASSUNÇÃO-MIRANDA³, G. NEVES², C. PINTO FIGUEIREDO¹, J. ROSSAURO CLARKE¹

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Abstract: Zika virus (ZIKV) infection during pregnancy has been largely associated with microcephaly and neurodevelopmental disorders. The epidemic of ZIKV which started in 2015 was considered a public health emergency of international concern by the World Health Organization. However, how ZIKV affects the developing brain is poorly understood and most of the studies performed so far are based on infection performed in immunodeficient mice. This study aims to investigate whether early neonatal ZIKV infection leads to histological or behavioral changes related to brain function in immunocompetent, wild type mice throughout their lifespan. For such, both male and female Swiss mice were infected subcutaneously with ZIKV (strain isolated from a Brazilian patient) at post-natal day 3 (PND3) and early neonatal reflexes were assessed. ZIKV-infected group showed no impairment in reflex development compared to Mock mice. Viral replication in the brain and cytokine expression were assessed by qPCR. ZIKV successfully replicated in the brains of immunocompetent mice. Motor dysfunction were found in pre-weaning pups infected with ZIKV compared to Mock animals. ZIKV-infected group showed persistent decrease in body weight gain and by PND70 mortality rates were approximately 60% in ZIKV-infected group (0% in Mock group). Finally, histological and immunohistochemical analysis will be made in the animals to evaluate the organization of cortical structure and brain inflammation. We believe our study will help guide public health policies for ZIKV-exposed babies and children around the world, positively contributing to the field. Ethical approval: CEUA/CCS/UFRJ protocol no. 075/15.

Disclosures: I. Nem De Oliveira Souza: None. P. da Silva Frost: None. R. Leão Silva Neris: None. S. Teixeira Ferreira: None. F. Guarino De Felice: None. A. Thompson Da Poian: None. I. Assunção-Miranda: None. G. Neves: None. C. Pinto Figueiredo: None. J. Rossauro Clarke: None.

Poster

304. Neuroinflammation in Neurodegenerative Diseases

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CNPq

FAPERJ

FINEP

Title: Zika virus replicates in adult human and mouse brains, leading to brain inflammation, synapse loss and memory impairment in mice

Authors: *F. G. BARROS-ARAGÃO¹, C. P. FIGUEIREDO², R. L. S. NERIS², P. S. FROST², D. C. ZAMBERLAN³, L. M. S. ANTONIO², C. S. P. S. MELO², A. S. SOUZA², A. L. A. GUIMARÃES², J. M. SOUZA², S. ALVES-LEON², F. G. DE FELICE², J. R. CLARKE², I. B. ASSUNÇÃO-MIRANDA², A. DA POIAN², S. FERREIRA² ¹Biomed. Sci. Inst., Federal Univ. of Rio De Janeiro, Rio De Janeiro, Brazil; ²Federal Univ. of Rio de Janeiro, Rio de Janeiro, Brazil; ³Federal Univ. of Santa Maria, Santa Maria, Brazil

Abstract: Zika virus (ZIKV) infection during pregnancy is associated to severe neurological complications in newborns, including microcephaly and cortical calcifications. Initially considered a self-limited illness in adults, ZIKV infection was recently linked to various neurological manifestations, including acute myelitis, encephalitis and meningoencephalitis. Whereas several lines of evidence indicate that human neural precursor cells are affected by this infection, whether human adult neuronal tissue is susceptible to it remained to be determined. We aimed to determine the ability of ZIKV to replicate in human and mice adult brain tissue and its possible effects on memory and cognition. Initially, ex-vivo human adult cortical tissue was infected with 10⁷ plaque-forming units (PFU) of a ZIKV strain isolated from a Brazilian patient, or the corresponding volume of virus-free C6/36 cells conditioned medium (mock). Culture media were collected for determination of the number of infectious viral particles by plaque assay and levels of cytokines by ELISA, and slices were processed for quantification of viral RNA by qPCR. ZIKV RNA in tissue, infectious particles and interleukin-6 (IL-6) released to the medium increased in a time-dependent manner, demonstrating that ZIKV successfully replicates in adult human mature neural tissue. To study the consequences of ZIKV infection in the adult brain, we performed intracerebroventricular injections of ZIKV (10⁵ PFU) in young adult male Swiss mice. Brain infusion of ZIKV was not associated with increased mortality of mice, but caused a persistent reduction in body weight when compared to mock-infused mice. Brains of ZIKV-injected mice were collected at different time points for determination of viral RNA by qPCR. Increasing viral RNA levels were found, peaking at 6 days post-infection (dpi). Viral mRNA was higher in the hippocampus and frontal cortex when compared with others brain regions. No fluorojade-positive cells were found in the brain of ZIKV-infected mice, but we found an increase in Iba-1 (microglial) positive cells and a decrease in synaptophysin (synaptic marker) levels in their hippocampus. Brain IL-6 and tumor necrosis factor-α mRNA levels were markedly increased at 6 dpi. Using the novel object recognition task, memory impairment was detected as early as 1 dpi, persisting for up to 30 dpi. Our findings indicate that ZIKV replicates in human and mice adult brain in sites unrelated to neurogenesis, and that viral infection induces brain inflammation, synapse loss and memory impairment.

Disclosures: F.G. Barros-Aragão: None. C.P. Figueiredo: None. R.L.S. Neris: None. P.S. Frost: None. D.C. Zamberlan: None. L.M.S. Antonio: None. C.S.P.S. Melo: None. A.S. Souza: None. A.L.A. Guimarães: None. J.M. Souza: None. S. Alves-Leon: None. F.G. De Felice: None. J.R. Clarke: None. I.B. Assunção-Miranda: None. A. Da Poian: None. S. Ferreira: None.

Poster

304. Neuroinflammation in Neurodegenerative Diseases

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R21 NS081418-02

NIH R37 NS041435

Title: Intraventricular administration of TH2 cells into the CNS ameliorates the inhibitory effects of IFNg on remyelination

Authors: *M. J. ALEXIS, L. R. HERBST, H. STRAUSBURGER, K. MARTIN, L. KIRBY, P. CALABRESI

Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Multiple sclerosis is an immune mediated demyelinating disease of the CNS. One potential cause of remyelination failure is chronic inflammation, therefore understanding the molecular mechanisms by which CNS inflammation inhibits the oligodendrocyte precursor cells (OPC) is important. While effector T-cells of the TH1 and TH17 phenotype are understood to be critical for the pathogenesis of MS, effector T-cells of the TH2 phenotype may have an opposing role by promoting remyelination and repair especially in an inflammatory setting. To investigate the role of TH2 cells we utilized the GFAP/tTA; TRE/IFNg transgenic mouse line in which CNS expression of IFNg from GFAP positive astrocytes is achieved upon doxycycline withdrawal. In order to determine whether TH2 cells promote remyelination after cuprizone induced demyelination we first directly administered TH2 cells into the CNS via intraventricular microinjection. In three separate experiments remyelination was significantly improved in TH2 injected mice compared to PBS injected control animals (mean percent difference = 18.91; pvalue = 0.0107) as determined by black gold staining in corpora callosa tissue. Interestingly, the TH2 effect of improved remyelination was only detected in cuprizone fed mice induced to express IFNg in the CNS and there was no significant difference between TH2 and control mice in which IFNg expression was suppressed two weeks TH2 injection. After thorough examination at the one week time point we did observe a significant increase in remyelination from TH2 cells compared to control. The fact that TH2 cells can reproducibly overcome the strong and potent

inhibitory effect of IFNg and promote remyelination is notable. In addition, it was found that arginase 1, a marker for anti-inflammatory microglia, in corpora callosa tissue was elevated in the presence of TH2 cells. To investigate by what mechanism the TH2 cells produce this effect, we directly treated OPCs with TH2 cells, but observed no significant response. Due to the fact that TH2 cells do not seem to promote OPC differentiation directly but have an effect on microglia activation, we hypothesize the at effect on remyelination may be mediated indirectly through microglia. In order to further probe the question of how TH2 cells are mediating their effect, further work will involve an OPC differentiation assay to examine microglial secreted factors in response to TH2 supernatants on OPC differentiation.

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Poster

304. Neuroinflammation in Neurodegenerative Diseases

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NCAT 1TL1 TR001431

Roger D. Semerad Award for Research in the Neurosciences, TurnFirst Foundation

Title: Interleukin-4 induced protein 1 as a biomarker & treatment option in multiple sclerosis

Authors: *S. DAVIS¹, H. OFT¹, E. VIETSCH³, F. AMJAD⁴, A. WELLSTEIN³, J. HUANG² ²Dept. of Biol., ¹Georgetown Univ., Washington, DC; ³Lombardi Comprehensive Cancer Ctr., ⁴Dept. of Neurol., MedStar Georgetown Univ. Hosp., Washington, DC

Abstract: Analysis of interleukin-4 induced protein 1 in human peripheral blood mononuclear cells

Multiple sclerosis (MS) is a chronic inflammatory neurodegenerative disease of the central nervous system that, with an average age of onset of 34, afflicts over 2.3 million individuals worldwide during many of the most productive years of their lives. The pathogenesis of MS, though known to involve unresolved inflammation and autoimmune destruction of myelin, is poorly understood. Accurate biomarkers, which could predict disease progression, are yet to be identified and would provide valuable information to patients and their treating clinicians. Likewise, while disease modified drugs have been developed to combat the symptoms of relapsing remitting MS, none prevent progression to, or symptoms of, the progressive phase of MS.

Interkeukin-4 induced protein 1 (IL4I1), an endogenously secreted L-amino acid oxidase

expressed by immune cells is a promising candidate for both roles. Recently demonstrated by our lab, IL4I1 is upregulated during CNS remyelination, and functions to reduce inflammation to enable CNS remyelination in mice after experimental demyelinating injury. The goal of the present study is to evaluate the effect of IL4I1 on inflammatory cytokines in human peripheral blood mononuclear cells (PBMCs).

Our preliminary data show that addition of recombinant human IL4I1 to CD3/CD28-activated PBMCs modulates MS-related inflammatory cytokines expression, including *IFNg* and *IL17*. Moreover, we found that recombinant IL4I1 altered the expression of *TOB1*, an anti-proliferative protein whose levels are known to correlate with progression from clinically isolated syndrome (CIS) to clinically definitive MS. These results suggest that the immunomodulatory effects of IL4I1 in mouse-models of MS are also found in human populations, and that the mechanism of this effect may involve a previously identified correlate of MS development. These findings from healthy control PBMCs justifies our next steps to further explore IL4I1 in the clinical patient population.

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Poster

304. Neuroinflammation in Neurodegenerative Diseases

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01NS093057

Title: The development of inflammatory responses in degenerative thalamic injury after stroke

Authors: *Z. CAO, M. CHENG, A. FOLTZ, G. STEINBERG Neurosurg., Stanford Univ., Stanford, CA

Abstract: Background: After stroke, the primary infarct site can cause secondary degeneration in neighboring and connected regions. In particular, a secondary degenerative injury has been observed in the connected thalamus. This progressive secondary injury causes neurological dysfunction and impedes stroke recovery. Inflammation in the injured thalamus is considered to be a hallmark of thalamic injury. However, the dynamics of inflammatory activation during the development of thalamic injury is unclear. We aim to investigate the development of microglial/astrocyte activation in secondary thalamic injury after stroke. **Material and Methods**: Cortical ischemic stroke was generated by permanent occlusion of left middle cerebral artery in male C57BL6J mice (12-15 weeks). Time course analysis of Inflammatory responses were assessed by immunostaining in brain sections collected on day 1, 3, 7, 14 and 28 after stroke.

Immunostaining was performed with antibodies specific for activated microglia/macrophage (CD68) and astrocytes (GFAP). Images were captured by confocal imaging with z-stack and activated microglial/astrocytes at different time points were analyzed. Results: Primary infarction was observed mainly in the somatosensory cortex after stroke. Time course analysis of microglial/astrocyte activation revealed differential patterns after stroke. At the primary infarct site, activated microglia were initially detected on post-stroke day 1 surrounding the infarct, and gradually appeared in the infarct core after day 14. Activated astrocytes were detected at poststroke day 3, surrounding the infarct with a stellate morphology. Interestingly, astrocytes became densely packed and elongated after day 7. At the secondary thalamic injury site, activated microglia were sparsely detected as early as post-stroke day 1, while activated astrocytes were not observed until day 3. Clustered activated microglia/astrocytes with clear boundary were observed starting day 7, and gradually accumulated and became denser at later time points (day 14 and 28). Conclusion: Our study suggests that microglial and astrocyte activation exhibit differential patterns after stroke, in both timing of activation and morphology. Microglial activation occurs earlier than astrocyte activation in both the primary cortical infarct and the degenerative ipsilesional thalamus. Current studies investigate the subtypes of activated microglia and astrocytes in the thalamus after stroke. Identifying specific subtypes of astrocyte/microglia and their involvement in thalamic injury will provide potential targets for stroke recovery.

Disclosures: Z. Cao: None. M. Cheng: None. A. Foltz: None. G. Steinberg: None.

Poster

304. Neuroinflammation in Neurodegenerative Diseases

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 304.10/X32

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Conacyt

Title: Determination of endogenous antioxidant capacity and profile of proinflammatory cytokines in patients with metabolic syndrome: Risk for neurodegenerative diseases

Authors: *R. G. RESENDIZ GUTIERREZ, I. IBARRA VALVODINOS, B. UGALDE VILLANUEVA, M. SALGADO SALGADO, M. RAMOS GÓMEZ, N. HERNÁNDEZ CHAN, H. L. HERNÁNDEZ MONTIEL

Facultad de Medicina, Univ. Autónoma De Querétaro, Santiago de Querétaro, Mexico

Abstract: Introduction: Metabolic Syndrome (MS) has become a public health problem because of its association with increased risk of cardiovascular disease. The International Diabetes Federation (IDF) has reported that it least 25% of the world's population suffers from it.

Several studies have shown that a basis of its pathophysiological is insulin resistance (IR) the common with neurodegenerative diseases such as Alzheimer's and Parkinson's, sharing more risk factors than previously thought. The body has endogenous antioxidant systems that can be altered by the presence of MS. This pathophysiological connection suggests that the increase in the prevalence of MS will also increase the prevalence of neurodegenerative disorders, and metabolic problems are associated with oxidative stress and inflammatory status. **Objective**: To determine the profile of inflammatory markers (TNF α , IL-6, IL-1 β) and the endogenous antioxidant system (CAT, SOD and GSH) in patients with MS, which are common in neurodegenerative diseases. **Results**: It will show it share profile of endogenous antioxidant enzymes and proinflammatory cytokines in patients with MS, analyzing the similarity of this behavior with that of neurodegenerative disorders.

Disclosures: R.G. Resendiz Gutierrez: None. I. Ibarra Valvodinos: None. B. Ugalde Villanueva: None. M. Salgado Salgado: None. M. Ramos Gómez: None. N. Hernández Chan: None. H.L. Hernández Montiel: None.

Poster

304. Neuroinflammation in Neurodegenerative Diseases

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: RAS LR7/2007

Title: Up-regulation of the neurotrophin receptor p75NTR by valproic acid in human neuroblastoma cells

Authors: *S. DEDONI, M. C. OLIANAS, P. ONALI Univ. of Cagliari, Dept Biomed. Sci., Cagliari, Italy

Abstract: The anticonvulsant and mood-stabilizer drug valproic acid (VPA) has been shown to adversely affect neuronal differentiation, growth and survival through mechanisms still not completely understood. We have previously reported that exposure to VPA down-regulates the expression and signalling of the brain-derived neurotrophic factor (BDNF) receptor TrkB in neuronal cells, suggesting that VPA may impair central BDNF activity. In the present study we show that in human neuroblastoma cells VPA causes a marked up-regulation of p75NTR, a common neurotrophin receptor that belongs to the tumor necrosis factor receptor superfamily. Prolonged treatment with VPA (1.0 mM) and other histone deacetylase (HDAC) inhibitors, such as trichostatin A (TSA), sodium butyrate (NaBut), MS-275 and MC 1568, enhanced the protein levels of p75NTR in either undifferentiated or retinoic acid (RA)-differentiated SH-SY5Y neuroblastoma cells. VPA also significantly increased the expression of sortilin, a member of the

Vps 10p-domain receptor family which complexes with p75NTR to mediate neuronal cell death. Under similar experimental conditions, the levels of TrkA, the receptor of nerve growth factor (NGF), were not significantly affected by VPA in RA-differentiated SH-SY5Y cells. A marked increase of the cellular levels of p75NTR was also detected in LAN-1 human neuroblastoma cells following treatment with VPA. In these cells the enhanced p75NTR expression was associated with stimulation of apoptotic cell death induced by either VPA, NaBut and MS-275. Collectively, our data demonstrate that VPA and other HDAC inhibitors alter the balance between neurotrophin receptors by down-regulating TrkB and up-regulating the p75NTR/sortilin complex and suggest that this action may contribute to the deleterious effects of these agents on neuronal survival.

Disclosures: S. Dedoni: None. M.C. Olianas: None. P. Onali: None.

Poster

304. Neuroinflammation in Neurodegenerative Diseases

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Program#/Poster#: 304.12/Y1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Changes in the blood-brain barrier under toxic demyelination

Authors: *J. SHELESTAK¹, R. CUKELJ¹, N. K. SINGHAL², J. MCDONOUGH¹, R. CLEMENTS³

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Abstract: The cuprizone animal model is widely used to study toxic demyelination and subsequent remyelination in the central nervous system. A copper chelator, cuprizone affects oligodendrocytes, leading to their degeneration and the loss of myelin sheathing. Studies suggest the cuprizone model appears to maintain an intact blood-brain barrier throughout the treatment. In the present study, we aim to assess the integrity of the blood brain barrier after 6 weeks of cuprizone administration followed by 2 weeks without to allow for remyelination. Mice were imaged using MRI every two weeks starting on week 0 of administration. Measurements of demyelination and ventricle enlargement were taken with T1, T2, and diffusion weighted protocols. A portion of mice were intraperitoneally injected with Evans Blue 3 days prior to sacrifice in order to stain the vasculature as well as investigate any potential areas of integrity breakdown in the BBB. Immunohistochemistry was utilized to stain brain sections after sacrifice. The tissue sections were stained for neurons, myelin, astrocytes, microvessels, oligodendrocytes, immune cells, as well as aspects of the extracellular matrix to assess the integrity of the bloodbrain barrier. Cuprizone treatment caused significant weight loss, as well as a measurable change in ventricle size seen after 6 weeks of treatment. There was a significant increase in gliosis, as well as morphological changes indicative of activation. BBB integrity was assessed using

computational image analysis to measure changes in vessel size, astrocyte ensheathment, and degeneration.

Disclosures: J. Shelestak: None. R. Cukelj: None. N.K. Singhal: None. J. McDonough: None. R. Clements: None.

Poster

304. Neuroinflammation in Neurodegenerative Diseases

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Program#/Poster#: 304.13/Y2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CONACYT

Title: Evaluation of the electrophysiological effect in patients with diabetic polyneuropathy treated with thiamine pyrophosphate

Authors: *I. IBARRA VALDOVINOS, B. UGALDE VILLANUEVA, R. G. RESENDIZ GUTIERREZ, M. SALGADO SALGADO, M. RAMOS GÓMEZ, N. G. HERNANDEZ CHAN, H. L. HERNANDEZ MONTIEL

Facultad de Medicina, Univ. Autonoma De Queretaro, Santiago de Querétaro, Mexico

Abstract: Introduction: Diabetes mellitus type 2 (DM2) is one of the diseases of high prevalence in the Mexican population. Included in the microvascular complications associated with this pathology are diabetic polyneuropathy (DPN), which affects the quality of life of diabetic patients. Currently, treatment for DM2 focuses on optimizing adequate glycemic control with oral hypoglycemic agents and insulin to prevent or delay the development of DPN. When the patient develops DPN, the treatment focuses on providing relief of symptoms; however, there is little evidence that any of the drugs used modifies the underlying pathophysiology of DPN. **Objective**. Analyze the effect of thiamine pyrophosphate (TPP) on the nerve conduction velocity of lower limbs of patients with DPN. Material and methods. We studied 26 patients with DPN, organized into 2 study groups: Group I (n=14) patients with 1 to 10 years of evolution of diabetes mellitus type 2 (DM2) and Group II (n=12), with 10 to 20 years of DM2. Both groups were treated with TPP (10 mgrs/kg body weight) for intramuscular injection once a week for three months. The effect of the therapy was evaluated by the Michigan Neuropathy Screening Instrument (MNSI), biochemical analysis and nerve conduction tests, before and after treatment. Results: After 3 months of treatment, the patients of Group I showed improvement in the speed of conduction of the lower limbs while Group II patients had a lower response of improvement with treatment. For both groups, prolonged latencies, reduction in amplitude of the potential and increase of its duration were determined. Conclusions. The results show a marked improvement

in the nerve conduction velocity of the lower limbs in Group I, with a shorter evolution time of the disease.

Disclosures: I. Ibarra Valdovinos: None. B. Ugalde Villanueva: None. R.G. Resendiz Gutierrez: None. M. Salgado Salgado: None. M. Ramos Gómez: None. N.G. Hernandez Chan: None. H.L. Hernandez Montiel: None.

Poster

304. Neuroinflammation in Neurodegenerative Diseases

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Program#/Poster#: 304.14/Y3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: DTRA Grant #CB5730

Title: Knockout mice with inhibited IL-1 and TNF signaling pathways have significantly reduced seizure activity, morbidity and mortality following exposure to soman

Authors: ***E. A. JOHNSON**¹, J. F. IRWIN¹, K. LAITIPAYA¹, J. K. CHANDLER¹, D. D. PALMER¹, T. M. FERRARA-BOWENS¹, C. L. HONNOLD², M. D. WEGNER³ ¹Pharmacol., ²Comparative Pathology, US Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD; ³Armed Forces Res. Inst. of Med. Sci., Bangkok, Thailand

Abstract: Exposure to acetylcholinesterase inhibitors such as soman (GD) can initiate status epilepticus (SE) that can lead to progressive brain damage and behavioral impairment. Current treatments targeting the GABA system can ameliorate GD-induced SE, though treatment effectiveness diminishes rapidly following exposure. Therefore, new treatment strategies that do not rely on the GABA system are needed to reduce seizure activity, tissue loss and cognitive deficits. One strategy involves modulation of the pro-neuroinflammatory response, a prominent feature in GD-induced brain injury, which can exacerbate seizure susceptibility, generation and frequency. We have previously shown that reducing interleukin -1 receptor 1 (IL-1R1) or tumor necrosis factor receptor 1A (TNFR1A) signaling can moderately reduce many deleterious physiological effects after SE. This study focused on inhibition of both IL-1 and TNFα signaling as an anticonvulsant and neuroprotective strategy. Using an IL-1R1/ TNFR1A double knockout (KO) mouse, seizure profiles, neuropathology, mortality, cognitive performance and other relevant physiological responses were compared to a wild type background mouse strain. Inhibiting both IL-1 and TNF α signaling pathways had a synergistic effect on reducing deleterious physiological effects of GD-induced SE such as neuropathology, seizure activity and mortality. These results show that IL-1R1/TNFR1A double KO mice are remarkably resilient to the effects of GD exposure to include reduced seizure incidence, morbidity, mortality and cognitive deficit. These data suggest that inhibition of specific pro-inflammatory pathways may

be a viable addition to standard therapies to treat GD-induced SE.

The views expressed in this talk are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. These studies were funded by the Defense Threat Reduction Agency (DTRA).

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Poster

304. Neuroinflammation in Neurodegenerative Diseases

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Title: Exosomally secreted miR-146a dysregulates brain energy metabolism during neuroinflammation

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Abstract: Mitochondrial dysfunction is associated with the aging process and with the pathogenesis of a variety of disorders such as metabolic syndrome and neurodegenerative diseases including Alzheimer's disease (AD). Interestingly, miRNAs can modulate mitochondrial activity, and a discrete miR set has recently been identified in mitochondria of different species and cell types (mitomiRs). Among them, miR-146a is involved in important cell

functions including inflammation, immune responses, invasion, metastasis and cell death. The impact of miR-146a on mitochondrial function and glucose metabolism has not been previously analyzed. Since a single microRNA (miRNA) is capable of deregulating many genes' function, we hypothesized that increased miR-146a by inflammatory stimuli including LPS, TNF- α and Aβ may influence mitochondrial function and glucose metabolism, especially glycolysis through regulating multiple target proteins involved in these processes in AD. We found that the level of miR-146a was significantly associated with Braak and Braak (B&B) stage not only in temporal cortex but also in frontal cortex and cerebellum of AD patients. We also found that increased miR-146a levels by inflammatory signaling may not be limited to the cellular level but also secreted exosomes in multiple brain cell types, including neurons, glial cells, and brain endothelial cells. Through an *in silico* bioinformatics analysis study, we identified four putative target genes related to mitochondrial electron transport chain as well as two glycolysis related genes. Furthermore, overexpression of miR-146a significantly decreased ATP production, spare capacity, and maximum respiration as measured in rat primary mixed glial cells as measured by an XF^e96 extracellular flux analyzer. MiR-146a also significantly decreased glycolysis and glycolytic reserve. The overexpression of miR-146a in HT22 and C8B4 also reduced level of proteins involved in electron transport chain complex II (SDHC) and glycolysis (PGK1). Our results indicate that the increased level of miR-146a in brain cells may play a direct role in controlling mitochondrial function and energy metabolism by regulating mitochondrial protein expression as well as proteins regulating glycolysis. The modulation of miR-146a could thus mediate the loss of mitochondrial integrity and function in various brain cells, inducing or contributing to the brain's inflammatory response in diseases such as AD and other neuroinflammatory disorders.

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Poster

304. Neuroinflammation in Neurodegenerative Diseases

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Efficacy of subcutaneous route of administration of gm1 ganglioside in the r62 mouse model of huntington disease

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Abstract: Huntington's Disease (HD) affects nearly 30,000 people in the United States. This disease is an inherited disease characterized by the progressive degeneration of nerve cells within

the nervous system. The disease was first documented in 1872 by George Huntington. Since then, we have come to learn that the symptoms of HD usually appear between the ages of 30 and 50. These symptoms include chorea (involuntary movements), impared cognitive function, personality changes, progressive weight loss and eventually death. There is no current cure for HD, just palliative treatment. In the later stages of the disease, the patient will be unable to walk, talk or care for themselves.

One possible treatment that has been looked at as of recently is the use of the substance GM1. GM1 is a ganglioside and resides within the outer leaflet of our cells. It has been shown to have neuroprotective effects and is important in the development of our neurons In neurodegenerative diseases like HD, Parkinson's Disease, and Alzheimer's Disease, GM1 is significantly reduced within the cells. Recent studies have used GM1 as a treatment for HD and have seen positive results. In this study, we have looked at the efficacy of subcutaneous administration of GM1 in the R6/2 mouse model of HD. Previous studies have only looked at intraventricular administration of GM1. We have chosen subcutaneous administration due to the fact that it is much more practical. We hypothesize that the administration of GM1 will help to reverse the effects the disease has on behavior and cognition.

Support for this study was provided by the Neuroscience program, the College of Medicine, Department of chemistry and biochemistry, and the Field Neurosciences Institute. Neuroscience at CMU

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Poster

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CNPq Grant 300552/2013-9

CAPES

Title: Dimethyl fumarate accelerates axonal regeneration following peripheral nerve axotomy by modifying macrophage response post-injury

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Abstract: Following peripheral nerve injury, distal stump degeneration takes place, allowing tissue clearance, and remodeling, mostly driven by Schwann cells and macrophages. Dimethyl fumarate (DMF) is a fumaric acid ester that presents antioxidant properties, anti-inflammatory, and mmunomodulatory activity, shifting the overall response towards an anti-inflammatory (Th2) profile. However, DMF activity on peripheral nerve regeneration is poorly studied. Thus, unilateral sciatic nerve crushing in C57BL/6J mice was followed by DMF (100mg/Kg, orally) or vehicle (methylcellulose) daily treatment, 3 days after lesion (3 dal, when there is a high influx of macrophages in the nerve) up to 28 dal (when regeneration is established). Motor recovery was recorded daily (3 - 28 dal), using a computerized walking track test system (CatWalk system). Animals were euthanized at 7, 14 and 28 dal (n=5/group/time point) and nerves were analyzed regarding the expression of neurofilaments (axonal marker), growth associated protein 43 (GAP-43, regrowing axons) and Iba1 (macrophage marker). Intact nerves were used as the baseline control. Under DMF treatment, macrophage immunolabeling peaked at 7 dal (2,420%, p<0.001), being kept constant up to 14 dal and then decreasing, while in the control group it peaked at 14 dal (2,400%, p<0.001) and then dropped. In both groups, axon labeling rose at 7 dal (134%, p<0.05, DMF; 122%, ns, control), possibly as the sum of degenerating and growing axons. However, while axon labeling progressively decreased in the DMF group, it abruptly diminished in the control at 14 dal (67%, p<0.05), possibly due to a massive macrophage infiltration. Neurofilament baseline levels were restored at 28 dal in both groups. GAP-43 kinetics in the control group resembled that of neurofilament, with smaller expression at 14 dal (59%), however, peaking at 28 dal (384%, p<0.001). On the contrary, in DMF group it rose at 14 dal (477%, p<0.001), decreasing at 28 dal. Motor recovery was fully reestablished in both groups at 28 dal, with no significant difference between then. Overall, the present data indicates that DMF accelerates axon regeneration, besides extending macrophage permanence in the regenerating nerve microenvironment, possibly in a Th2, anti-inflammatory fashion.

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Poster

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Title: Neurotoxic reactive astrocytes in neurodegenerative disease

Authors: *S. A. LIDDELOW¹, T. PETERSON², R. N. ELDANAF¹, A. M. MÜNCH¹, K. A. GUTTENPLAN¹, A. D. HUBERMAN³, M. S. BUCKWALTER⁴, B. A. BARRES¹ ¹Neurobio., ²Neurol. and Neurolog. Sci., ³Dept. of Neurobio., ⁴Neurol., Stanford Univ., Stanford, CA

Abstract: Although reactive astrocytes are rapidly generated following brain injuries and neurodegenerative and neuroinflammatory diseases, their role in trauma and disease states is not well understood. Previously we distinguished two reactive astrocyte subclasses based on the kind of inducing injury. We named these classes "A1" and "A2". Based on their gene profiles we hypothesized that they were harmful and helpful respectively. We have shown that the harmful A1 reactive astrocytes are induced by classically-activated neuroinflammatory microglia. Specifically, we found that activated microglia induce A1s by secreting II-1 α , TNF α , and C1q, and that these factors together are necessary and sufficient to induce A1s both in vitro and in vivo. A1s have little ability to promote neuronal survival, outgrowth, synaptogenesis or phagocytosis and instead are powerfully toxic to neurons and oligodendrocytes. We further showed that A1s are present in human Alzheimer's disease, Huntington Disease, Amyotrophic Lateral Sclerosis, and Multiple Sclerosis, and that death of axotomized CNS neurons is prevented when A1 formation is blocked with neutralizing antibodies to Il-1a, TNFa, and C1q. We now show the role of A1 neurotoxic reactive astrocytes in the context of neurodegeneration in both acute (ischemia) and chronic (glaucoma) mouse models. Taken together our findings strongly suggest that A1s drive death of neurons in neurodegenerative disorders, and point the way forward for developing new treatments for these diseases.

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Poster

304. Neuroinflammation in Neurodegenerative Diseases

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Effect of intra-nigral injection of endotoxin on neuronal homeostasis in the hippocampus

Authors: B. DARWISH¹, F. CHAMAA², J. ISMAIL², E. AL-CHAER², N. SAADE², *W. ABOU-KHEIR²

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Abstract: Background Neurogenesis in the hippocampus has been shown to be prone to alterations in response to a variety of physiological and pathological factors, one of which is neural inflammation. Inflammation has been implicated in changes observed in neurodegenerative diseases such as Parkinson's and Alzheimer's. Previous studies are in favor of the hypothesis that overt or discrete brain inflammation can result in reduced neurogenesis and/or neuronal death associated with cognitive decline and other functional disorders. We aim to show that mild inflammation induced by unilateral Endotoxin injection in the substantia nigra (SN) and ventral tegmental area (VTA) can alter neurogenesis in the Dentate Gyrus (DG) of the hippocampus. Methods Adult Sprague Dawley female rats (250-300g) received stereotaxic unilateral injection of Endotoxin (2ug/2ul saline) or sterile saline (2ul) in the right SN. Rats then received 3 consecutive intraperitoneal injections of 5'-Bromo-2'-deoxyuridine (BrdU) (66mg/kg/injection) separated by 2 hour intervals on the day of the surgery. The rats were perfused on day 3, 6, or 9. Behavioral observations included cylinder test, y-maze and Rotarod tests. The fractionator method was used together with confocal immunofluorescent analysis to probe for BrdU and NeuN positive cells in the left and right DG. In addition, Tyrosine hydroxylase (TH) immunostaining was performed and TH cells were quantified in the right and left SN. Results The cylinder test showed significant ipsilateral bias (right side) in the rats that received ET injection compared to the sham and Naïve rats at days 2, 3 and 6, with the most prominent effect at day 3 (p<0.0049). The Rotarod test showed significant decrease in the latency to fall for ET injected rats at day 2 (p<0.0059), which was partially reversed on day 3 and back to normal by day 6. ET injection in the SN led to a significant decrease of BrdU positive cells (p<0.05) in the DG at day 3 compared to sham and there was no significant difference between sham and naïve rats. This was followed by a trend decrease in neurogenesis on days 6 and 9 post injection. Interestingly, there was no prominent difference between left and right DG and the decrease appears to be a bilateral decrease. Moreover, at day3 post injection, SN showed a 35% decrease of TH in the right side compared to the left and this decrease was no longer present at day 9. Conclusion This study shows that neurogenesis is altered following localized inflammation and represents one process that can be further investigated in neurodegenerative diseases.

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Poster

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: In vivo analysis of calcium-initiated axon degeneration in an animal model of MS

Authors: *M. M. WESOLOWSKI¹, M. A. SCHUMACHER¹, K. KNELLES¹, I. KLEIN¹, M. KERSCHENSTEINER^{1,2}

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Abstract: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system and a major cause of neurological disability in young adults. There is robust evidence showing that immune-mediated axon damage is the major cause for progression of permanent neurological deficits. Using in vivo imaging in a mouse model of MS we identified focal axonal degeneration as a correlate of inflammatory axon loss. It is characterized as a sequential process, beginning with focal swellings and progressing to multi-focal axon fragmentation. This process is observed in axons with intact myelin sheaths and its early stages can recover spontaneously. We were able to show that elevated calcium levels influence the axonal fate to degenerate in EAE lesions, however the degenerative process triggered via elevated calcium is not understood. In this work, we investigated which mechanism is involved in the neurodegeneration in experimental autoimmune encephalomyelitis (EAE), an animal model of MS. We assessed the effect of delayed Wallerian degeneration genotypes (Δ NLS-Wlds and Sarm1 knock-out), which did not result in a reduction of clinical disability or reduction of axonal fragmentation in acute or chronic lesions in vivo. The inhibition of Calpain, a calcium-activated protease, reduces axonal fragmentation in acute lesions in vivo. These results suggest a mechanism of axonal destruction which appears to be distinct from Wallerian degeneration and opens possibilities for neuroprotective therapies targeting the calcium-calpain axis.

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Poster

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Alterations of neurochemical, inflammatory and behavioral parameters in hyperphenylalaninemic female rats

Authors: *P. F. SCHUCK¹, J. F. AGOSTINI¹, F. MALGARIN¹, M. L. GOMES¹, B. K. FERREIRA², M. L. GARCEZ³, M. MICHELS⁴, F. S. VUOLO⁴, F. DAL-PIZZOL⁴, J. BUDNI³, E. L. STRECK⁵, G. C. FERREIRA²

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Abstract: Phenylketonuria (PKU) is an autosomal recessive inborn error of amino acid Lphenylalanine (Phe) metabolism caused by deficiency of phenylalanine hydroxylase (PAH) activity. Biochemically, it is characterized by hyperphenylalaninemia (HPA). Clinically, patients present with psychomotor impairment and severe intellectual disability. The pathogenesis of brain alterations related to PKU is based on HPA neurotoxicity, which is not completely understood. In this context, the aim of this study was to evaluate neurochemical, inflammatory and behavioral parameters in rats submitted to an experimental HPA model. For this, five-dayold female Wistar rats received 2 daily subcutaneous injections of Phe (5.2 µmol/g; 12 hours interval between administrations) and daily subcutaneous administration of pchlorophenylalanine (0.9 µmol/g), a PAH inhibitor, from the 5th to the 30th day of life. Control group received saline solution under the same conditions. Twenty-four hours after the last administration, behavioral tasks (open field and radial maze) were evaluated. Immediately after, animals were euthanized and cerebral cortex, hippocampus and striatum were dissected and used for the determination of synaptophysin immunocontent and brain derived neurotrophic factor (BDNF), interleukin (IL)-1beta (IL-1 β), IL-6, IL-10 and tumoral necrosis factor alpha (TNF α) levels. HPA caused cognitive deficit in animals, as seen in the open field test, without any alteration in the radial maze. HPA also decreased synaptophysin immunocontent, a marker of synaptic integrity, and increased levels of the IL-6 (proinflammatory interleukin) and IL-10

(anti-inflammatory interleukin) only in cerebral cortex of hyperphenylalaninemic animals. On the other hand, IL-1 β , TNF α and BDNF levels were not altered in HPA animals. The present study demonstrated that chronic HPA causes cognitive damage, which could be subsequent to synaptic changes and neuroinflammation. These results may contribute to the understanding of the pathological mechanisms of cognitive damage observed in phenylketonuric patients.

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Poster

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ARSEP

ANR "NeuroInflamDyn"

Title: Dynamics of spinal cord axonal degeneration and innate immune cell phenotypes in multiple sclerosis

Authors: *C. CARAVAGNA¹, A. JAOUËN¹, K. K. FENRICH², S. JEGO-DESPLAT³, M. MALISSEN⁴, G. ROUGON¹, F. DEBARBIEUX¹

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Abstract: Multiple sclerosis (MS) is dependent on an interplay between the adaptive and innate immune cell subsets. Activation of resident microglia together with infiltrating leucocytes are key elements in the myelin and axons' degradation during disease initiation and progression, responsible for clinical symptoms. However, the precise interdependence between these elements remains unknown. We developed a mouse preclinical model of MS in order to dynamically analyze the relationships between inflammation, neurodegeneration and disease progression. We induced experimental autoimmune encephalomyelitis (EAE) in Thy1-CFP//LysM-EGFP//CD11c-EYFP transgenic reporter mice¹. Animals carrying a spinal glass window² were subjected to recurrent two photon imaging and the *in vivo* spatio-temporal distribution as well as the morphology of LysM- and CD11c-expressing cells were correlated to the observed axonal

degeneration, weight losses and clinical scores. We validated a 19 parameters flow cytometry panel³ compatible with the simultaneous detection of the two EGFP and EYFP fluorescent reporter proteins. LysM-expressing cells were identified as infiltrating monocytes and neutrophils and CD11c-expressing cells as microglia. Our data define a precise pathology timeline, with primary infiltration of neutrophils and monocytes through the meningeal compartment, secondary *in situ* differentiation of monocytes into monocyte-derived dendritic cells, and finally recruitment of microglial cells in the entire spinal cord followed by migration to plaques. The occurrence of monocyte-derived dendritic cell subset and the diminution of EGFP-expressing cells characterized a stabilization of axon degradation and clinical signs and the predominance of microglia to process axon debris in plaques. Our technical set up and animal model should help identifying targets for efficient anti-inflammatory drugs as well as the opportunity windows for their administration.¹ Fenrich et al., 2013, J Vis Exp, (82):e50826² Fenrich et al., 2012, J Physiol, 590(16):3665-75³ Tamoutounour et al., 2013, Immunity, 39(5):925-38

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Poster

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Support: NIH Grant 2R15NS060117-02

Title: Modification of gut microbiota through Brassicaceae and Asteraceae plants (Kale, Arugula, Dandelion) modulate neurodegeneration and memory in diet-induced obese prediabetic C57BL/6 mice

Authors: *B. TENG¹, D. FOSTER², D. G. HICKS¹, A. A. OYETUNDE¹, N. OCFEMIAA¹, G. E. FLORES¹, L. R. BANNER¹ ¹Biol., ²California State Univ. Northridge, Northridge, CA

Abstract: World obesity rates have increased in the last few decades. An increase in high fat consumption along with a decrease in physical activity is the primary driver leading the obesity epidemic. Diet-induced obesity predisposes individuals to type-2 diabetes and contributes to complications including those of the nervous system and the immune system. Diabetes is associated with an elevated risk for neurodegeneration and dementia and changes in hippocampal plasticity. Studies have shown that neurodegeneration caused by diabetes is related in part, to elevated levels of inflammatory cytokines involved in brains of animals fed a high fat diet

(HFD). In addition, it is well documented that a high fat diet causes changes in gut microbiota. The dysbiosis of gut microbiota triggers a pro-inflammatory response and may also disrupt neuronal signaling. While diabetes caused by diet-induced obesity is largely preventable by reducing high fat intake, individuals often find it difficult to radically change unhealthy aspects of their diet. Instead, we are proposing to alter the imbalance of gut microbiota through supplementation of kale, arugula (f. Brassicaceae) and dandelion (f. Asteraceae) plants to mediate the inflammatory response, potentially taper neurodegeneration, and thus stymie cognitive decline.

To address this issue, C57BL/6 mice were fed either a control or high-fat diet (HFD)(60% fat) for 18 weeks until the high-fat group reached a pre-diabetic stage. After 18 weeks, mice on a HFD weighed significantly more than the control mice, displayed elevated blood glucose levels, and showed deficits in spatial learning. During weeks 18 to 40, the diets of all the mice were supplemented daily with 1.0 gram of kale, arugula, or dandelion. Consumption of the greens had no effect on the weights of either groups. During the 22-weeks when the mice were fed their supplemental kale, arugula, or dandelion diets, the mice were subjected to multiple repetitions of the Morris Water Maze, Barnes Maze, and Nonconditioned Social Discrimination Procedure, to probe for changes in their memory. Fecal samples before and after supplementation of Brassicaceae and Asteraceae plants were collected and changes in gut microflora were characterized by 16S rRNA gene sequencing for bacterial identification using the Illumina MiSeq platform. Analyses are ongoing; the brains of the subject animals will also be analyzed for inflammatory, neuronal markers and changes in dendritic spine morphology.

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Poster

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Title: Chronic inhibition of HDACs for the treatment of neurodegenerative diseases

Authors: M. S. ALAM¹, *K. HALDAR²

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Abstract: Histone deacetylase inhibitors (HDACi) are approved for treating rare cancers and are of great interest as potential therapies for both mono- and polygenetic neurological disorders.

However, the toxicity and poor permeability of HDACi across the blood-brain barrier (BBB) have been major barriers for their use in neurological disorders. We developed a triple combination formulation (TCF) comprising the FDA-approved HDACi vorinostat, the caging agent 2-hydroxypropyl-beta-cyclodextrin (HPBCD), and polyethylene glycol (PEG) for treating a mouse model of Niemann-Pick type C (NPC) disease a difficult-to-treat, neurodegenerative disorder. Vorinostat alone showed activity in cultured primary cells derived from Npc1^{nmf164} mice but did not improve animal survival. However, low-dose, once-weekly intraperitoneal injections of the TCF containing vorinostat increased histone acetylation in the mouse brain, preserved neurites and Purkinje cells, delayed symptoms of neurodegeneration, and increased mouse life span by over 100%. We demonstrate that the TCF boosted the ability of HDACi to cross the blood-brain barrier with no metabolic toxicity or neuroinflammation in the brain, even when used long term. TCF enable dose reduction, an additional challenge in HDACi therapy. Mechanistically, TCF enables epigenetic resetting and rescue the function of the misfolded NPC1 protein in the brain. Data will also be presented on adapting TCF therapy for multiple neurological disorders.

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Poster

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Quillen College of Medicine at ETSU

Title: Blood vitronectin robustly induces LIF and pro-inflammatory IL-6 expression *In vitro* and in the mouse brain through integrin-FAK signaling

Authors: *M. P. KEASEY¹, C. JIA², L. PIMENTEL², R. SANTE², C. LOVINS², T. HAGG³ ¹East Tennessee State Univ., Johnson City, TN; ²Dept. of Biomed. Sci., East Tennessee State Univ., Mountain Home, TN; ³Dept. of Biomed. Sci., East Tennessee State Univ., Johnson City, TN Abstract: We have identified a novel function for vitronectin (VTN), an abundant blood protein, as a rapid and potent inducer of leukemia inhibitory factor (LIF) and pro-inflammatory interleukin-6 (IL-6) both in vivo and in vitro. In contrast to VTN, laminin-111, fibrinogen, fibronectin or collagen-1 had no effect on either cytokine after 4 hours in cultured C6 astroglioma cells. Also in vitro, plasma from VTN -/- mice was a less effective inducer of LIF and IL-6 relative to wild type plasma. In adult mice, intra-striatal injection of VTN but not heatdenatured VTN significantly increased LIF and IL-6. Conversely, VTN -/- mice had reduced LIF and IL-6 in response to intracerebral haemorrhage relative to wild type mice. In vitro, VTN induction of LIF and IL-6 were suppressed by RGD-integrin blocking peptides, including one specific for $\alpha v\beta 3$ integrin. Pharmacological blockade of the urokinase plasminogen activator receptor (uPAR), which can bind VTN, also reduced LIF and IL-6 induction by VTN. Further, pharmacological blockade of focal adhesion kinase (FAK) activation and siRNA against FAK, but not the related PYK2, suppressed both LIF and IL-6 in endothelial cells. Overexpression of mutated FAK (Y397F) in cultured cells reduced mechanical injury-mediated LIF and IL-6 induction. We propose that VTN leakage from the blood is an important mediator of cytokine and inflammatory signalling. Integrin-FAK activation may represent a novel target for the regulation of both LIF and IL-6 with implications for the stem cell and inflammation fields.

Disclosures: M.P. Keasey: None. C. Jia: None. L. Pimentel: None. R. Sante: None. C. Lovins: None. T. Hagg: None.

Poster

304. Neuroinflammation in Neurodegenerative Diseases

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 304.26/Y15

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIAAA AA019767

NIAAA AA11605

NIAAA AA007573

NIAAA AA021040

Title: Ethanol activates 'death receptor' signaling to cause neurodegeneration

Authors: *L. G. COLEMAN, JR¹, J. Y. ZOU², F. T. CREWS³

¹Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; ²Univ. North Carolina, Chapel Hill, Chapel Hill, NC; ³Prof Pharmacol & Psychiat, Skipper Bowles Ctr. Alcohol, Chapel Hill, NC

Abstract: Alcohol addiction is associated with neurodegeneration in the hippocampus and cortex, which is associated with functional deficits. We investigated the role of TNF receptor family, i.e. the Death Receptor Family, in the pathology of alcohol-induced neurodegeneration. Ethanol, both in vivo and in vitro caused activation of the death receptor pathway. The TNFrelated apoptosis-inducing ligand (TRAIL), in particular was activated by ethanol. TRAIL binds two receptors, DR4 and DR5, resulting in caspase-dependent cell death. TRAIL was increased in brain by nearly two-fold 24 hours after acute ethanol in vivo (6g/kg, **p<0.01). TRAIL was also increased in the mouse plasma by 4-fold 12 hours after ethanol. In BV2 microglia, TRAIL was also increased 2.3-fold (**p<0.01) and SH-SY5Y neurons (1.5-fold, *p<0.05) at 24 hours after ethanol (100mM). Ethanol caused a 140% increase in DR5 expression in BV2 microglia at 12 hours after ethanol. Immunofluorescent co-labeling found the majority of TRAIL was present in neurons in vivo. In SH-SY5Y neuronal cultures, DR4 and DR5 were increased by 125% and 155% respectively 12 hours after ethanol treatment. Interestingly, neurons secreted TRAIL into the media in response to ethanol, suggesting autocrine-paracrine signaling. In order to assess the effect of ethanol on TRAIL-mediated neurodegeneration, hippocampal-entorrhinal cortex (HEC) slice culture was employed. Ethanol (100mM) caused increased TRAIL in HEC at 48 hours (1.5fold, p<0.05). DR4 and DR5 were also increased after 48 hours of ethanol exposure (150%). Pre-treatment of slices with ethanol (48h) followed by addition of TRAIL resulted in a robust enhancement of neurodegeneration, evidenced by a near 3-fold increase in propidium iodine uptake greater than either TRAIL or ethanol alone (*p<0.05). Thus, ethanol increases TRAIL signaling and enhances TRAIL mediated neurodegeneration. TRAIL mediated neurodegeneration represents a novel mechanism of ethanol-induced neurodegeneration. This may represent a novel target for the developmental degeneration associated with alcohol abuse.



Ethanol Enhances TRAIL Neurotoxicity (Propidium Iodine)

Disclosures: L.G. Coleman: None. J.Y. Zou: None. F.T. Crews: None.

Poster

304. Neuroinflammation in Neurodegenerative Diseases

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 304.27/Y16

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Intracellular calcium channel expression in autoimmune encephalomyelitis

Authors: *R. GUMMI, S. L. GRILLO, H. JOHNSON, C. L. MONTGOMERY, P. KOULEN Basic Med. Sci. & Ophthalmology, Univ. of Missouri Kansas City, Kansas City, MO

Abstract: Multiple sclerosis (MS) is a common cause of autoimmune demyelination of the central nervous system (CNS) leading to motor and vision deficits. The exact mechanism of MS remains unknown, though disruption in calcium homeostasis is thought to play a role. MS can be modeled in experimental animals through generation of experimental autoimmune encephalomyelitis (EAE) by inducing an autoimmune response against the myelin sheath, an insulating layer covering the nerve fibers. This causes perivascular inflammatory infiltrates and lesions of demyelination in the spinal cord. We hypothesize that intracellular calcium channels (ICCs) are altered during MS development, resulting in abnormal calcium signaling, resulting in neuronal dysfunction, and therefore contributing to loss of axons. This study measured the expression levels of inositol 1,4,5-trisphosphate receptors (IP₃Rs) in EAE-induced animals to determine if calcium channel complexes contribute to neurodegeneration in EAE and might ultimately represent a target for the development of novel pharmaceutical treatments of MS. EAE was induced in female Dark Agouti rats through immunization with myelin oligodendrocyte glycoprotein (MOG) protein. Immunoblotting was used to quantify ICCs in the CNS. Immunohistochemistry was used to visualize and quantify expression of ICCs, as well as of markers for cell types and structures affected by EAE, including oligodendrocytes, somata and axons of neurons, as well as astrocytes. Image analysis software was used to quantify levels of protein immunoreactivity. Six out of eight rats immunized with MOG protein exhibited signs of EAE and presented with increased cell infiltration and demyelination in CNS lesions. Increased expression of some, but not all ICCs was found. Expression of neuronal ICCs was increased in CNS neurons after EAE-induction. Increase in ICC expression could lead to apoptosis of neurons and axon damage during autoimmune encephalomyelitis disease progression due to calcium toxicity. Our findings of altered ICC calcium expression in a model of MS provides a strong rationale for further investigations determining the role of calcium regulation in MS as the basis for novel therapy development to prevent impairment of motor function and vision.

Disclosures: R. Gummi: None. **S.L. Grillo:** None. **H. Johnson:** None. **C.L. Montgomery:** None. **P. Koulen:** None.

Poster

305. Inflammation in Ischemia

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 305.01/Y17

Topic: C.07. Ischemia

Title: Nasal Associated Lymphoid Tissue ablation does not affect infarct volume or immune cell infiltration after stroke

Authors: *D. BREA¹, C. POON², M. MURPHY³, C. IADECOLA³, J. ANRATHER⁴ ¹Weill Cornell Med. Col., Joan and Sanford I Weill Med. Col. of Cornell Univ., New York, NY; ²BMRI, Weill Cornell Med. Col., New York, NY; ³Weill Cornell Med., New York, NY; ⁴Weill Cornell Med., NEW YORK, NY

Abstract: Stroke is a devastating disease with a strong inflammatory component partially mediated by meningeal IL17⁺ $\gamma \delta T$ cells^{1,2}. $\gamma \delta T$ cells constitute a lymphocyte population with innate features that populate epithelial surfaces, such as the nasal-associated lymphoid tissue (NALT). We have shown that, in the context of stroke, T cells migrate from the small intestine to the meninges but whether $\gamma\delta T$ cells can migrate from other epithelial surfaces is still unknown. Because of its close proximity, one possible source of stroke-associated IL17⁺ $\gamma\delta T$ cells could be the NALT from which T cells could migrate along olfactory nerve sheaths into the brain or the meninges. Indeed, it has been shown that Th17 cells are able to do it after intranasal infection with group A Streptococcus³. The role of NALT as a source for immune cells participating in the inflammatory response and in stroke outcome are still unknown. In order to study this, 25 C57bl/6 mice (male, 8-10 weeks old) were randomized to NALT ablation or sham surgery. 14 days later mice were subjected to transient middle cerebral artery occlusion (tMCAO). Infarct volumes were determined on Nissl stained coronal sections 3 days after reperfusion. Infarct volume analysis did not show any significant difference between sham $(39.99 \pm 6.46, n=12)$ and NALT-ablated mice (47.85 ± 7.13 , n=13, p>0.05). Although NALT ablation does not affect infarct volume, we sought to investigate its effect on immune cell infiltration, and specifically IL17⁺ T cells, in the brain or meninges after stroke. For this purpose, 14 IL17-eGFP mice were randomized to NALT ablation or sham surgery, and subjected to tMCAO. At 16 hours after reperfusion, at a time when IL17+ $\gamma\delta T$ cells are increased in the meninges after stroke¹, immune cells were isolated from brain and meninges and analyzed by flow cytometry. No significant differences were found in total infiltrated CD45⁺ cells (3318 ± 892 vs 3095 ± 933 ; p=0.87, n=7 per group), CD11b⁺ cells (2389 \pm 739 vs 2122 \pm 865; p=0.82), TCR β^+ cells (234 \pm 51 vs 203 \pm 35; p=0.62), TCR $\gamma\delta^+$ cells (27 ± 6 vs 31 ± 6; p=0.67), Th17 cells (16 ± 6 vs 10 ± 3; p=0.32) or IL17- $\gamma\delta$ T cells (13 ± 3 vs 15 ± 4; p=0.78) in the brain. No significant differences were found in total CD45⁺ cells (4485 \pm 1599 vs 2303 \pm 741; p=0.24), CD11b⁺ cells (2625 \pm 1119 vs 1245 \pm 422; p=0.27), TCR β^+ cells (376 ± 114 vs 260 ± 99; p=0.46), TCR $\gamma\delta^+$ cells (57 ± 16 vs 40 ± 11;

p=0.36), Th17 cells (16 ± 6 vs 11 ± 4 ; p=0.46) or IL17- $\gamma\delta$ T cells (57 ± 16 vs 40 ± 11 ; p=0.36) in the meninges of sham surgery and NALT-ablated animals. We conclude that NALT ablation does not affect ischemic brain damage or immune cells infiltration of the meninges or brain after stroke. ¹ Nat Med 22: 516 ² Blood 120: 3793 ³ J Clin Invest 126: 303

Disclosures: D. Brea: None. **C. Poon:** None. **M. Murphy:** None. **C. Iadecola:** None. **J. Anrather:** None.

Poster

305. Inflammation in Ischemia

Location: Halls A-C

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Topic: C.07. Ischemia

Support: the National Natural Science Foundation of China (21402241)

the Natural Science Foundation of Jiangsu Province (BK20160032)

Title: Natural product balasubramide derivative ameliorates brain inflammation and brain ischemic stroke via AMPK-mediated microglia polarization

Authors: *T. PANG¹, Y. WANG¹, W. RUAN¹, H. WANG¹, H. LIN² ¹China Pharmaceut. Univ., Jiangsu, China; ²Col. of Pharm., Guangzhou, China

Abstract: Brain inflammation plays an important role in the pathophysiology of brain ischemic stroke, psychiatric and neurological diseases. During brain inflammation, microglia cells are activated and show pro-inflammatory M1 and anti-inflammatory M2 phenotypes, producing neurotoxic molecules and neurotrophic factors, respectively. We discovered a natural product balasubramide derivative 3C exhibiting anti-inflammatory effects in microglia cells, but the underlying mechanisms and its beneficial effects on brain inflammation and brain ischemia are unknown. We found that 3C inhibited M1 polarization and promoted M2 polarization in LPS-stimulated BV2 and primary microglia cells, and these effects are mediated by CaMKKβ/AMPK/JNK signaling pathway. Furthermore, 3C prevented M1 gene expression and enhanced M2 gene expression in a mouse model of LPS-induced neuroinflammation, and reduced the LPS-induced sickness behavior. In addition, 3C significantly reduced infarct volume, improved the neurological deficit, and reduced neuroinflammation in rats with acute focal cerebral ischemia. Our results indicate that the natural product balasubramide derivative 3C suppresses microglia activation by promoting M2 polarization and may provide a novel therapeutic approach to treat brain ischemic stroke associated with enhanced brain inflammation.

Disclosures: T. Pang: None. Y. Wang: None. W. Ruan: None. H. Wang: None. H. Lin: None.

Poster

305. Inflammation in Ischemia

Location: Halls A-C

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Program#/Poster#: 305.03/Z1

Topic: C.07. Ischemia

Support: PRESTO, JST

Title: Mafb prevents excess inflammation after ischemic stroke by accelerating clearance of danger signals through MSR1

Authors: *T. SHICHITA¹, H. OOBOSHI², A. YOSHIMURA³

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Abstract: Inflammation is an essential step for the pathology of ischemic stroke. Since no pathogen exists within brain, post-ischemic inflammation will be triggered by some endogenous molecules (DAMPs: danger associated molecular patterns), which were released from dying brain cells. High mobility group box 1 (HMGB1) and peroxiredoxin (PRX) have been recently identified as DAMPs in the ischemic brain. HMGB1 exaggerates the disruption of blood brain barrier; on the other hand, PRX directly activates macrophage/neutrophil and induces the inflammatory cytokine production through the stimulation of Toll-like receptor (TLR2/4). Both the extracellular release of PRX and the infiltration of immune cells reach the peak within 1 to 3 days after the onset of ischemic stroke, and thereafter they decrease. This will lead to the resolution of post-ischemic inflammation.

We clarified the molecular meshanisms for the clearance of DAMPs from ischemic brain. Using random mutagenesis method, MSR1 (scavenger receptor) and *Mafb* (transcription factor) were idetified as key factors for DAMP clearance. MSR1 expression levels in infiltrating mononuclear phagocytes increased from day 1 to day 3 after stroke onset, which was dependent on *Mafb*. These MSR1-high cells removed DAMPs efficiently and produced IGF-1, a neurotrophic factor, and therefore revealed pro-resolving phenotype. The deficiency of *Msr1* or *Mafb* in infiltrating immune cells resulted in the exacerbation of inflammation and neuronal damages. Thus, *Mafb*-MSR1 pathway is important for the resolution of cerebral post-ischemic inflammation. We also found that Am80, a retinoic acid receptor agonist, enhanced MSR1 expression in infiltrating immune cells through *Mafb* and revealed neuroprotective effects against ischemic brain injury. Our results indicate that DAMPs regulate not only the induction but also the resolution of post-ischemic inflammation (Nat Med 2017 DOI: 10.1038/nm.4312). The novel neuroprotective strategy for ischemic stroke can be developed by accelerating the endogenous pro-resolving mechanisms to prevent the excess inflammatory responses.

Disclosures: T. Shichita: None. H. Ooboshi: None. A. Yoshimura: None.

Poster

305. Inflammation in Ischemia

Location: Halls A-C

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Program#/Poster#: 305.04/Z2

Topic: C.07. Ischemia

Support: NS095359

Title: Infiltrated peripheral macrophages expresses elevated phagocytic markers in the stroked brain during recovery

Authors: *K. PARK¹, M.-S. WOO², S. CHO³

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Abstract: Infiltrating monocyte-derived macrophages (MMs) have been implicated in strokeinduced inflammation and injury. Although the inflammatory nature of MMs in acute stroke has been well documented, their role during the resolution phase of stroke is less clear. The present study investigates the changes of infiltrated MMs subsets and phagocytic markers at sub-acute (7 days (d)) and chronic recovery periods (2 month (m)). C57 male mice (12 weeks old) were subjected to transient focal ischemia. Brain immune cells were collected and CD11b+ cells were further distinguished by CD45^{Hi} and CD45^{Low} subset. Stroke caused the appearance of CD45^{Hi} subset at 7d and 2m after stroke. CD45^{Low} populations were presented both hemispheres with significantly more in the stroked hemisphere. Adoptive transfer of GFP+ splenocytes 24 hour before sacrifice showed the presence of GFP+ cells in CD45^{Hi} and CD45^{Low} subsets at 7d and 2m, Protein expression of CD36 and lysosomal acid lipase (LAL), M2 and phagocytic markers, were increased at 7d and 2m with a greater extent at 2 m. Immunohistochemical analyses showed CD36 expression in infiltrated GFP+ MMs in the ipsilateral hemisphere. The present study showed persistent infiltration of MMs during sub-acute and long-term stroke recovery phase. The accumulation of CD45^{Low} MMs in the stroked hemisphere suggests a potential conversion of infiltrated CD45^{Hi} subset to CD45^{Low} subset. Additionally, elevated CD36 and LAL in MMs at 7d and 2m indicate an involvement of infiltrating MMs in injury repair through phagocytosis.



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Poster

305. Inflammation in Ischemia

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Topic: C.07. Ischemia

Support: NIH Grant HL091541

NIH Grant HD079123

Title: Inflammatory profile in a canine model of Hypothermic Circulatory Arrest

Authors: S. TORRES-ODIO¹, J. G. ALLEN², E. S. WEISS², G. J. ARNAOUTAKIS², P. CARR¹, C. C. TALBOT², *M. E. BLUE¹, M. V. JOHNSTON¹, W. A. BAUMGARTNER², M. A. WILSON¹

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Abstract: A particularly important technique for neuroprotection in complex cardiac surgery is hypothermic circulatory arrest (HCA). However, HCA leads to multifocal brain injury and neurobehavioral alterations. Inflammatory cytokines are increased in response to brain injury and targeting neuroinflammation is neuroprotective in some brain injury models. We characterized the inflammatory response in a large animal model at different time points after HCA. Twentyfour dogs underwent 2-hour HCA at 18 degrees and were sacrificed 2, 8 or 24 h later (n=8 per group). 8 normal dogs were used as gene expression controls. The hippocampus is highly vulnerable to injury after HCA, and therefore changes in mRNA expression were evaluated in the hippocampus using cDNA microarrays. Gene ontology (GO) analysis was completed using Spotfire Decision Site® and Ingenuity® Pathway Analysis was used to examine regulated

pathways. Selected proteins were further analyzed in hippocampal homogenates using ELISA. Gene ontology analysis indicated that the expression of inflammatory response genes was altered after HCA, with 91 of 430 genes regulated at 2h, 152 at 8h, and 67 at 24h (p<10⁻⁵ at each time point). Several pro-inflammatory cytokines (IL-6, IL-8, CCL2, IL-1b) were upregulated at all three time points, with peak expression at 8h post HCA. The signaling pathways iNOS Signaling, Nf-kb Signaling and Dendritic Cell Maturation signaling pathways were upregulated at 2 and 8h post HCA. Trem-1 Signaling, Granulocyte Adhesion and Diapedesis, and Glucocorticoid Receptor Signaling were upregulated at all time points. IL6 and IL8 were examined at the protein level and expression peaked at 8h, consistent with the temporal pattern of mRNA expression (85 and 115 pg/mg of protein respectively: significantly greater than normal 2 h and 8 h after HCA. Brain inflammation occurs rapidly in response to HCA, with detectable increases in mRNA for multiple inflammatory cytokines and chemokines at 2 h, strikingly elevated expression at 8 h and with some inflammatory mediators remaining elevated 24 h after HCA. This pattern was confirmed at the protein level for selected cytokines. These results support the hypothesis that inflammation contributes to brain injury after HCA, and suggest that therapeutic approaches modulating this response may be beneficial.

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Poster

305. Inflammation in Ischemia

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Topic: C.07. Ischemia

Support: National Natural Science Foundation of China (grant number 81471780)

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Title: Interferon-gamma as a double-edged sword: Its roles on neural stem cells therapy for ischemic stroke model

Authors: *G. ZHANG, *G. ZHANG, B. LI, L. CHEN Dept. of Neurosurgery, Zhongda Hosp., Sch. of Medicine, Southeast Univ., Jiangsu, China

Abstract: The inflammatory and immune responses induced by ischemic stroke commonly affect the functional or structural recovery of nerve system. The pro-inflammatory cytokine interferon gamma (IFN- γ) plays a vital role on both inflammatory and immune responses, and IFN- γ also influences on the role of neural stem cells (NSCs). The aim of this study was to

examine the effects of IFN- γ on NSCs in vitro and ischemic stroke model in vivo. Here it showed that low concentration IFN- γ (10 or 20 ng/ml) superior to other cytokines (BDNF, VEGF, TGF-β1 and IGF-1) could increase the proliferation and differentiation of NSCs in vitro. After treated by NSCs with IFN- γ , it significantly increased the level of inflammatory oxidative stress of NSCs cultured, but it did not induce NSCs abnormality. At in vivo experiment, we built cerebral ischemic model of Sprague Dawley (SD) rats by transient middle cerebral artery occlusion. Two hundred and ten rats were randomized into seven groups as PBS group, high concentration IFN- γ (500ng/rat) group, low concentration IFN- γ (50ng/rat) group, NSCs group, NSCs combined with high concentration IFN-y (500ng/rat) group, NSCs combined with low concentration IFN-y (50ng/rat) group and sham group. It was observed that IFN-y groups did not improve the mNSS scores of ischemic rats, but the repairing function of NSCs group or NSCs combined with IFN- γ groups had been improved compared to PBS group, and the repairing ability of NSCs combined with low concentration IFN-y group was better than NSCs group. TTC staining showed the same results with mNSS, and rats in NSCs combined with low concentration IFN- γ group had smaller infarct volume than rats in other groups. And more brdu/nestin, brdu/DCX positive cells were found in NSCs combined with low concentration IFN- γ group. However, CD4 and CD8 T cells significantly infiltrated into the ischemia sites of rats in high IFN- γ combination group, and the expression of SOD and IFN- γ were significantly increased in high concentration IFN- γ and PBS groups. ELISA showed that the levels of anti-inflammatory cytokines IL-10 and TGF-B1 in cerebrospinal fluid (CSF) and proteins in different time of ischemic rats were increased in NSCs or combined treatment groups compared to IFN-y and PBS groups, but the expressions of pro-inflammatory cytokines (IL-1 β , IL-6, IFN- γ , and TNF- α) were decreased. Thus, transplantation of NSCs combined with low concentration IFN-y can promote the recovery of ischemic stroke rats and reduce the inflammatory response. In conclusion, low concentration IFN-y can promote the functions of transplanted NSCs and change the ischemic microenvironment, so as to facilitate neurological recovery.

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Poster

305. Inflammation in Ischemia

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Topic: C.07. Ischemia

Support: AHA grants 16GRNT31300011

AHA grant 12SDG11970010

Title: Sexual dimorphism in inflammasome activation: Possible cause of exacerbated ischemic brain damage in reproductively senescent female rats

Authors: *A. P. RAVAL¹, J. DE RIVERO VACCARI²

¹Neurol., ²Dept. of Neurolog. Surgery and The Miami Project to Cure Paralysis, Univ. of Miami, Miami, FL

Abstract: A woman's risk of a stroke increases exponentially following the onset of menopause, and underlying mechanisms remains unknown. The current study tests the hypotheses that: (1) inflammasome activation is significantly higher in the brain of RS females as compared to their young counterparts and senescent male rats, (2) RS triggers an innate immune inflammatory response in the ovaries that spreads to the brain, making the brain more susceptible to ischemic damage. We tested our hypotheses using Sprague-Dawley rats of both sexes (6-7 and 9-12 months). The estrous cycles of female rats were monitored for 14-20 days prior to experimentation by daily examination of vaginal smears. Rats that remain in constant diestrus were considered RS. Rats (n = 4-7) of both sexes and ages were sacrificed and hippocampus, gonads, serum and cerebrospinal fluid (CSF) were collected. Additionally, cerebrospinal fluid (CSF) of women (<40 and >50 age) was obtained. Extracellular vesicles (EV) were isolated from serum and CSF using an Invitrogen kit. Inflammasome proteins caspase-1, apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and IL-1ß significantly increased in the hippocampus, serum, and ovaries of RSF as compared to YF (p<0.05). This was not observed in the hippocampus or gonads of age-matched males. Importantly, EV obtained from RSF contains significantly higher levels of the inflammasome proteins as compared to YF (p<0.05). EV containing inflammasome proteins originates in the ovaries of RSF and then are carried to the brain via blood. The observed increase in ovary-derived EV containing inflammasome proteins in the brain contributes to the inflammation present in the brain of RSF, and it might exacerbate ischemic brain damage. Future studies investigating the role of ovarian EV in post-ischemic inflammation are underway to understand how modulating EV trafficking can reduce the incidence and impact of cerebral ischemia in post-menopausal women.

Disclosures: A.P. Raval: None. J. de Rivero Vaccari: None.

Poster

305. Inflammation in Ischemia

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Program#/Poster#: 305.08/Z6

Topic: C.07. Ischemia

Support: SNRP U54NS083924

Title: Neuroprotective effect of a single injection of losartan in male and female rats subjected to ischemic stroke
Authors: *S. MARTINEZ¹, M. N. GONZALEZ VEGA², A. H. MARTINS³ ¹Neurosci., Univ. Central Del Caribe, Bayamon, Puerto Rico; ²Univ. Central del Caribe, Bayamon, PR; ³Univ. of Puerto Rico Med. Sci. Campus, San Juan, PR

Abstract: Stroke is the fifth leading cause of death in the US, affecting 80,000 people with new or recurring stroke every year. Ischemic stroke, which accounts for 87% of all types of strokes, is caused by a blockade of blood vessels in the brain by a blood clot. Hence loss of oxygen causes apoptosis and necrosis, giving rise to inflammatory conditions. This inflammation affects neurons, astrocytes and consequently the blood brain barrier (BBB). The BBB then becomes more permeable and allows inflammatory molecules into the brain, which exacerbates ischemic brain damage in a positive feedback fashion. Losartan is an angiotensin type 1 receptor blocker commonly used to decrease blood pressure but also has anti-inflammatory properties. In recent studies, losartan has been observed to help maintain BBB integrity. But these studies have been performed with chronic administration of losartan in male rodents for several days to weeks. The efficacy of a single acute injection of losartan in stroke has yet to be quantified. We propose that a single dose of losartan will decrease the neuronal damage and at least partially restore BBB integrity in male and female rats subjected to ischemic stroke. In order to verify this hypothesis we performed middle cerebral artery occlusion (MCAO) to induce an ischemic stroke in male and female Sprague Dawley rats weighing 250-300g. Losartan 1mg/Kg or sterile saline injection was given intravenously. For BBB integrity analysis, Evans Blue (EB) 2% was injected intraperitoneal 1hr after stroke onset. Rats were perfused with sterile saline 24hrs after stroke induction and brain was removed for analysis. For infarct damage analysis, rats were decapitated 24hrs after stroke induction and brain was submerged in tetrazolium chloride (TTC) solution. Both EB dye infiltration and infarct damage was quantified using ImageJ software. Results showed that losartan reduces infarct damage and BBB permeability in losartan-treated male and female Sprague Dawley when the angiotensin receptor 1 blocker was injected 5 min before stroke (p<0.05). These results are reproduced in our preliminary data using rats injected with losartan 2hrs after the stroke onset. Therefore, these results suggest that even a single injection conserves the neuroprotective effects of losartan against stroke and could prove to be a useful alternative therapeutic approach in stroke therapy.

Disclosures: S. Martinez: None. M.N. Gonzalez Vega: None. A.H. Martins: None.

Poster

305. Inflammation in Ischemia

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Topic: C.07. Ischemia

Support: NS37853

Title: The apolipoprotein E4 allele induces neurovascular dysfunction mediated by perivascular macrophages

Authors: *Y. HATTORI¹, K. UEKAWA¹, K. KOIZUMI¹, L. ZHAO³, S. M. PAUL⁵, C. IADECOLA², L. PARK⁴

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Abstract: The apolipoprotein E4 (ApoE-ε4, ApoE4) allele is a major risk factor for Alzheimer's disease and white matter ischemic lesions (Neurology 81:292, 2013). However, the mechanisms underlying the increased risk for cerebrovascular diseases and neurodegeneration associated with ApoE4 remain unclear. We sought to determine if the ApoE4 genotype is associated with alterations in the regulation of the cerebral microcirculation that may render the brain more susceptible to ischemic brain injury and neurodegeneration. Cerebral blood flow (CBF) was recorded by laser-Doppler flowmetry in the somatosensory cortex of urethane-chloralose anesthetized human ApoE targeted replacement mice (ApoE4-TR) (Sullivan et al., JBC, 272:17972, 1997) and wild-type (WT) mice (males; age 3-4 months; n=5/group). Blood pressure and blood gases were monitored and controlled. In ApoE4-TR mice, the increase in CBF produced by whisker stimulation (WS) or by topical application of the endothelium-dependent vasodilator acetylcholine (ACh, 10 µM) was attenuated (WS, -49±3%; ACh, -38±3%; p<0.05; mean±SE) compared WT mice, while the CBF increase evoked by the smooth muscle relaxant adenosine (400 μ M) was not affected (p>0.05). The neurovascular dysfunction is mediated by oxidative stress because neocortical superfusion of the reactive oxygen species (ROS) scavenger MnTBAP (100 µM) reversed the suppression of WS and ACh responses in ApoE4-TR mice (p>0.05 form WT). Next, we sought to determine if Nox2 is the enzymatic source of the ROS responsible for the dysfunction. Neocortical superfusion of the Nox2 peptide inhibitor gp91ds-tat (1µM), but not its control peptide, counteracted the neurovascular dysfunction in ApoE4-TR mice (p<0.05 from control peptide). Perivascular macrophages (PVM) are closely associated with cerebral resistance vessels, are enriched with Nox2, and mediate neurovascular dysfunction in models of hypertension (JCI, 126:4674, 2017). Therefore, we tested if ApoE4 in PVM is required for the neurovascular dysfunction in ApoE-TR mice. To this end, after lethal irradiation, we transplanted ApoE4 BM in WT mice to repopulate their perivascular space with ApoE4 PVM. In WT mice transplanted with ApoE4 BM, the increase in CBF induced by WS or by ACh was attenuated compared to WT mice receiving WT BM (WS, -45%; ACh, -35%; p<0.05 from WTOWT). These data indicate that the ApoE4 genotype is associated with profound alterations in neurovascular regulation mediated by oxidative stress and dependent on ApoE4 expression in PVM. Such alterations are likely to play a role in the increased susceptibility to ischemic brain injury and neurodegeneration conferred by the ApoE4 genotype.

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Poster

305. Inflammation in Ischemia

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 305.10/Z8

Topic: C.07. Ischemia

Support: KAKENHI

Title: Suppression of nitric oxide level from microglia by co-culturing with human mesenchymal stem/progenitor cells

Authors: *H. OHTAKI¹, J. WATANABE⁴, K. YAGURA¹, K. MIYAMOTO², Y. HIRAIZUMI³, K. HONDA¹, K. DOHI²

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Abstract: We have reported that transplant of human mesenchymal stem/progenitor cells (hMSCs) decreased ischemic neuronal cell death after ischemia. We also have determined that hMSCs modified several transcriptomes which related to immune and inflammatory responses. In particular, hMSCs modified microglial activating phenotype in the ischemic brain. However, it is still unclear that how hMSCs regulate microglial activation in detail. In present study, we determined that mixed cultured hMSCs reduced nitric oxide production from mice microglial cell line, BV-2. Several number of passage 3 hMSCs $(1.25 - 20 \times 10^4)$ were plated on six-well plate and were cultured with 20% FBS in α-MEM (20% completed cultured medium (CCM)). The next day, the media were replaced to 1% CCM and added $1x10^{6}$ BV-2 and then added vehicle, 10ng/mL IFNy or 10ng/mL IL-4. At 24 and 48 hours after cultivation, the media were collected and measured nitric oxide (NO) level by Griess method. The cells were measured inducible nitric oxide synthase (iNOS) level by western blotting. Moreover, the BV-2 were cultured with inviable hMSCs and human dermatomal fibroblast (HDF) to determine the contribution of cell-cell communication and of xenoreaction. BV-2 increased NO in the media and iNOS in the cells with IFNy application. The BV-2 also increased medial TNFa and cellular gp91^{phox}. These are the IFNy applied BV-2 activated to classical cytotoxic phenotype. On the other hands, IL-4 applied BV-2 decreased medial TNFα and increased cellular Ym1 and arginase activity, suggesting anti-inflammatory phenotype. The phenotypic BV-2 did not produced NO in media. Cultured BV-2 with hMSCs decreased NO in media and iNOS in cells with hMSCsnumber dependent fashion. Four different kinds of inviable hMSCs did not show any NO reduction, suggesting a cell-cell communication required to the response. Moreover, although cultured BV-2 with HDF (20 x 10⁴) slightly decreased NO, the reduction was obviously weaker than that with hMSCs. These results suggested that hMSCs suppressed microglial inflammatory responses with direct cell-cell communication.

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Poster

305. Inflammation in Ischemia

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Topic: C.07. Ischemia

Support: Office of Research ans Sponsored Programs at CMU

College of Medicine at CMU

College of Humanities and Social and Behavioral Sciences at CMU

Field Neurosciences Institute

Title: Transplantation of mesenchymal stem cells which overexpress interleukin 10 as treatment for stroke-induced behavioral deficits in rats

Authors: *M. M.-M. ANDREWS¹, S. PERUZZARO², J. ROSSIGNOL⁴, G. L. DUNBAR³ ¹Neurosci., ²Central Michigan Univ., Mount Pleasant, MI; ³Dept Psychol, Central Michigan Univ., Mt Pleasant, MI; ⁴Field Neurosciences Inst. Lab., Mount Pleasant, MI

Abstract: Stroke is a leading cause of death and long-term disability worldwide. There is currently only one FDA approved pharmacological treatment and due to strict exclusion criteria, it is administered to only a few stroke patients. Initial damage from stroke occurs from loss of oxygen and nutrients while damage continues even after blood flow is restored, due to the inflammation that ensues. Interleukin 10 is an anti-inflammatory cytokine that may help to improve functional recovery following stroke. Rats underwent the middle cerebral artery occlusion procedure to produce a 75-min stroke. Two days after stroke, rats were given two intracerebral injections as follows: Group 1 received mesenchymal stem cells that were engineered to over-express interleukin 10; Group 2 received unaltered mesenchymal stem cells; Group 3 received Hanks balanced salt solution (HBSS); and Group 4 received sham surgery and HBSS. Following intracerebral injections, rats were given a battery of behavioral tests. For the first five weeks, rats underwent cylinder testing and rotorod testing weekly. Open field testing and ladder testing occurred bi-weekly during the same period. Following these tests rats were then trained to lever press for sucrose pellets in an operant chamber. After training, a behavioral flexibility operant task was put into place and five consecutive lever presses on the active lever were required to earn a pellet. The active lever was assigned at random at the beginning of the session and each time a pellet was earned the active lever would alternate between the two levers, although no signal identified this switch. The behavioral flexibility task was administered for 7 days. At the conclusion of operant testing, rats were euthanized (day 45 post-stroke). Brain tissue was collected for Hematoxylin and Eosin staining to quantify infarct size and levels of inflammation and interleukin 10 was also examined. Preliminary results indicate stroke-induced deficits, but no significant treatment effect has been observed thus far.

Disclosures: M.M. Andrews: None. **S. Peruzzaro:** None. **J. Rossignol:** None. **G.L. Dunbar:** None.

Poster

305. Inflammation in Ischemia

Location: Halls A-C

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Topic: C.07. Ischemia

Support: NIH grants NS085568 (LW/SPY), NS091585 (LW), NS062097 (LW), and NS075338 (LW)

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Title: The role of GPR37 in microglia/macrophage activation and proinflammatory responses after focal ischemic stroke

Authors: X. HUANG^{1,4}, M. R. MCCRARY¹, M. Q. JIANG¹, X. H. GU¹, R. A. HALL², M. FAN⁴, L. WEI^{1,3}, *S. YU¹ ¹Anesthesiol., ²Pharmacol., ³Neurol., Emory Univ. Sch. of Med., Atlanta, GA; ⁴Dept. of Cognitive Sci., Inst. of Basic Med. Sci., Beijing, China

Abstract: Post-ischemic inflammation and uncontrolled glial responses could contribute to longterm damage of the brain. GPR37, an orphan G protein-coupled receptor, is highly expressed in the mammalian central nervous system, but its function remains obscure. We observed previously that the GPR37 KO mouse shows deficient reactive astrocyte and significantly reduced glia scar formation after cerebral ischemia, evidenced by reduced number of GFAP+ astrocytes in the peri-infarct region as well as the mean GFAP fluorescence intensity across the ischemic boundary regions (Jiang et al., 2016). Compared to WT controls, elevated autophagic cell death and apoptosis were observed in the peri-infarct region of GPR37 KO animals at early time points of 6 to 48 hrs after stroke (McCrary et al, 2016). The current investigation hypothesized that GPR37 KO elevated microglia/macrophage activation that contributes to enforced inflammatory responses. Adult WT and GPR37KO mice were subjected to a focal cerebral ischemia affecting the right sensorimotor cortex. Immunohistochemical staining was performed to identify Iba-1+ microglia/macrophage in the post-stroke brain of WT and GPR37 KO mice. qPCR identified M1 and M2 microglia/macrophage phenotypes by quantifying the expression levels of related genes. Our data showed that there were more Iba-1+ cells in the periinfract region of GPR37 KO animals compared to WT at all inspected time points. iNOS, an M1type gene, was significantly upregulated in the GPR37 KO brain whereas Ym1 and Fizzl, M2type genes, remained unchanged. The expression of interleukin (IL)-1 β , IL-6, monocyte chemoattractant protein (MCP)-1 and macrophage inflammatory protein (MIP)-1 α significantly increased in GPR37 KO mice compared to WT mice. These results indicate that deletion of GPR37 would increase microglia/macrophage activation and augment injurious inflammatory responses after stroke. This study may further suggest GPR37 as a potential regulator of microglia/macrophage activation and long-term inflammation after ischemic stroke.

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Poster

305. Inflammation in Ischemia

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 305.13/DP05/Z11 (Dynamic Poster)

Topic: C.07. Ischemia

Support: NIH grant

Title: TREM1 signaling in peripheral infiltrating myeloid cells increases stroke severity

Authors: *Q. LIU, J. WANG, Q. WANG, H. YE, K. ANDREASSON Stanford Univ., Stanford, CA

Abstract: TREM1 is an amplifier of maladaptive innate immune responses in peripheral models of inflammatory disease and is exclusively expressed in myeloid cells, including macrophages, monocytes, neutrophils, and microglia. Cerebral ischemia causes a significant and well characterized immune response in the days and weeks following stroke, beginning with infiltration of myeloid cells from the periphery, including macrophages and neutrophils. This post-stroke immune response contributes to stroke injury, however cellular mechanisms underlying this effect are not fully understood. We tested the hypothesis that TREM1, which signals through DAP12 to amplify pro-inflammatory responses in peripheral models of inflammation, may drive injury elicited by the post-stroke inflammatory response in the murine middle cerebral artery-reperfusion (MCAo-RP) model. Following MCAo-RP, TREM1 surface expression was highly induced in infiltrating CD11b/CD45hi macrophages at 48h and subsequently declined by 6d. Systemic administration of a decoy peptide that inhibits TREM1 ligand binding reduced infarct volume, improved neurological scores and was associated with reduced TREM1 expression in infiltrating myeloid cells. Consistent with this, genetic ablation of

TREM1 reduced stroke injury and improved neurological scores. In previous studies, we determined that TREM1 expression was highly induced by inflammatory PGE₂ EP2 signaling, a major suppressor of beneficial microglial functions. In Cd11bCre; EP2 ^{lox/lox} mice lacking myeloid EP2 receptor, as well as in Rosa26CreERT2; EP2 ^{lox/lox} mice, TREM1 surface expression and numbers of infiltrating myeloid cells were significantly decreased. Taken together, these studies suggest that modulating the innate immune response after stroke, via inhibition of TREM1 activity, improves stroke outcome, and peripheral targeting of TREM1 may be effective in reducing injury and accelerating recovery in a model of transient focal cerebral ischemia.

Disclosures: Q. Liu: None. J. wang: None. Q. wang: None. H. ye: None. K. andreasson: None.

Poster

305. Inflammation in Ischemia

Location: Halls A-C

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Program#/Poster#: 305.14/Z12

Topic: C.07. Ischemia

Support: INSERM

UNAFTC

Normandy region

IRME

Title: Alcohol-induced inflammatory priming worsens outcome after ischemic stroke

Authors: ***A. DRIEU**¹, M. NAVEAU², A. QUENAULT¹, D. VIVIEN¹, M. RUBIO¹ ¹INSERM U1237, ²UMS 3408, GIP Cyceron, Caen, France

Abstract: Alcohol consumption is as risk factor for stroke. Furthermore, recent clinical and fundamental studies have shown that chronic alcohol consumption aggravates ischemic stroke outcome (Ducroquet et al., 2013; Lemarchand et al., 2015). Our hypothesis is that long-term alcohol consumption provokes a silent inflammatory status (i.e., inflammatory priming) that could be responsible of exacerbated inflammatory responses in the brain after ischemic stroke. In this study, we used adult male Swiss mice exposed to water (control group) or to 10% ethanol *ad libitum* (alcohol group) for 6 weeks. Inflammatory status was evaluated by original techniques such as the *in vivo* measurement of microglial phagocytosis, *in vivo* vascular adhesion molecular MRI and intravital two-photon microscopy as well as immunohistochemistry. Alcohol exposure provoked a silent inflammatory status (i.e., inflammatory priming) in the brain characterized by a

significant increase in (i) microglial phagocytosis, (ii) the number of perivascular macrophages, (iii) the vascular adhesion molecule P-selectin and (iv) leukocyte rolling and adhesion to blood vessel walls. These results suggest an alcohol-induced inflammatory priming that was confirmed by the exacerbated inflammatory brain reaction after the systemic injection of lipopolysaccharide (LPS). The consequence of this alcohol-induced inflammatory priming is the worsening of ischemic stroke outcome (induced by thrombin injection directly into the middle cerebral artery). Our results demonstrate that alcohol-induced inflammatory priming markedly worsens the outcome after ischemic stroke. Inflammatory priming could thus be an essential target to avoid worsening in the outcome of stroke patients.

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Poster

306. Stroke: Imaging Assessments

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 306.01/Z13

Topic: C.08.Stroke

Support: Wellcome Trust Grant 106161/Z/14/Z

Title: The role of the left inferior frontal cortex in cognitive challenge

Authors: *D. NARDO¹, K. PAPPA¹, J. DUNCAN³, P. ZEIDMAN⁴, M. CALLAGHAN², A. P. LEFF², J. CRINION⁵

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Abstract: Background. The left inferior frontal cortex (including Broca's area) is traditionally regarded as a core hub for spoken language processing. However, whether its role is domain-specific (i.e., linguistic), or domain-general (i.e., involved in multiple challenging cognitive functions) is a matter of debate [Fedorenko & Thompson-Schill, Trends Cogn Sci 2014]. **Method.** In an fMRI study we investigated how the left inferior frontal cortex is modulated by task and task challenge using a $2 \times 2 \times 2$ (*Task x Challenge x Modality*) factorial design. 11 healthy right-handed native English speakers performed both a linguistic (picture naming) and a non-linguistic (size judgment) task. Stimuli were 480 black and white pictures of monosyllabic objects. Task challenge (low vs. high ambiguity) was manipulated by varying: 1) visually, the amount of visual noise superimposed to a picture (5 vs. 15 black squiggly lines); 2) aurally, the cue delivered concurrently with the picture (initial phoneme vs. noise control). Subjects' overt spoken responses (object names, yes/no replies for size judgment) were recorded online, and

reaction times (RT) were computed. **Results.** RT data showed no significant main effect of *Task* (i.e., tasks were behaviourally matched overall), significant *Challenge x Modality* interactions (visual, p<.001; auditory, p<.001), and significant Task x Challenge x Modality interactions (visual, p=.038; auditory, p<.001). Higher ambiguity resulted in slower RT across both tasks. fMRI data (p<.05 FWE-corrected) revealed that both tasks recruited a common widespread network including the left inferior frontal cortex and supplementary motor area, as well as bilateral visual, auditory and premotor cortices. There was a main effect of Task with the naming task activating bilateral superior temporal cortices more than the non-naming task. The Challenge x Modality interactions showed significant effects in bilateral visual cortices for the visual modality, and in bilateral temporal cortices and in the left precuneus for the auditory modality. The Task x Challenge x Modality interaction showed no significant activation for the visual modality, but significant activations in the left inferior frontal cortex, supplementary motor area, and premotor cortex for the auditory modality. Conclusions. Our data suggest that the left inferior frontal cortex is domain-general (i.e., involved in both cognitive tasks), yet modulated by complex interactions between task, cognitive challenge and sensory modality. Connectivity analyses will enable further characterisation of the neural mechanisms underlying its dynamic role within these task-engaged networks.

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Poster

306. Stroke: Imaging Assessments

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 306.02/Z14

Topic: C.08.Stroke

Title: Dynamic rat MCA stroke evolution in relation to reperfusion and spreading depolarizations

Authors: U. HOFFMANN¹, *D. A. TURNER² ¹Anesthesia, ²Neurosurg., Duke Univ. Med. Ctr., Durham, NC

Abstract: MCA stroke evolution centers on the evolution of the dynamic relationship between penumbra and ischemic core over time, with infarct size determined by collaterals, reperfusion and spreading depolarizations (SDs). Our hypothesis is that MCA infarcts can be dynamically tracked using imaging and physiological markers, and that infarct consolidation occurs over several days, ameliorated by increasing collateral blood flow, reperfusion and avoidance of SDs. Our methods include filament occlusion of MCA in adult rats, direct infarct monitoring via craniotomy in isoflurane and propofol anesthetized animals, and reperfusion using filament removal at specific times post occlusion. We performed intrinsic optical imaging (IOS), blood flow, DC recordings, tissue pO2, direct fluorescent angiograms, and KCl-induced SD spread to assess collateral flow and infarct size. For long term observations over several days we also measured spontaneous SDs and cortical activity in vivo using high density cortical µECoG arrays in awake animals, before and after MCA infarct.

KCl-induced SD propagation was reliable (from anterior to posterior) in exposed rat cortex with clear IOS wavefront propagation and physiological SD measurement (n = 36 SD episodes). After MCA infarct (n = 7) the blood flow was reduced with decreased angiographic vessel filling, both improved with reperfusion at 2 hr. KCl-induced SDs did not propagate into the core infarct region, instead deviating through the penumbra, and spontaneous SDs showed circuitous cortical paths around the highlighted core infarct region. Reperfusion significantly enhanced collateral flow and reduced infarct size. Isofluorane suppressed most spontaneous SD occurrences whereas propofol did not; KCl-induced SD episodes occurred equally in both anesthesia conditions. Ongoing longer-term recordings in awake rats using implanted high resolution arrays to define SD occurrence and infarct size will substantially enlarge our observation time window and avoid the confounding effects of anesthesia.

These novel experiments reveal the ongoing, dynamic nature of stroke boundaries as well as the role of reperfusion and SD occurrences in secondary stroke expansion. Thus, MCA infarct size may be tracked dynamically with IOS, blood flow and tissue pO2, SD propagation and angiographic vessel filling complemented by chronic recordings in awake freely moving rats after stroke. This dynamic MCA infarct assessment will assist in identifying additional treatment mechanisms.

Disclosures: U. Hoffmann: None. D.A. Turner: None.

Poster

306. Stroke: Imaging Assessments

Location: Halls A-C

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Topic: C.08.Stroke

Support: NIH R01NS080839

TIRR Rehabilitation Innovations Pilot Grant

Memorial Hermann Foundation

Title: Relative and absolute reliabilities of the MScanFit MUNE

Authors: *X. LI¹, Y. ZONG², P. ZHOU³

¹Univ. of Texas, Houston, TX; ²UTHealth, Houston, TX; ³PM&R, Univ. of Texas Hlth. Sci. Ctr. At Houst, Houston, TX

Abstract: This study examined the reliability of a novel motor unit number estimation (MUNE) technique based on the compound muscle action potential (CMAP) scan. Six healthy subjects (28-45 y) without any known neurological disorders participated in the same experiments at two different days. CMAP scans were recorded from extensor digitorum bevis (EDB) and abductor hallucis (AH) muscles by stimulating the peroneal nerve and tibial nerve respectively. Motor unit population from two test sessions was quantified using the MScanFIT MUNE technique. Relative and absolute reliability of the technique was assessed by calculation of the intraclass correlation coefficient (ICC), standard error of measurement (SEM), smallest real difference (SRD) and Bland-Altman's 95% limits of agreement (LOA). MUNE of EDB and AH muscles were averaged on different sessions and the estimations were 110±12 and 44±11 respectively. MUNEs of the two muscles were pooled and the ICC, SEM% and SRD% were calculated as0.967, 0.099%, and 0.049%, respectively. The Bland-Altman test indicated no systematic bias between most of the repeated measurements. These findings suggest that MScanFit is a reliable technique in assessment of motor unit counts.

Disclosures: X. Li: None. Y. Zong: None. P. Zhou: None.

Poster

306. Stroke: Imaging Assessments

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Topic: C.08.Stroke

Support: NHRI Grant 05A1-BNPP15-014

MOST Grant MOST-105-2221-E-400-006

Title: Understanding neurovascular mechanisms during cortical spreading depression in ischemic stroke rats

Authors: *L.-D. LIAO, H.-C. PAN, S.-P. SUN Inst. of Biomed. Engin. and Nanomedicine, Natl. Hlth. Res. Inst., Miaoli County, Taiwan

Abstract: Brain functions, such as motor and sensory functions, are based on neuronal activities and also highly associated with the hemodynamic supporting for energy and metabolic homeostasis. Therefore, the corresponding neurovascular mechanism plays an important role in the complex brain activities. To elucidate the neurovascular mechanisms underlying physiological or pathological conditions, we develop a novel multimodality imaging system for simultaneously recording the electrocorticography (ECoG) signals for brain activities and hemodynamics responses by laser speckle contrast imaging (LSCI), so-called ECoG-LSCI system, to collecting the sufficient information of neurovascular functions with high

spatiotemporal resolution. The photothrombotic ischemia (PTI) rat model is used and induced the stroke over the motor cortex of right hemisphere at the coordinate of anterior-posterior: 0 mm and medial-lateral: +1.5 mm to the bregma. Interestingly, the PTI stroke leaded to contralateral somatosensory evoked potential (SSEP) attenuations of primary motor cortex (M1) and primary sensory cortex forlimb (S1FL), resting-state evoked potential (RS-EP) decline, cortical spreading depression (CSD)-like surge induction and ischemic infarct damages in motor cortex which confirmed by 2,3,5-triphenyltetrazolium chloride (TTC) staining. Taken together, the ECoG-LSCI system provides *in vivo*, full-field, real-time, multi-functional and useful detection for the neurovascular mechanism of ischemia-induced CSD study.

Disclosures: L. Liao: None. H. Pan: None. S. Sun: None.

Poster

306. Stroke: Imaging Assessments

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 306.05/Z17

Topic: C.08.Stroke

Title: Sequential restoration patterns of cerebral autoregulation in different ischemic rat models

Authors: *E. CHOI, G. PARK, Y. KWON, K.-E. LEE, M. CHOI, J. LEE, J. HONG Dept. of Neurology, Ajou Univ. Sch. of Med., Suwon, Korea, Republic of

Abstract: BACKGROUND: Cerebral autoregulation (CA) is the ability to maintain sufficient and stable cerebral blood flow (CBF) despite changes in cerebral perfusion pressure (CPP). Theoretically, CA has been known as an initiative surrogate for cerebrovascular dysfunction and subsequent brain damage. Early prognostication ways with various CA measurements for accurate prediction of the brain injury would be a useful screening tool to triage a candidate for numerous neurovascular protectives. For this reason, we are to assess the feasibility of various CA measurements for early prognostication in ischemic rat models according to the different mode of brain damage. **METHODS:** Cerebral ischemia were assessed in male Sprague-Dawley rats (250-280g). The temporary focal ischemia was performed by transient middle cerebral artery occlusion (tMCAO) for 90 minutes. The transient global ischemia with four-vessel occlusion (4-VO) was performed by an electrocauterization of two vertebral arteries and temporary clamping of two common carotid arteries (CCA) for 10 minutes. Sequential changes of cerebral blood flow (CBF) by intravenous acetazolamide (50mg/kg) administration was measured using PeriScan PIM 3 System at baseline (T0) and 1 (T1), 3 (T3), 5 (T5) and 7 (T7) days after surgery and expressed as cerebrovascular reserve capacity (CVRC). We analyzed infarction volume, neuronal death, and glial activation with immunohistochemistry. We assessed the neurological behavior with modified neurological severity score (18 points). RESULTS: The focal tMCAO had an immediate ipsilateral CBF reduction to 41±17% from baseline (100%), representing with

post-stroke neurological deficits (8.4 ± 0.6 point) and infarct volume (321 ± 20 mm³) at 7days. As compared with CVRC on contralateral hemisphere (T1: $18\pm3\%$, T2: $18\pm5\%$, T5: $10\pm7\%$, T7: $4\pm4\%$), CVRC on ipsilateral hemisphere was initially decreased (T1: $-4\pm4\%$, T3: $-3\pm6\%$) and restored after 5 days (T5: $3\pm6\%$, T7: $11\pm4\%$). The global 4-VO model showed a marked decrease in CBF to $12\pm2\%$ from baseline after temporary clapping of CCAs. An immediate overflow ($139\pm16\%$ of baseline) after recirculation was shown and its flow maintained below baseline until 7 days (T1: $87\pm7\%$, T7: $85\pm5\%$). The global 4-VO model also showed a selective neuronal death at CA1 region of the hippocampus. CVRC on bilateral hemispheres remained above baseline. **CONCLUSIONS:** Our data shows that CVRC has a temporary reduction and restoration in focal tMCAO, and it also increases above baseline in global 4-VO models. Such findings can be feasible as biological surrogates to mitigate subsequent brain damage for new drug development in post-cardiac arrest care.

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Poster

306. Stroke: Imaging Assessments

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Topic: C.08.Stroke

Support: NHRI-BN-PP-06

NHRI/Central Government S & T grant, Taiwan (106-1901-01-10-02)

Title: Using diffusion kurtosis magnetic resonance imaging to monitor iPS-MSCs treatment response in stroke

Authors: *C.-W. CHIANG¹, K.-J. WU², S.-Y. LIN^{1,4}, K.-H. CHO¹, B. YEN³, Y. WANG², L.-W. KUO^{1,5}

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Abstract: After stroke onset, the degenerative reactions were activated leading to cell death and affecting neural repair. Stem cell administration shows an attractive therapeutic approach that reduces inflammation and enhances brain reorganization. Diffusion kurtosis imaging (DKI), an extension of diffusion tensor imaging (DTI), not only computes diffusion tensors but also allows to evaluate the non-Gaussianity of water diffusion. DKI has also been demonstrated to provide

complementary and sensitive imaging biomarkers in many neurological diseases. For example, an increase of mean kurtosis (MK) may be represented as an increase of cell infiltration. Recently, many therapies were focused on pre-treatment study which limits the application of many stroke patients. Therefore, it is important to expend the therapeutic window as well as to apply appropriate neuroimaging to monitor the ischemic damage and recovery. In this study, we aimed to apply DKI to evaluate the stem cell treatment response at different time points and further investigate the underneath pathology. Adult Sprague-Dawley rats were underwent right middle cerebral artery occlusion (MCAo) for 60 minutes. Our recently-developed induced pluripotent stem cells from human mesenchymal stromal cells (iPS-MSCs) was administrated. Cyclosporine was employed as anti-inflammation agent. A total of 12 stroke rats was underwent iPS-MSCs (n=4), iPS-MSCs with cyclosporine (n=5), or vehicle (n=3) grafting on contralateral corpus callosum of rat brain on day 2 post-stroke. T₂-weighted imaging (T2WI) and diffusion weighted imaging (DWI) were acquired on day 3, 10 and 16, using a 7T/30cm animal scanner (Bruker Biospec, Germany). T2WI was acquired for examination of infarct volume. Diffusion tensors and diffusion kurtosis tensors were reconstructed with DKI model analysis. Regions-ofinterest (ROIs) of cortex and external capsule on both lesioned and contralateral sites of rat brain around bregma were selected. ROI analysis was performed on DKI-computed MD and MK maps for cortex as well as on FA maps for white matter. Two sample t-test was performed for statistical analysis. Our preliminary results suggest, by DKI, transplantation of iPS-MSCs poststoke shows the efficacy to resolve inflammation and potentially enhance neural repair at chronic. DKI shows the ability to provide sensitive imaging biomarkers for neuronal pathologies in stroke non-invasively. Histology will be further examined for pathological validation.

Disclosures: C. Chiang: None. K. Wu: None. S. Lin: None. K. Cho: None. B. Yen: None. Y. Wang: None. L. Kuo: None.

Poster

306. Stroke: Imaging Assessments

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 306.07/Z19

Topic: C.08.Stroke

Title: Determination of stroke volume using multi-parametric analysis based on *In vivo* MRI measurements in rats

Authors: *K. J. PATZWALDT¹, P. KATIYAR¹, Y. WANG², X. YANG², R. K. LERCH¹, J. A. DISSELHORST¹, S. POLI³, S. CASTANEDA VEGA¹, B. J. PICHLER¹ ¹Werner Siemens Imaging Center, Dept. of Preclinical Imaging & Radiopharmacy, ²Herti Inst. for Clin. Brain Res., Eberhard Karls Univ. of Tuebingen, Tuebingen, Germany; ³Dept. of Neurol. & Stroke, Univ. Hosp. Tuebingen, Tuebingen, Germany

Abstract: Ischemic Stroke (IS) is the third most common cause of death affecting 15 million people each year worldwide. Magnetic resonance imaging (MRI) allows non-invasive in vivo evaluations of stroke volume (SV), edema and perfusion. However, reliable computational methods for measuring SV are still warranted since subjective analysis of multi-parametric MRI data often leads to an overestimation of SV, which is partly due to the lack of specificity (edema vs stroke) in T2 weighted images (T2WI). In order to develop personalized therapies, it is essential to accurately assess the extent of ischemic injury using automated algorithms analyzing multi-parametric data. Therefore, in this work we aimed to determine the spatial distribution of ischemic lesions and other biological compartments using a Gaussian mixture model (GMM) and the following MRI features: apparent diffusion coefficient (ADC) maps, perfusion weighted images (PWI) and T2WI. We induced IS on rats using the middle cerebral artery temporary occlusion stroke model (MCAO). MRI was measured longitudinally at multiple time points until 2 weeks post-stroke using ADC, PWI and T2WI and behavioral tests (BT) were performed per time point. ADC maps and PWI were co-registered to the T2WIs and volumes of interest (VOIs) were drawn around the brain. The 24 h time point MRI data of 16 rats were divided into training (n=12) and testing (n=4) group. A GMM was applied on the training set to identify different biological compartments, including stroke and non-stroke areas. Testing was performed using random forest and classification maps were corroborated using histology. A strong spatial correspondence was present between the stroke areas identified using the proposed approach and the corresponding histology. The BTs also indicated the severity of the symptoms correlating to the clustering results. Stroke compartment was characterized in the training step with an average ADC value of $[401.82 \pm 64.11] \times 10^6 \text{ mm}^2/\text{s}$, a perfusion value of $[38.0 \pm 17.93] \text{ ml}/100\text{g/min}$. Likewise, the average ADC and perfusion values for the stroke cluster in the test phase were $[369.32 \pm 70.44] \times 10^6$ mm²/s and $[31.9 \pm 15.16]$ ml/100g/min, respectively. The proposed algorithm identified clusters with radiological characteristics classical of IS, which we were able to corroborate with histology at 24 h. Using this machine learning approach on multi-parametric MRI data, we aim to model the behavior of the stroke region over the remaining time points and subsequently, to objectively characterize and evaluate the efficacy of treatments at different time points.

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Poster

306. Stroke: Imaging Assessments

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: C.08.Stroke

Support: The Dunhill Medical Trust [R38J/114]

Title: An introduction to 'F2': A quantitative MRI classifier for treatment stratification of acute ischaemic stroke patients with unknown onset time

Authors: *B. L. MCGARRY¹, M. J. KNIGHT¹, P. CLATWORTHY², R. BOSNELL², D. CARONE³, G. HARSTON³, J. KENNEDY³, P. JEZZARD⁴, R. A. KAUPPINEN¹ ¹Sch. of Exptl. Psychology and Bristol Clin. Res. and Imaging Cent, Univ. of Bristol, Clifton, United Kingdom; ²North Bristol NHS Trust, Bristol, United Kingdom; ³Radcliffe Dept. of Med., Acute Vascular Imaging Ctr., University of Oxford, United Kingdom; ⁴Nuffield Dept. of Clin. Neurosci., Oxford Ctr. for Functional MRI of the Brain, University of Oxford, United Kingdom

Abstract: Unknown onset time is a major contraindication to hyper acute ischaemic stroke treatment. If MRI could identify patients within the therapeutic time window or with enough salvageable tissue, many more patients would receive IV thrombolysis. Weighted MR images are unreliable due to shading effects from strong brain surface signals and bias field problems resulting from multi-element receiver systems. Quantitative T_2 (qT_2) relaxation time images avoid these problems. Elevated qT_2 is a potential proxy for stroke timing and tissue status ^{1,2,3}, however the increase is small (1-2 ms/hr) and cerebral qT₂ distributions are broad, reflecting heterogeneity of ischaemic lesions^{3,4}. We propose a classifier 'F2' to identify potentially treatable strokes^{3,4}. F2 is the proportion of voxels with elevated qT_2 within regions of decreased apparent diffusion coefficient (ADC); F2 increases with time in a rat stroke model⁴. **Objectives:** We determine qT_2 and F2 time dependencies in ischaemic stroke patients. **Methods:** In this ongoing study, ischaemic stroke patients (onset < 9hrs) are scanned at 3T at 2 hospitals. Protocol includes ADC, multi-echo T_2 and $3D-T_1$. Ischaemic tissue = ADC voxels 1MAD < whole-brain ADC distribution. Elevated $qT_2 = qT_2$ voxels > modal non-ischaemic qT_2 by HWHM. F2 =100*(high qT₂ voxels/low ADC voxels). Potential issues considered include lesion size and location, and natural regional variability in qT_2 including low qT_2 in iron rich

regions.

Results (Figure 1): Initial observations of the first 7 patients with ADC lesions > 2cm³ are consistent with rat stroke³ as elevated qT₂ is heterogeneous within low ADC volumes and larger F2s occur in later onsets.

Conclusions: F2 may identify patients within the therapeutic window and reveal the extent of potentially salvageable tissue. Thus, F2 is a potential classifier for treatment stratification of stroke patients with unknown onset.

¹ Rogers et al. Neuroreport.2014. 25(15):1180-5

² Siemonsen et al. Stroke.2009.40(5):1612-6

³ McGarry et al. Int J Stroke.2016.11(6):677-82

⁴ Knight et al. J Cereb Blood Flow Metab. 2016.36(7):1232-43



Figure 1. A. F2 values of ischaemic stroke patients with different onset times. Bottom right hand graph shows average F2s over time in rats from data published in McGarry et al³. B. ADC & elevated qT_2 in a typical rat up to 4 hours post stroke³. C. Reduced ADC and elevated qT_2 in 3 stroke patients scanned at different times from onset (TFO – rounded to nearest 5 mins).

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Poster

306. Stroke: Imaging Assessments

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 306.09/Z21

Topic: C.08.Stroke

Support: IZKF Promotionskolleg

Title: A theranostic approach in stroke imaging: The benefit of using an intranasal erythropoietin-tracer for multiparametric imaging

Authors: *R. K. LERCH, K. J. PATZWALDT, P. KATIYAR, R. STUMM, N. ALTMEYER, A. MAURER, S. CASTANEDA VEGA, B. J. PICHLER Dept. of Preclinical Imaging and Radiopharmacy, Werner Siemens Imaging Ctr., Tübingen, Germany

Abstract: Erythropoietin (EPO) is a glycoprotein that mediates neuroprotective effects through various pathways. It was shown, that there is an upregulation of the EPO-receptor in the brain under hypoxic conditions, which makes it a specific target for brain imaging and neuroprotection in stroke research. However, EPO administered intravenously can lead to

increased systemic side effects due to altered blood parameters. Therefore, an alternative administration route which reduces systemic delivery and simultaneously increased brain delivery is warranted. In this project, we investigated the therapeutic potential of Cu-64 radiolabelled EPO, as well as its applicability for imaging when administered intranasally. Sprague Dawley rats were induced a stroke using the middle cerebral artery occlusion stroke model. The rats were randomly divided into groups, with each rat receiving a dose of radiolabelled EPO intranasally three hours after stroke on-set at different dosages. Stroke progression was examined repeatedly up to three months after on-set of the stroke using behavioural tasks and brain imaging consisting of T2-weighted magnetic resonance imaging (MRI), diffusion weighted MRI, perfusion weighted MRI and positron emission tomography (PET). Furthermore, we performed autoradiography and biodistribution after 24 hours. The biodistribution of intranasal EPO showed a remarkably different biodistribution pattern when compared with intravenous administration, specially in the kidney. Results of the autoradiography showed, that an increased uptake in the brain and the stroke area, is achievable but rather inconsistent. PET-images showed inhomogeneous brain uptake which we attribute to a not long enough half-life of Cu-64 to demonstrate brain penetration. However, the behavioural and the stroke volume data showed a tendency for a dose dependent effect of the EPO-tracer on stroke-outcome.

Our work shows that EPO presented a promising reduction of stroke volume in a dose-dependent manner without increasing the biodistribution in non-target organs, hence potentially decreasing systemic side effects. The findings are consistent with literature, where it has been shown small amounts of EPO mediate an effect when administered intranasally . Ongoing work using a longer half-life tracer will likely allow a better visualization of the distribution of EPO in the stroke area and present neuroprotection without systemic side effects.

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Poster

306. Stroke: Imaging Assessments

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Program#/Poster#: 306.10/Z22

Topic: C.08.Stroke

Title: A novel infarct-avid agent ¹⁸F-fluorodeoxyglucaric acid (FGA) for imaging brain stroke by positron emission tomography (PET)

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Abstract: Background: Stroke is a common cause of death worldwide and leads to considerable disability among survivors. Current management of stroke patients is aimed at preventing the progression of at-risk tissue towards infarction by restoring blood supply to ischemic penumbra. Diffusion-perfusion mismatch in magnetic resonance or computed tomography images is routinely employed for discerning non-viable and viable tissue and optimize treatment in brain stroke patients. However, diffusion-perfusion mismatch is not always a reliable indicator of salvageable tissue; some lesions may show acute reversal, whereas others may fail to infarct. The objective of this study was to synthesize ¹⁸F-FGA and demonstrate its potential for PET imaging of infarct in the brain stroke. Methods: Radiopharmaceutical ¹⁸F-FGA was synthesized in onepot by 3 min controlled oxidation of commercially available ¹⁸F-fluorodeoxyglucose (FDG). Quality control of radiopharmaceutical was performed by ion-exchange HPLC and thin-layer chromatography. Brain stroke was induced in the left cerebral hemisphere of male CD-1 mice (20-25 g) by occluding middle cerebral artery and allowing reperfusion after 1 h of occlusion (transient MCAO model). Post-stroke PET imaging was performed on the day of occlusion as well as 24 h after occlusion. Approximately 100-500 µCi of ¹⁸F-FGA was injected intravenously and PET data was acquired for 20 min after 1 and 2 h of injection. In some instances, right and left cerebral hemispheres were separated upon necropsy and radioactivity counting was performed in a well counter to compare ipsilateral versus contralateral accumulation of ¹⁸F-FGA. Results: Synthesis of ¹⁸F-FGA was accomplished in a quantitative manner from rapid oxidation of ¹⁸F-FDG. The synthesized ¹⁸F-FGA was well tolerated by mice when injected intravenously. Ex vivo counting of the brain hemispheres showed that ipsilateral tissue accumulated more than 2.5 times ¹⁸F-FGA as compared to the contralateral tissue (p < 0.05, Student's t-test). However, in vivo images were difficult to interpret because of the small volume of injury surrounded by non-specific accumulation of ¹⁸F-FGA in nasal secretions. To circumvent this, we isolated the intact brain together with skull for imaging; these ex vivo images clearly showed higher ipsilateral accumulation of ¹⁸F-FGA as compared to the contralateral hemisphere. Conclusions: Results show that infarct-avid agent ¹⁸F-FGA can be synthesized from ubiquitously available ¹⁸F-FDG in a simple reaction. As the first infarct-avid PET agent, ¹⁸F-FGA has a potential to enable extremely sensitive and high resolution neuroimaging of the brain infarct pathologies.

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Poster

306. Stroke: Imaging Assessments

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Title: Genomic analysis revealed changes of inflammation-related genes in a mice model of focal cerebral ischemia

Authors: *Z. CHENGJIE^{1,2,3}, Y. B. ZHU³, S. WANG², Z. Z. WEI², M. Q. JIANG², Y. B. ZHANG¹, Y. H. L. PAN³, S. X. TAO³, J. M. LI¹, L. WEI² ¹Neurol. Beijing Friendship Hosp.Capital Med. Univ., Beijing City, China; ²Anesthesiol., Sch. Med., Emory Univ., Atlanta, GA; ³Labs. Stem Cell Biol. Regen. Med., Exp. Res. Ctr., Beijing Friendship Hosp., Beijing, China

Abstract: Ischemic stroke remains one of the leading causes of human death and disability in the world, caused by a cascade of pathological processes including increased inflammation involving inflammatory cell activation and filtration in and around ischemic lesion areas. However, our understanding of these complicated cellular and molecular events following ischemic stroke are still incomplete. In this investigation, we used RNA sequencing to analyze the whole genomic gene expression profiles at different days (D1, D3, D7, D14, D21, D60, D90) in a mice model of focal cerebral ischemia. The result showed that the number of differentially expressed genes (DEGs) increased over time after ischemia. There were 1967 DEGs at D1, 2280 DEGs at D3, 2631 DEGs at D7, 5516 DEGs at D14, 7093 DEGs at D21, 3971 DEGs at D60, and 4471 DEGs at D90. By performing gene ontology enrichment and network analyses, 87 DEGs significantly related to inflammation were identified. Among them, pro-inflammatory genes CD86, tumour necrosis factor (TNF)- α , and interleukin (IL)-1 β were all upregulated and peaked at D14; antiinflammatory genes arginase 1 (Arg1) and Chitinase-like 3 (Ym1) peaked at D1 while IL-10, transforming growth factor (TGF)-β and CD206 peaked by D7 to D14. Our study provided new insights and detailed information on the molecular pathology of experimental ischemic stroke. The genomic data analysis-revealed regulatory mechanisms would be useful in developing therapeutic interventions targeting inflammation of ischemic stroke in humans.

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Poster

306. Stroke: Imaging Assessments

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Title: Regional diffusion differences in people with severe upper limb impairment post-stroke: A multi centre study

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Abstract: Background: Severe upper limb (UL) impairment post-stroke often results from significant structural damage to the corticospinal tract (CST), the primary motor output pathway. In individuals with severe impairment with movement limited to proximal musculature, structural integrity of supporting motor pathways may be important. Aim: To determine the unique contribution of CST, alternate motor (corticorubrospinal and corticoreticulospinal), and corpus callosum (CC) tracts to motor outcome in individuals with severe as compared to mild-moderate impairment post-stroke and non-stroke older adults. We hypothesise: 1) tract order of importance for motor outcome in the severe group will be CST followed by alternate motor and CC tract integrity; 2) time post-stroke may alter the pattern of importance in the severe group; and 3) an overall effect of group by pathway, such that people with severe have the poorest tract integrity. Methods: Data was pooled from four centres across three participant groups defined

apriori: individuals with 1) severe impairment (Fugl-Meyer Upper Limb, [FM-UL] score: \leq 30/66); 2) mild-moderate impairment (FM-UL score: >30/66); 3) no history of stroke. All individuals underwent diffusion weighted imaging (DWI). Participants with stroke completed a motor impairment (FM-UL) and/or function (Wolf Motor Function Test [WMFT] WMFT-rate) assessment. Hand-drawn regions of interest were delineated to reconstruct the CST, alternate motor, and CC tracts, and extract mean fractional anisotropy (FA). Planned analysis are: 1) stepwise linear regression model for each dependent motor outcome (FM-UL, WMFT-rate) for each stroke group (severe, mild-moderate), with mean FA of reconstructed tracts entered as independent variables; 2) one-way ANOVA with between-subject factor of TIME (subacute, chronic) in severe group only; and 3) two-way mixed model ANOVA of GROUP (severe, mildmoderate, healthy) x MOTOR PATHWAY (FA of CST, alternate motor, CC). Results: We have DWI data from 65 individuals with severe UL impairment (30 subacute, 35 chronic), 30 with chronic mild-moderate impairment and 30 non-stroke adults. Preliminary analysis of 10 chronic individuals from one centre indicated that CC was more strongly correlated with impairment than CST or alternate motor tracts. Conclusions: This pooled analysis of DWI in people with severe UL impairment after stroke will advance our understanding of the regional importance of white matter status to motor outcome across severity levels; inform studies of stroke recovery; and contribute to more precise targeting of treatments to individual patients based on white matter status.

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Poster

306. Stroke: Imaging Assessments

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Program#/Poster#: 306.13/Z25

Topic: C.08.Stroke

Support: NIH R01-HD-065438

Title: Quantification of corticospinal tract using DTI in chronic stroke survivors

Authors: *B. KIM¹, S. CHOI², D. B. KAY², N. SCHWEIGHOFER¹, J. P. HALDAR³, R. M. LEAHY³, B. E. FISHER¹, C. J. WINSTEIN¹ ¹Div. of Biokinesiology and Physical Therapy, ²Neurosci. Grad. Program, ³Ming Hsieh Dept. of Electrical Engin., USC, Los Angeles, CA **Abstract:** There are a number of different DTI-based methods that have been used to quantify corticospinal tract (CST) structure in the context of stroke rehabilitation research. However, there is no gold standard method that has been shown to provide the most accurate estimate of CST structure in chronic stroke survivors. This study aims to compare different DTI-based approaches to quantify CST structural characteristics in chronic stroke survivors, and to determine which, if any, reveals the strongest brain-behavior relationship. We used DTI and clinical motor behavior data from a phase-I clinical trial. Participants were chronic stroke survivors with mild-tomoderate arm and hand motor impairment (N=37, average chronicity=3 years). We processed the Imaging data using BrainSuite16a (http://brainsuite.org/), and used the Wolf Motor Function Test log mean time score for distal control items (WMFT-distal) as the primary motor behavior measure. We calculated mean Fractional Anisotropy (FA) for CST of each tract (L, R) with 7 different approaches: 1) manually drawn 2-D posterior limb of the internal capsule (PLIC) region, 2) manually drawn 2-D cerebral peduncle (CP) region, 3) 3-D PLIC template volume from a JHU white matter atlas, 4) 3-D CP template volume from a JHU white matter atlas, 5) 3-D CST individual volume from each participant's tractography, 6) 3-D CST template volume from a standard white matter atlas, and 7) 3-D CST template volume generated from participants' contra-lesional CST. We compared CST FA between the two tracts for each method using a paired *t*-test; calculated a CST FA asymmetry index between the two hemispheres from each approach, and performed partial correlation analyses between each CST FA asymmetry index and WMFT-distal time score, controlled for age, chronicity, and severity. The mean ipsilesional CST FA was significantly lower than the mean contralesional CST FA for all 7 methods. Only CST FA asymmetry from the 3-D CST individual volume from each participant's tractography significantly correlated with WMFT-distal (r=0.46, p=0.005). Further only range of CST FA asymmetry from individual tractography met the criterion of CST FA asymmetry for those who have mild-to-moderate arm motor impairment. These findings suggest that compared to the six other methods, CST FA asymmetry based on the individual's CST tractography is the most accurate estimate of CST structural characteristics in chronic stroke survivors with mild-tomoderate motor impairment. We recommend this method for future studies in chronic stroke that aims to investigate the relationship between CST structural characteristics and motor behavior.

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Poster

306. Stroke: Imaging Assessments

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Topic: C.08.Stroke

Support: the Strategic Research Program for Brain Science of BMI Technologies for Clinical Application carried out under the Strategic Research Program for Brain Sciences

Title: Estimation of motor impairment in sub-cute stroke patients using least absolute and selection operator (LASSO) analysis of resting state functional connectivity

Authors: *T. NAKAMURA¹, F. HOTTA², K. SHINDO², M. HIROSAWA², J. USHIBA³, T. HANAKAWA⁴

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Abstract: Functional imaging as a prognostic biomarker for post-stroke motor recovery would help understanding of neural mechanisms in post-stroke recovery. Among various types of imaging modalities, resting-state fMRI (rs-fMRI) is one of the emerging methods yielding biomarkers of brain conditions. In this research, we tested association between rs-fMRI and clinical motor impairment scale using least absolute and selection operator (LASSO) analysis.Fifty-three post-stroke hemiplegic patients in the subacute stage participated in this study after giving written informed consent. Clinical motor impairment scale, Fugl-Meyer Assessment (FMA) motor score, and rs-MRI scans were obtained at the admission, 8 weeks after admission, and the discharge. In total, 96 rs-fMRI scans were used for the analysis. MRI data were acquired using a 3-T scanner (GE Discovery MR750w). T1 weighted image was acquired for the localization of the lesions and for adjunct data for rs-fMRI analysis.rs-fMRI data were pre-processed with slice timing correction, motion correction, spatial normalization to the standard brain space and smoothing, with functional connectivity toolbox and SPM12. rs-fMRI was flipped between right and left when a patient had impairment in the left-side impairment. Functional connectivity matrix was estimated as a correlation coefficient of MRI signals for each pair of anatomical volumes of interest (AAL). Features of the functional connectivity matrix that statistically correlates with FMA were selected by LASSO model.Figure 1 shows functional connectivity selected by LASSO for the estimation of FMA in all the patients. Functional connection including the ipsilateral cerebellum and fronto-parieto-temporal opercular areas contralateral to the impaired hemibody are positively associated with FMA. The present study suggests that rs-fMRI can be used as a biomarker of motor impairment in post-stroke hemiplegia.



Fig. 1 Functional connectivity between AAL ROIs. (a) connectivity with cerebellum area. (b) extract only ipsilateral area

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Poster

306. Stroke: Imaging Assessments

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Topic: C.08.Stroke

Support: RPC- Cleveland Clinic

Title: Ascending cerebello-cortical projection viewed by manganese-enhanced MRI

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Abstract: Pre-clinical studies in rodent models of stroke suggest that deep brain stimulation (DBS) of the lateral cerebellar nucleus (LCN) may be a promising treatment to enhance recovery after stroke.Our aim here was to map, both anatomically and functionally, the cerebellar-cortical

projection in vivo using manganese-enhanced MRI (MEMRI). In the first part of the study the cerebellar-cortical projections were mapped by tracking manganese chloride (MnCl₂) transport over a 48-h period. For this, male Long-Evans rats a single injection of MnCl₂ into LCN (500 mM, 1 uL). MRI scans were performed at 2-, 8-, 24- and 48-h post injection utilizing a 3D T1-FLASH protocol with the following parameters: TE=6.37 ms, TR=50 ms, SA=3, Flip angle = 20, bandwidth = 20,000 Hz, and FOV = $200 \times 200 \times 200$ (Bruker Biospec Avance 70/20 (Bruker Biospin) system with a 72 mm volume coil setup). MRI processing and quantification were performed using FSL and AFNI toolkits. To visualize the LCN-cortical projections at 8h, 24H and 48H after injection, a whole brain statistical map (z map) was quantified for each time point by comparisons with the 2h scan. The voxel that survived FDR correction at a threshold of p<0.05 on the z map were included in the final visualization map. Our results indicate that MEMRI is a suitable and accurate way to study cerebello-cortical projections in vivo; 8H after LCN manganese chloride injection the contrast can be visualized in cortical areas, mainly contralateral. The peak of contrast was obtained 24H post-injection with bilateral distribution and faded by 48H. In the second part of the study, we tested the functionality of this projection via DBS activation. Animals underwent surgical implant of MRI-compatible electrode into the LCN. One week later, these animals received a systemic injection of MnCl₂ (60 mg/kg). Twelve hours post injection, the animals were scanned using the same MRI protocol as above. Immediately after the first scan, the animals received one hour of DBS with the following parameters: 100- 200 uA, 30 Hz, 90 us. LCN-DBS was then followed by a second MRI scan. Similar manganese-induced enhancement in contrast was observed in animals that underwent LCN DBS after systemic manganese chloride injection. Differences between pre and post-DBS MRI are higher than 20% for most studied brain areas. In contrast, animals who did not receive DBS showed no increase the in the MEMRI contrast. Overall our data suggest that the LCN-DBS can be functionally evaluated by means MEMRI in longitudinal in vivo studies.

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Poster

307. Stroke: Neuroprotection

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 307.01/Z28

Topic: C.08.Stroke

Support: Department of Biotechnology (DBT), Govt. of INDIA

Title: Monomethyl fumarate confers neuroprotective effect against experimental model of ischemic stroke in rat by inhibiting TNF- α and IL-1 β

Authors: *D. SINGH¹, R. KH², U. SHARMA³, J. NR³, Y. K. GUPTA² ²Pharmacol., ³NMR &MRI Facility, ¹All India Inst. of Med. Sci. (AIIMS) Ne, New Delhi, India

Abstract: Despite advances in the understanding the pathophysiology of cerebral ischemia, no drug is available to protect the brain from stroke-induced neuronal injury. Increasing evidences suggest that inflammatory cytokines plays an important role in progression of stroke. The objective of this study was to evaluate the effect of an ester of fumaric acid, monomethy fumarate (MMF) on cerebral infarct and levels of inflammatory cytokines TNF- α and IL-1 β . Middle cerebral artery was occluded using a 3.0 monofilament in male Sprague Dawley rats (260-290 g) for 90 min followed by 24 h of reperfusion. Laser Doppler flow meter was used to confirm middle cerebral artery occlusion (MCAo). MMF (10 mg/kg) was administered at two time points, 30 min post ischemia and 5 min post reperfusion. Twenty four hours later, neurological deficits score and time spent on rota rod were evaluated. Cerebral infarct was estimated by MRI-T2 imaging using 7.0T animals MRI. After MRI, rats were sacrificed; cortex and striatum were separated and homogenized. The levels of TNF- α and IL-1 β were estimated using ELISA kits. Post occlusion, cerebral blood flow reduced by $82.8 \pm 1.8\%$ of baseline and post reperfusion 71.1 \pm 2.7% of blood flow was found to be restored. Treatment with MMF improved neurological deficit score and the time spent on rota rod significantly (p<0.01) when compared to MCAo group. Further, in MMF group, cerebral infarct $(23.5 \pm 4.3 \%)$ was significantly (p<0.01) reduced when compared to MCAo group (36.6 ± 2.0 % of ipsilateral area). The levels of inflammatory cytokines were increased significantly in MCAo group when compared to sham group. MMF treatment significantly (p<0.05) reduced the levels of both TNF- α (115 ± 25 to 83 ± 6.5 pg/mg of protein) and IL-1 β (130 ± 10 to 99 ± 6.1 pg/mg of protein) in cortex when compared to MCAo group. However, in striatum, there was no significant decrease in the levels of cytokines when compared to MCAo group. Our results indicate that MMF limits the progression of brain infarct from striatum to cortex by regulating the levels of inflammatory cytokines.

Disclosures: D. Singh: None. R. Kh: None. U. Sharma: None. J. Nr: None. Y.K. Gupta: None.

Poster

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Topic: C.08.Stroke

Support: NHRI intramural grant

Title: CXCR4 antagonist CX549 induces neuroprotection in stroke brain

Authors: *Y. WANG, K.-J. WU, S.-J. YU, E. BAE, K.-S. SHIA Natl. Hlth. Res. Inst., Zhunan, Taiwan

Abstract: C-X-C chemokine receptor type 4 (CXCR4) is a receptor for a pleiotropic chemokine CXCL12. The purpose of this study was to characterize the neuroprotective and neurotrophic effect of a novel CXCR4 antagonist CX549. We demonstrated that CX549 had a higher affinity for CXCR4 and was potent to inhibit CXCL12 -mediated chemotaxis in culture. CX549 effectively reduced the activation of microglia and improved neuronal survival after injury in primary rat cortical neuron and BV2 microglia co-cultures. The protective effect of CX549 was further examined in an animal model of stroke. Adult male rats were subjected to a transient (60 min) distal middle cerebral artery occlusion. CX549 or vehicle was administered to the animals at 10 min after the onset of reperfusion and then daily for 4 days. Early post-stroke treatment with CX549 significantly improved behavioral function, reduced brain infarction, and suppressed the expression of inflammatory markers TNFa and IL6. Our data support that CX549 is a potent anti-inflammatory and neuroprotective agent against ischemic brain injury and may have clinical implications for the treatment of stroke.

Disclosures: Y. Wang: None. K. Wu: None. S. Yu: None. E. Bae: None. K. Shia: None.

Poster

307. Stroke: Neuroprotection

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Support: NIH Grant R00EY021624

NIH Grant UH2NS100121

cureCADASIL Association research grant

Title: Therapeutic antibody targeting of Notch 3 signaling prevents mural cell loss in CADASIL

Authors: *J. ARBOLEDA-VELASQUEZ, A. I. MACHUCA-PARRA, A. A. BIGGER-ALLEN, A. V. SANCHEZ

Schepens Eye Res. Inst., Boston, MA

Abstract: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a neurological syndrome characterized by small vessel disease (SVD), stroke, and vascular cognitive impairment and dementia caused by mutations in NOTCH 3. No therapies are available for this condition. The prevalence of CADASIL mutations in NOTCH 3 is estimated at 3.4/1,000. Loss of mural cells, which encompass pericytes and

vascular smooth muscle cells, is a hallmark of CADASIL and other small vessel diseases including diabetic retinopathy, resulting in vascular instability. We report the characterization of a mouse model of CADASIL in which mural cell coverage in arteries depends upon human Notch 3 function. Here we showed that Notch 3 signaling is both necessary and sufficient to support mural cell coverage in arteries using genetic rescue in Notch 3 knockout mice. Furthermore, we show that systemic administration of an agonist Notch 3 antibody prevents mural cell loss and modifies plasma proteins associated with Notch 3 activity including endostatin/collagen 18a1, and Notch 3 ectodomain in mice with the C455R mutation, a CADASIL variant associated with Notch 3 loss-of-function. These findings open opportunities for the treatment of CADASIL by modulating Notch 3 signaling and establish that the levels of Notch 3 ectodomain and endostatin/collagen 18a1 in liquid biopsies could be used as surrogate markers of Notch 3 activity in vivo. Figure shows (A) Timeline of A13 antibody injections in N3KO (light gray) and CADASIL mutant mice (black). (B) Immunofluorescence images of retinal whole mounts showing SMA staining in green and collagen IV (ColIV) in white for N3KO and C455R mutant mice treated with IgG or A13 antibodies (scale bar=1mm). Right panels show merged images of green and white signals (scale bar = 100μ m). (C) Quantification of the effect of antibody injection on SMA coverage in retinal vasculatures. *p<0.05, data were analyzed via ANOVA. (D) Images of brain tissue from C455R injected with A13 or IgG antibody stained with V1662 antibody. Scale bar = $100 \,\mu m$.



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Poster

307. Stroke: Neuroprotection

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Topic: C.08.Stroke

Support: Institutional support

Title: Green tea polyphenols and cAMP induce internalization of the Nogo-A receptor NgR1 and desensitize neuronal cells to axonal growth inhibitor Nogo-A

Authors: *R. GOPALAKRISHNA¹, U. GUNDIMEDA², S. ZHOU², H. BUI², C. LE², A. DAVIS², T. MCNEILL², W. MACK² ¹Cell and Neurobio., ²USC Keck Sch. of Med., Los Angeles, CA

Abstract: Recovery from injury to the mammalian CNS requires axonal regeneration from the remaining neurons. However, conditions in the CNS are impermissive to this regeneration because of the presence of axonal growth inhibitors such as Nogo-A. NgR1, a cell-surface protein, serves as a receptor for Nogo-A and other axonal growth inhibitors. Currently, various pharmacological agents are being evaluated for the ability to block NgR1 or its downstream signaling to enhance axonal sprouting and functional recovery. Inexpensive and safe natural products such as green tea polyphenols (GTPP) are well suited for this purpose and elucidating their mechanism of action will help optimize their use and develop drugs with greater efficacy. In the current study, we found that GTPP, especially epigallocatechin-3-gallate, induced the internalization of NgR1 in Neuroscreen-1 neuronal-like cells and mouse primary cortical neurons. This NgR1 internalization was mediated by cAMP, which was generated in response to GTPP binding to a high affinity 67kDa laminin receptor. Consistent with this finding, exogenous dibutyryl-cAMP and agents that elevate intracellular cAMP such as forskolin, an adenylyl cyclase activator, and rolipram, a cyclic nucleotide phosphodiesterase inhibitor, induced substantial internalization of NgR1. This GTPP- and cAMP-induced internalization also occurred in protein kinase A(PKA)-deficient cells. Furthermore, N⁶-benzoyl-cAMP, which selectively activates PKA, did not induce NgR1 internalization. In addition, KT 5720, a specific PKA inhibitor, failed to block the GTPP- and cAMP-induced internalization of NgR1. Conversely, 8-pCPT-2'-O-methyl-cAMP, which selectively activates another effector of cAMP, EPAC (exchange protein directly activated by cAMP), induced internalization of NgR1. Moreover, ESI-09, an EPAC-specific inhibitor, blocked GTPP- and cAMP-induced internalization of NgR1. These results suggest that EPAC may play a greater role than PKA in

this process. This internalization was mediated by both clathrin- and lipid raft-mediated mechanisms. Internalization of NgR1 by GTPP and cAMP, correlated with their ability to prevent Nogo-A from causing growth cone collapse and inhibiting neurite outgrowth. Desensitization of neurons through the internalization of NgR1 may be one of the intracellular mechanisms to influence axonal growth and may be exploited for drug development to enhance functional recovery after stroke and other neuronal injuries.

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Poster

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Louisiana State University Research Council

Title: Palmitic acid methylation provides neuroprotection against cerebral ischemia

Authors: *A. DO COUTO E SILVA¹, R. H.-C. LEE², C. Y.-C. WU², H. POSSOIT², R. AZIZBAYEVA³, J. NEUMANN³, H. LIN² ¹Cell. Biol. and Anat., ²Neurol., LSU Hlth. Sci. Ctr. Shreveport, Shreveport, LA; ³West Virginia Sch. of Osteo. Med., Lewisburg, VA

Abstract: Cardiopulmonary arrest (CA) remains one of the leading causes of death and disability in the USA. Whole body ischemia disrupts cerebral blood flow (CBF) that results in severe and selective brain damage. CA-induced hypoperfusion (decrease in CBF) contributes to neuronal cell death leading to cognitive impairment. Our goal is to identify novel neuroprotective therapies that will modulate CBF and provide neuroprotection with beneficial functional outcomes after ischemia. We previously discovered that palmitic acid methyl ester (PAME) is a novel vasodilator/neuroprotective agent derived from the superior cervical ganglion (sympathetic) innervating major cerebral arteries enhanced in the presence of arginine derivatives. Arginine is a substrate to activate protein arginine methyltransferases (PRMTs), which can act as a "switch" to turn "on or off" pre or post-transcriptional/translational modifications. Thus, our hypothesis is that methylation of palmitic acid (PA) via PRMT to form PAME, enables PAME's therapeutic actions against ischemia, providing enhancements in CBF, neuroprotection, and functional recovery. Methylation of PA to form PAME but not PA alone, is

necessary to enhance CBF, neuroprotection, and cognitive function after asphyxial CA (ACA, 6 min). To investigate the neuroprotective properties of PA v. PAME, hippocampal slices were subjected to oxygen glucose deprivation (OGD), and visualized using propidium iodide staining for cell death. Our results indicate that PAME, but not PA, provides neuroprotection in the CA1 region of the hippocampus after OGD [vehicle OGD (0.538 ± 0.022) and PAME OGD (0.232 ± 0.055)]. Furthermore, we utilized a rodent model of global cerebral ischemia (ACA, 6 min) to investigate if the methylation is crucial for the enhancement of CBF after ACA. Rats received PA/PAME treatment (0.02mg/kg, IV) 30 min before ACA. The rat skull was thinned to visualize red blood cell speed (an indicative measure of CBF) in microvessels of the neocortex via intravital two-photon laser scanning microscopy. PAME enhanced cortical CBF while maintaining systemic blood pressure in vivo. Functional cognitive/memory outcomes were tested post-ACA via spontaneous alternation test (T-maze). Posttreatment of PAME improved functional outcomes after ACA [improvements in both alternation ratio ACA (0.261±0.049), ACA+PAME (0.487±0.039) and side-bias preference, ACA (0.821±0.046), ACA+PAME (0.641 ±0.025)]. Overall, our data suggest that methylation of PA to form PAME, rendered PAME's biological activity under normal and pathological conditions to enhance CBF, provide neuroprotection, and improve functional outcomes after CA.

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Poster

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Topic: C.08.Stroke

Support: R01NS046400

R01AT007429

UF-CTSI

Title: Role of prostaglandin D₂ DP1 receptor on post-stroke sleep disturbance and stroke outcomes

Authors: *A. S. AHMAD^{1,2}, P. KAMAT^{1,2}, D. HERNANDEZ^{1,2}, S. DORE^{1,2,3} ¹Dept. of Anesthesiol., Univ. of Florida, GAINESVILLE, FL; ²Ctr. for Translational Res. in Neurodegenerative Dis., ³Departments of Neurology, Psychiatry, Pharmaceutics, Psychology, and Neurosci., Univ. of Florida, Gainesville, FL

Abstract: Post-stroke sleep disturbance is reported to be an indicator of poor stroke outcomes. The strategic location of the PGD₂ DP1 receptor in specific brain regions regulating cerebrospinal fluid and sleep led us to hypothesize that the DP1 receptor may attenuate the deleterious effects associated with post-stroke sleep disturbance. First, to test the effect of sleep deprivation on locomotor activity, wildtype mice were individually housed and their activity was monitored 20h/d for 3d. Day zero was used to obtain baseline, while for the next two days, mice were subjected to sleep disturbance for 10h during the sleep phase followed by 10h of no sleep. Second, to test whether a selective DP1 receptor agonist decreases locomotor activity, mice were given an intraperitoneal injection of the agonist and locomotor activity was monitored. Third, to test the effect of post-stroke sleep disturbance on stroke outcomes, wildtype and DP1-/- mice were subjected to 45min of MCAO and 7d of reperfusion. After MCAO, mice were subjected to post-stroke sleep disturbance for 12h/d for 3d during the mouse sleep phase. Sleep disturbance resulted in higher activity (awake like) during the sleep phase and lower activity during the awake phase. The distance travelled during the awake phase after sleep deprivation was significantly lower (P<0.001). These data suggest that sleep deprivation attenuated locomotor activity. The total ambulatory distance covered by DP1 agonist-treated group 4h post-treatment was 2161.2±846.4cm compared with the vehicle group activity of 7093.5±1953cm (P<0.01), suggesting that the selective pharmacological agent induced sleep. Analysis of the Cresyl violetstained sections revealed a significantly larger (P<0.05) infarction volume and neurologic deficit in post-stroke, sleep-disturbed DP1^{-/-} mice compared to similarly treated WT mice. These data show that the PGD₂ DP1 receptor plays a vital role in attenuating the deleterious effects of sleep disturbance on stroke outcomes. Additional studies are underway to further investigate mechanisms and optimal therapeutic conditions.

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Poster

307. Stroke: Neuroprotection

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Topic: C.08.Stroke

Support: R01NS085272

R01NS42617

Title: Microrna-9 inhibition upregulates glutamate oxaloacetate transaminase attenuating stroke injury

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Abstract: Elevated extracellular glutamate causes ischemic brain injury. The current study stems from our key observation demonstrating that induction of glutamate oxaloacetate transaminase (GOT), an oxygen-sensitive glutamate-metabolizing enzyme in the brain, can attenuate strokeinduced injury. GOT metabolizes glutamate to generate energy in the ischemic neural tissue. Here, we report that inducible GOT expression in the brain is regulated by miR-9. This work demonstrates that GOT is subject to post-transcriptional gene silencing by miR-9. Computational prediction, target reporter luciferase assay, and Western blot analysis provided first evidence demonstrating that miR-9 targets and silences GOT. Delivery of miR-9 inhibitor to HT4 neural cells upregulated GOT protein expression. Consistently, delivery of miR-9 mimic lowered the abundance of GOT protein. To validate whether miR-9 dependent binding exerts translational repression, GOT 3'UTR reporter assay was performed using HT4 neural cells. Delivery of miR-9 mimic suppressed GOT 3'UTR reporter luciferase activity. To test the role of miR-9 in glutamate neural toxicity, miR-9 mimic was delivered to the cells followed by glutamate challenge. Delivery of miR-9 mimic significantly exacerbated the toxic effects of glutamate as measured by loss of cell viability. To evaluate the role of miR-9 in stroke in vivo, miR-9 inhibitor lentiviral transduction particles or non-targeting scrambled control were delivered to S1 cortex of C57/BL6 mice by stereotaxic injection. After 72h of delivery, mice were subjected to middle cerebral artery occlusion (MCAO) for 90 minutes followed by reperfusion. At 48h post-stroke, brain was collected to measure miR-9 and GOT levels. Mice that received the miR-9 inhibitor exhibited increased GOT protein expression at the stroke site and reduced stroke lesion volume when compared to control mice. Thus, inhibition of miR-9 at the stroke site de-silenced GOT and attenuated brain injury.

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Poster

307. Stroke: Neuroprotection

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Topic: C.08.Stroke

Support: NIH NINDS 1R21NS087157

Title: Xenon improves hemorrhagic stroke outcome in spontaneous hypertensive rats

Authors: *H. SHENG¹, H. SUN², B. YAN³, D. S. WARNER⁴

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Abstract: Introduction: Xenon, as a glutamate NMDA receptor antagonist, has neuroprotective effects in several types of brain injury including ischemic stroke. We hypothesized that xenon would also serve to improve intracerebral hemorrhage (ICH) outcome. If xenon either has no adverse effect on ICH or is found to improve ICH outcome, this may allow early therapy onset, prior to neuroimaging diagnostics.

Methods: Male SHR rats (8-10 weeks old) were anesthetized with isoflurane, ventilated and mounted on a stereotaxic frame. Pericranial temperature was controlled at 37 °C. Via a midline scalp incision, a small burr hole was made at the coordinates of 0 mm anterior to bregma and 3 mm left lateral to midline. A 5 µl Hamilton syringe needle was inserted 6 mm below the cortical surface and 2.5 µl collagenase solution (0.1875 units/µl) was slowly injected into the left striatum area. Rats then were randomly assigned to groups and transferred into a gas exposure system where they were treated with either 30% O₂ or 30% Xe in 30% O₂ for 20 hours beginning 2 hours after injection (n=10). Sham rats received underwent procedures except collagenase injection and were treated with 30% O₂ (n=6). Oxygen and xenon concentrations were monitored and automatically regulated by computer software. Pericranial temperature was controlled at 37 °C Normal saline was infused IV at 1 ml/h during the treatment period. Body weight, blood pressure and rotarod performance were measured prior to surgery and 3, 7 and 28 days after surgery. Neurological score was evaluated at 7 and 28 days. Morris water maze was tested at 29 to 33 days post-ICH. Brain histology was examined after completing the water maze. Results: Male SHR rats subjected to ICH had a transient body weight loss. Hypertension was persistent at all time points. No intra-group (30% xenon versus 0% Xe) difference was found for either body weight or blood pressure. Latency to fall from the was decreased in both groups on days 3, 7 and 28 post-ICH. However, this latency was improved in the rats receiving 20 hours 30% xenon (p < 0.05). Hemorrhage also resulted in a significant neurological deficit that was improved by xenon treatment (p < 0.05). In Morris water maze test, hemorrhage induced a decline of latency to find the escape platform. Xenon treatment did not significantly prevent this decline. Brain histology still is in progress.

Conclusions: This long-term study demonstrated that xenon improves functional outcome in hemorrhagic stroke when 20 hours of 30% xenon exposure is given beginning at 2 hours poststroke. Brain histopathology will be evaluated to further verify this effect. Xenon has the potential to serve as a stroke therapeutic in near future.

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Poster

307. Stroke: Neuroprotection

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Program#/Poster#: 307.09/AA4

Topic: C.08.Stroke

Title: The endozepine ODN improves functional recovery after experimental stroke

Authors: *J. CHUQUET¹, R. LAMTAHRI¹, M. HAZIME¹, P. P. QUILICHINI², B. LEFRANC¹, D. VAUDRY¹, J. LEPRINCE¹ ¹Normandy University, UNIROUEN, INSERM U1239, Rouen, France; ²Neurosciences Systems Inst., INSERM U1106 INS, Marseille, France

Abstract: In stroke, the survival of neurons and their ability to re-establish connections to recover lost functions are strongly influenced by the balance between neuronal excitation and inhibition. In the acute phase of stroke, lethal hyperexcitability can be attenuated by positive allosteric modulators (PAM) of the GABAA receptor (GABAAR). In contrast, in the chronic phase, negative allosteric modulators (NAM) of the GABAAR can correct the sub-optimal excitability and improve cellular plasticity (Clarkson et al. 2010). Endozepines, known as the endogenous ligands of benzodiazepine-binding sites include the diazepam binding inhibitor (DBI) and its processing products, the triakontatetraneuropeptide (TTN, DBI₁₇₋₅₀) and the octadecaneuropeptide (ODN, DBI₃₃₋₅₀). Here, we hypothesized that ODN, an allosteric modulator of the GABA_AR synthetized by astrocytes, influences the outcome of ischemic brain tissue and its functional recovery. Local field potential analysis and calcium imaging confirmed that ODN acts like a NAM of the GABA_AR in the intact cortex. As expected, during the acute phase of brain ischemia, ODN dramatically exacerbated brain lesions, and enhanced hyperexcitation processes involved in the development of stroke damage (excitotoxicity and spreading depression waves). However, a daily ODN treatment starting 3 days after the onset of stroke safely enhanced the functional recovery over the following 4 weeks. To conclude, our results show that endozepines may have a significant role to tune the excitatory and inhibitory synaptic balance and reinforce the idea that an appropriate correction of GABAergic tone at the right time, can promote neuroprotection and improve functional recovery after stroke.

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Poster

307. Stroke: Neuroprotection

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Program#/Poster#: 307.10/AA5

Topic: C.08.Stroke

Title: Neuroprotective effects diphenyldihaloketone EF24 in brain stroke model

Authors: *A. MDZINARISHVILI¹, H. HOUSON², V. AWASTHI² ¹Dept. of Rehabil. Sci., Univ. of Oklahoma, Col. of Allied Hlth., Oklahoma City, OK; ²Pharmaceut. Sci., Univ. of Oklahoma, Col. of Pharm., Oklahoma City, OK

Abstract: <u>Background:</u> Brain stroke is the second most common cause of death worldwide. It is caused by an interruption of the blood supply to the brain which leads to the loss of neurologic function and death of the brain tissue. Current management of stroke patients is aimed at preventing the progression of at-risk cerebral tissue towards infarction by restoring blood supply to ischemic penumbra in a timely manner. Surgical removal of clot and thrombolytic therapy are the most common approaches to this effect, however they do not address local and systemic inflammation associated with stroke pathology. Our objective was to investigate a diphenyldihaloketone EF24 - an NF- κ B inhibitor, as a therapeutic agent in a mouse model of the brain stroke.

<u>Methods</u>: Stroke was induced in male CD-1 mice (20-25 g) by occluding middle cerebral artery and removing the occlusion after 1 h to allow reperfusion (transient MCAO model). EF24 treatment was given either 10-15min after reperfusion (Post-treatment) or 10-15min before occlusion (Pre-treatment). Control/stroke group received vehicle. Sham mice underwent the surgical procedure without artery occlusion. The surviving mice were euthanized 24 h after stroke to collect the brain and blood. The brain was sectioned for TTC-staining to determine histologic stroke volume. Inflammatory markers, IL-6 and TNF- α , and stress marker, corticosterone, were estimated in plasma samples.

<u>Results:</u> The 24 h survival was increased in both the EF24-treated groups compared to the untreated stroke group. Plasma corticosterone increased >10 fold in stroke; EF24-Post treatment prevented this increase in plasma corticosterone. Plasma IL-6 and TNF- α were not significantly altered by occlusion surgery or EF24 treatment. Compared to the untreated stroke group, the infarct size, as assessed by TTC-staining, was reduced by 41% and 52% in the Pre-treatment and Post-treatment groups, respectively. Additionally, the stroke-associated edema was significantly reduced in the both EF24-treated groups.

<u>Conclusion</u>: The results indicate therapeutic efficacy of anti-inflammatory molecule EF24 in controlling stroke pathology and improving survival. Especially notable was the reduction in stroke size even when EF24 was given after reperfusion. Although the biochemical basis of

EF24 action is still under investigation, its remarkable effects in the t-MCAO brain stroke model engender new therapeutic class of neuroprotective agents for brain ischemia and edema.

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Poster

307. Stroke: Neuroprotection

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Topic: C.08.Stroke

Title: Thrombolysis and antihypertensive agents in the functional and clinical outcome of acute ischemic stroke

Authors: *T. I. NATHANIEL¹, N. PATIL², C. UBAH³, B. BAILES³, T. FLEMING³, Z. CONN³, L. BRECHTEL³, M. SCALISE³, J. SULIVAN³, A. FRAZIER³, A. SHIPLEY³, L. WORMACK³, A. VENKATESH³, B. AVERKAMP³, S. STERNBERG², D. BLACKHURST² ¹Biomed. Sci., Univ. of South Carolina, Greenville, SC; ²Greenville Hlth. Syst., Greenville, SC; ³Univ. of South Carolina Sch. of Med. Greenville, Greenville, SC

Abstract: Background.

In general, antihypertensive treatment is generally known to reduce the risk of first and recurrent stroke, and poor adherence to antihypertensive treatment is directly linked to increased incidence of cerebrovascular events. However, no previous study has developed a tool to measure the functional outcome following treatment of stroke patients with prior treatment with antihypertensive agent. The objective of this study is to determine whether the functional ambulatory status of the patient can be used to assess functional outcome in stroke patients' treated with rt-PA with a prior history of anti-hypertensive medication.

Method.

We examined 1446 stroke patients that received antihypertensive agents prior to treatment with rt-PA for acute ischemic stroke, and analyzed functional ambulatory status as measurement of out outcome following treatment with rt-PA. Multiple logistic regression analysis was used to obtain adjusted odds ratios for functional ambulation at discharge for patients with history of taking antihypertensive agents vs the control group of patients with no history of taking antihypertensive agents. Adjustment was made for known risk factors and group differences. The backward selection method was used to exclude non-significant risk factors. Significance level was set at the probability level of 0.05.

Results.

Multiple logistic regression analysis for functional ambulation at discharge with adjustments for multiple confounding clinical factors showed significant association (OR, 0.63; P = .04) of improved ambulation at discharge compared to ambulation at discharge.

Conclusions

Functional ambulation can be used as the functional outcome tool to determine improved acutephase therapy outcomes for the use of rt-PA. Keywords: rt-PA, stroke, antihypertensive agents

Disclosures: T.I. Nathaniel: None. N. Patil: None. C. Ubah: None. B. Bailes: None. T. Fleming: None. Z. Conn: None. L. Brechtel: None. M. Scalise: None. J. Sulivan: None. A. Frazier: None. A. Shipley: None. L. Wormack: None. A. Venkatesh: None. B. Averkamp: None. S. Sternberg: None. D. Blackhurst: None.

Poster

307. Stroke: Neuroprotection

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 307.12/AA7

Topic: C.08.Stroke

Support: NIH grant NS081333

Title: Role of caspase-9 in blood brain barrier integrity in a mouse model of stroke

Authors: *E. CANEPA, K. JOHNSON, M. AVRUTSKY, B. CHRISTOPHE, E. CONNOLLY, C. TROY

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Abstract: The caspase family of death proteases has been implicated during ischemic stroke and neurodegeneration. Data from our laboratory demonstrate that specifically inhibiting caspase-9 (Casp9) abrogates edema and provides cellular and functional neuroprotection. Reperfusion injury after stroke can exacerbate damage to the blood brain barrier and lead to edema, an abnormal accumulation of fluids in the brain parenchyma. Cerebral edema, caused by a loss of vascular integrity, is the primary cause of mortality within the first three days following ischemia. Specifically targeting the molecular mechanisms underlying the development of edema is required for optimal therapeutic intervention. Preliminary data show that inhibition of Casp9 abrogates its own activation in blood vessels (BVs) and reduces Evans Blue (EB) extravasation at 4 hours post reperfusion (hpr), supporting a role for active Casp9 in early disruption of the BVs. It has been shown by others that matrix metalloproteinase-9 (MMP-9) null animals develop less edema and show less extravasation of EB during ischemia. It had been proposed that MMPs regulate edema and that activation of MMP-9 occur upstream of caspase activation. Our preliminary data show transient middle cerebral artery occlusion (tMCAo) induces Casp9 activation in wild-type and MMP-9 null mice and that the specific inhibition of Casp9 increases MMP-9 expression. This supports the hypothesis that Casp9 acts upstream of MMP-9 in the vasogenic pathway. Moreover, analysis of junctional proteins in BV fractions at 4 hpr from

ipsilateral cortices shows a significant reduction of vascular endothelial cadherin (VECad) and claudin-5 (Clau5) expression, and this loss is abrogated by Casp9 inhibition. These data suggest a novel Casp9-dependent process that regulates BBB dysfunction and vasogenic edema during cerebral ischemia. To assess endothelial function of Casp9 in ischemia- induced cerebral edema, we have generated inducible endothelial cell Casp9 null mice. Thus, targeting caspase-9 in stroke might improve vascular health and provide neuroprotection which could aid functional recovery.

Disclosures: E. Canepa: None. **K. Johnson:** None. **M. Avrutsky:** None. **B. Christophe:** None. **E. Connolly:** None. **C. Troy:** None.

Poster

307. Stroke: Neuroprotection

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Program#/Poster#: 307.13/AA8

Topic: C.08.Stroke

Support: NIH Grant NS094896

Title: Evaluation of systemic and cerebral safety profile of pro-hemostatic red blood cell microparticles

Authors: *K. DAVE¹, A. K. REHNI¹, C. BIDOT, Jr.², H. NAVARRO², Y. S. AHN², W. JY² ¹Neurol., Univ. Miami Sch. Med., Miami, FL; ²The Wallace H Coulter Platelet Laboratory, Div. of Hematology/Oncology, Dept. of Med., Univ. of Miami Sch. of Med., Miami, FL

Abstract: Red blood cells microparticles (RMPs) possess potent hemostatic activity. Procoagulant activity of RMPs has shown beneficial effect in a study on blood drawn from patients with coagulation disorders. Before efficacy of RMPs can be tested clinically, rigorous preclinical studies are warranted. The goal of this study was to test for any potential complications following RMPs administration. We specifically tested the effects of RMPs on physiological parameters and thrombosis following RMP injection as is relevant for a hemostatic agent. RMPs were prepared from Type O+ human RBCs using high pressure extrusion method. RMPs were characterized and quantified using flow cytometry. In vitro coagulant activity was confirmed using thromboelastogram. Male rats were treated with: I) $1 \times \text{bolus}$ (6.07 $\times 10^{10}$ particles / kg b.w.), II) $1/3 \times$ three bolus 7.5 minutes apart (6.07 $\times 10^{10}$ particles / kg b.w.), III) 1 \times over 30 min $(6.07 \times 10^{10} \text{ particles / kg b.w.})$, IV) 4 × over 60 min (2.43 × 10¹¹ particles / kg b.w.), V) 20 × over 60 min $(1.21 \times 10^{12} \text{ particles / kg b.w.})$, and (VI) vehicle (saline). We monitored physiological parameters viz. blood pressure; blood pH, pCO2, and pO2; body and head temperature; and hematocrit before and after RMPs injection. In a separate set of experiments, rats in groups IV and VI were perfused at 4 h post-RMPs injection using formaldehyde: acetic acid: methanol mixture. Brains were histologically assessed by an investigator blinded to

treatments for any thrombosed blood vessels or indications of toxicity. We did not observe any adverse effects of RMPs injection. Comparisons were made against baseline values. However, a minor but significant drop in blood pH (Δ pH=0.04 at 45 min of treatment, p<0.05), body temperature (0.35°C at 45 min of treatment, p<0.01), and increase in blood pO2 (21%, p<0.05 at 45 min of treatment) was observed for 1 × over 30 min regimen. We observed significant increase in blood pO2 for 20 × over 60 min regimen (24% at 75 min of treatment, p<0.05). Besides, we observed a significant drop in hematocrit values for 1/3 × three bolus (8, 12 and16% at 15, 30 and 45 min of treatment respectively, p<0.001), 1 × over 30 min regimen (11, 14, 16 and 20% at 30, 45, 60 and 75 min of treatment respectively, p<0.001). In general, we did not observe any side-effects of RMPs in rats. Moreover, RMP treatment did not produce any thrombosed blood vessels or indications of cerebral toxicity. Thus, we conclude that RMPs do not show systemic toxicity and thromboembolism in brain. Confirming safety profile of RMPs is critical in establishing their therapeutic potential.

Disclosures: K. Dave: None. A.K. Rehni: None. C. Bidot: None. H. Navarro: None. Y.S. Ahn: None. W. Jy: None.

Poster

307. Stroke: Neuroprotection

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 307.14/AA9

Topic: C.08.Stroke

Support: Program of Zhejiang Provincial Natural Science(LY14H270013)

Title: Huo-Luo-Xiao-Ling-Dan plays a protective role in ischemic brain in rats via inhibiting inflammatory responses after stroke

Authors: Z. JIN¹, W. SHEN², *H. ZHANG³

¹Sch. of Basic Medicine, Zhejiang Chinese Med. Univ., Hangzhou, China; ²The First Affiliated Hospita of Zhejiang Chinese Med. Univ., Hangzhou, China; ³Dept Psychiatry, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Huo-Luo-Xiao-Ling dan (HLXL), a traditional Chinese herbal mixture, can play an important role of anti- inflammation in the treatment of cerebral ischemia. In this study, we examined the effects of HLXL on brain injury of ischemic stroke in rats. Focal cerebral ischemia was induced by middle cerebral artery occlusion (MCAO) for 2 hours followed by 24 or 48 hours reperfusion in male rats. We treated ischemic stroke rats with HLXL (0.5, 2.0 and 6.0g/kg) by daily gavages beginning at the onset of stroke. The control group received the vehicle. HLXLD 2.0, 6.0/kg treatment significantly attenuated the infarct volume, neurological deficits,

upregulation of proinflammatory cytokines in the brain of rats after stroke compared with that of non-HLXLD group. HLXLD can induce the expression of MCPIP1 in brain tissue. Furthermore, the activation of NF- κ B signaling was significantly reduced in HLXLD treated rats after stroke compared to that of non-HLXLD group. Because MCPIP1 not only plays an important role in early cortical neurogenesis but also reveal an unexpected link between neocortical development, immune regulators, and epigenetic modification. Our data demonstrated that HLXLD can play an important protective role in brain injury after ischemic stroke via inhibiting inflammatory responses in the brain. We need further investigate the molecular mechanism of the HLXLD which via modulate the MCPIP1 expression.

Disclosures: Z. Jin: None. W. Shen: None. H. Zhang: None.

Poster

307. Stroke: Neuroprotection

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Program#/Poster#: 307.15/AA10

Topic: C.08.Stroke

Support: AHA 12SDG7360021

NIH NINDS R01NS085272

NIH NINDS NS42617

Title: Multimodal tocotrienol vitamin e protection against ischemic stroke

Authors: C. RINK¹, S. KHANNA¹, H. HARRIS¹, S. GNYAWALI¹, *C. K. SEN² ¹Surgery, The Ohio State Univ. Wexner Med. Ctr., Columbus, OH; ²Ohio State Univ. Med. Ctr., Columbus, OH

Abstract: At nanomolar concentration, lesser-characterized natural vitamin E tocotrienol (TCT), but not α-tocopherol, potently protects neural cells from glutamate induced neurotoxicity *in vitro* and attenuates ischemic stroke injury in a pre-clinical setting *in vivo*. While *neuro*protective mechanisms of TCT have been elucidated, neuroprotection alone is unlikely to explain the robust protection against stroke observed *in vivo*. Indeed, neuroprotective agents have largely failed in clinical trials where multimodal mechanisms (*i.e.* neuro and vascular) are recognized as necessary for protection against ischemic stroke. In the current work, we sought to test for and identify mechanisms of vascular protection in a pre-clinical setting of ischemic stroke. C57/BL6 mice (male, 5 wks) were orally gavaged daily with 50mg/kg body weight of TCT or volume matched placebo (n=12) for 10 wks prior to induction of ischemic stroke using the intraluminal thread method of middle cerebral artery occlusion (MCAO). While stroke-induced ischemia persisted, cerebrovascular perfusion was measured using laser speckle flowmetry. After imaging,

mice were euthanized and perfused with FITC-conjugated lectin to characterized cerebrovascular perfusion in the MCA territory of the stroke-affected hemisphere. Anastomotic connections (diameter and number) were quantified as CD31+/FITC-lectin+ arterioles in stroke-affected S1 cortex and collected by laser capture microdissection. Pro-arteriogenic mediators of vascular remodeling were queried by real-time PCR and histology. Compared to placebo, TCT treatment significantly increased perfusion (38.7%, p<0.05), collateral size (21.2%, p<0.05), and collateral number (5.7-fold, p<0.05) during MCAO. Furthermore, prophylactic TCT supplementation significantly induced TIMP1 expression 9.6-fold (p<0.05) in laser-captured collaterals as compared to placebo controls. In a separate experimental cohort, we observed TCT protection against ischemic stroke was lost in TIMP1 knockout mice. Taken together with known neuroprotective properties, the current work supports additional (multimodal) vascular-protective properties of TCT against stroke by inducing arteriogenesis in a TIMP1-dependent manner for functional collateral growth.

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Poster

307. Stroke: Neuroprotection

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Program#/Poster#: 307.16/AA11

Topic: C.08.Stroke

Support: NIH Grant NS077897

Title: Impact of immune receptor CD36 on post-stroke cognitive impairments

Authors: *M. BALKAYA, J. YANG, S. CHO Burke-Cornell Med. Res. Inst., White Plains, NY

Abstract: Post-stroke cognitive neuropsychological impairments (CNI) are common, affecting more than a half of stroke survivors. Despite the high prevalence and clinical significance, our knowledge on post-stroke cognitive deficits is limited. Using WT and CD36 KO mice (N= 7-11/group) subjected to transient focal ischemia, this study investigates long-term CNI and the impact of CD36 deficiency on CNI. A battery of tests for motor (rotarod, open field), depression (sucrose preference, forced swim test), anxiety and memory tests (Y maze) were performed before stroke, and 2 and 7 weeks after stroke. To evaluate spatial learning and memory, water maze tests were performed at 9W for hippocampal based learning: training (hidden platform) and probe trials (remove platform) and for hippocampal-striatal based trial (visible platform). At baseline CD36 KO animals displayed reduced exploration in open field and reduced motor performance on rotarod. Following stroke, WT mice showed impaired motor function in rotarod

and displayed increased activity in open field at 2W and 7W. Sucrose preference was markedly reduced at 2W, but returned to baseline at 7W. CD36 KO animals did not display any significant post-stroke behavioral difference from pre-stroke baseline. Water maze tests revealed that stroke caused impaired learning during the training phase and CD36 KO mice performed better although probe trial did not show a genotype difference. CD36 KO mice showed better performance in visible platform trials compared to WT mice (Fig 1). Our result showed that stroke induces a transient hedonic deficit, a persistent hyperactivity and memory impairments that hinders the striatal-based new rule learning and strategy switching. The detection of better cognition in CD36 KO mice further suggests a sensitive preclinical platform that can assess stroke-induced CNI in different genotype mice during recovery phase.



Time in former platfom quadrant

Figure 1: Latency to reach visible platform * WT Stroke vs all groups

Disclosures: M. Balkaya: None. J. Yang: None. S. Cho: None.

Poster

307. Stroke: Neuroprotection

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Support: AHA Mid-Atlantic Affiliate Grant-in-Aid 13GRNT15730001

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AHA Mid-Atlantic Affiliate Postdoctoral Fellowship Award 15POST25090114

Title: Pinocembrin protects hemorrhagic brain primarily by inhibiting toll-like receptor 4 and reducing M1 phenotype microglia in mice

Authors: *X. LAN, X. HAN, Q. LI, J. WANG Johns Hopkins Med., Baltimore, MD

Abstract: Neuroinflammation is a major contributor to intracerebral hemorrhage (ICH) progression, but no drug is currently available to reduce this response and protect against ICHinduced injury. Recently, the natural product pinocembrin has been shown to ameliorate neuroinflammation and is undergoing a phase II clinical trial for ischemic stroke treatment. In this study, we examined the efficacy of pinocembrin in an ICH model, and further examined its effect on microglial activation and polarization. In vivo, pinocembrin dose-dependently reduced lesion volume by ~47.5% and reduced neurologic deficits of mice at 72 h after collagenaseinduced ICH. The optimal dose of pinocembrin (5 mg/kg) suppressed microglial activation as evidenced by decreases in CD68-positive microglia and reduced proinflammatory cytokines tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-6. Pinocembrin also reduced the number of classically activated M1-like microglia without affecting M2-like microglia in the perilesional region. Additionally, pinocembrin decreased the expression of toll-like receptor (TLR)4 and its downstream target proteins TRIF and MyD88. The protection by pinocembrin was lost in microglia-depleted mice and in TLR4^{lps-del} mice, and pinocembrin failed to decrease the number of M1-like microglia in TLR4^{lps-del} mice. Inhibition of the TLR4 signaling pathway and reduction in M1-like microglial polarization might be the major mechanism by which pinocembrin protects hemorrhagic brain. With anti-inflammatory properties, pinocembrin could be a promising new drug candidate for treating ICH and other acute brain injuries

Disclosures: X. Lan: None. X. Han: None. Q. Li: None. J. Wang: None.

Poster

307. Stroke: Neuroprotection

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Title: Inhibition of neuronal ferroptosis protects hemorrhagic brain in mice

Authors: *Q. LI, X. HAN, X. LAN, J. WANG Anesthesiol. & Critical Care Med., Johns Hopkins Univ., Baltimore, MD

Abstract: Background and Purpose: Intracerebral hemorrhage (ICH) causes high mortality and morbidity, but our knowledge of post-ICH neuronal death and related mechanisms is limited. In this study, we aimed to investigate whether ferroptosis contributes to neuronal death post-ICH and whether inhibition of ferroptosis protects hemorrhagic brain in vivo. Methods: We injected collagenase to left striatum of mice and quantified mitochondrial area in neurons at perihematoma region. In addition, we injected ferrostatin-1 (a specific inhibitor of ferroptosis) 2h delay (intraventricular) post-ICH, and accessed the protection effects of ferrostatin-1 with Fluoro-Jade B staining, CV/Fast blue staining, neurologic deficit scoring, etc. Furthermore, we detected lipid ROS accumulation and cyclooxygenase-2 (COX-2) expression by MDA assay and Western blotting using 4-HNE and COX-2 antibodies. Results: We first demonstrated that ferroptosis, a newly identified form of cell death, occurs in the collagenase-induced ICH model in mice with evidence of shrunken mitochondria in neurons. We found that mice treated with ferrostatin-1 after ICH exhibited marked brain protection and improved neurologic function. Additionally, we found that ferrostatin-1 reduced lipid reactive oxygen species production and attenuated the increased expression level of COX-2 in vivo. Conclusion: These results indicate that ferroptosis contributes to neuronal death after ICH, that administration of ferrostatin-1 protects hemorrhagic brain, and that cyclooxygenase-2 could be a biomarker of ferroptosis. The insights gained from this study will advance our knowledge of the post-ICH cell death cascade and be essential for future preclinical studies.

Disclosures: Q. Li: None. X. Han: None. X. Lan: None. J. Wang: None.

Poster

307. Stroke: Neuroprotection

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Topic: C.08.Stroke

Support: AHA-13SDG1395001413

Evelyn F. McKnight Brain Institute

Louisiana State University Research Council

Title: Neuropeptide Y plays a detrimental role in cardiac arrest-induced brain injury

Authors: ***R. H.-C. LEE**¹, A. COUTO E SILVA², H. POSSOIT¹, C. Y.-C. WU¹, H. LIN¹ ¹Neurol., ²Cell. Biol. and Anat., LSU Hlth. Sci. Ctr. Shreveport, Shreveport, LA

Abstract: Cardiopulmonary arrest (CA, a form of global cerebral ischemia) is the leading cause of death and disability in the US. More than 350,000 victims/year suffer CA with only a 12% survival rate. The whole body ischemia following CA leads to brain damage causing severe neurological deficits such as learning/memory deficits. We previously showed that enhanced sympathetic nervous system (SNS) activity is one of the major contributors to CA-induced hypoperfusion (a decrease in cerebral blood flow, CBF), which plays a critical role in the progression of CA-induced neuronal cell death and learning/memory deficits. However, mechanisms underlying SNS-induced hypoperfusion and ischemic brain injury is unclear. It is important to note that neuropeptide Y (NPY, a 36-amino acid neuropeptide) is released upon SNS activation. The release of NPY induces a long-lasting and potent vasoconstriction (100-fold more potent than other sympathetic neurotransmitters, such as norepinephrine), which reduces the blood supply to the brain. We sought to attenuate NPY release from pre-synaptic sympathetic nerves via peptide YY (PYY)₃₋₃₆ (a pre-synaptic NPY2 receptor agonist) to investigate the impact of NPY inhibition on CA-induced hypoperfusion. A rat model of global cerebral ischemia (6 mins asphyxial cardiac arrest, ACA) was used in the present study. Results from intra-vital two-photon laser scanning microscopy suggest post-treatment with PYY₃₋₃₆ (56.87±4.48%; p<0.05 evaluated by Student's t test) attenuated cortical hypoperfusion (-33.20±2.65%) 24 hrs after ACA. Interestingly, post-treatment with PYY₃₋₃₆ inhibited neuronal cell death (via hematoxylin and eosin stain) in the CA1 region of the hippocampus. We further assessed cognitive/behavioral function (T-maze) to evaluate the rats' functional learning/memory after ACA. Rats post-treated with PYY₃₋₃₆ (0.5 ± 0.17) presented with better neurological outcomes than the control rats (0.26±0.05) after ACA. Here, we show that NPY plays a detrimental role in CA-induced brain injury. Inhibition of NPY release via PYY₃₋₃₆ provides neuroprotection against CA-induced hypoperfusion, neuronal cell death, and neurological deficits.

Disclosures: R.H. Lee: None. A. Couto e Silva: None. H. Possoit: None. C.Y. Wu: None. H. Lin: None.

Poster

307. Stroke: Neuroprotection

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Program#/Poster#: 307.20/AA15

Topic: C.08.Stroke

Title: The role of nitric oxide and dimethyl sulfoxide in formation of penumbra characteristics

Authors: *M. DEVDARIANI, L. DAVLIANIDZE, M. NEBIERIDZE, L. GUMBERIDZE, I. KVACHAKIDZE, N. SIKHARULIDZE, N. MITAGVARIA I.Beritashvili Ctr. of Exptl. Biomedicine, Tbilisi, Georgia

Abstract: Under the conditions of focal cerebral stroke the critical value, from the patient's output point of view has an optimal use of so called «therapeutic window". In particular this applies to preservation of Penumbra zone from the involvement in the ischemic processes as well as slowing of the processes taking place in the focus of stroke. The aim of this study was to obtain the specific data that will clarify the processes having place in development of focal stroke (and after its completion) in both ischemic focus and a zone of penumbra). From this point of view we tried to clarify: 1. The dynamics of local blood flow and oxygen tension changes in cerebral tissue as well as the level of Nitric Oxide in different time intervals. 2. The role of changes in the properties of blood rheology and presence of free radicals in defining of both geometric sizes of Penumbra and the levels of blood flow and oxygen tension in it. 3. To evaluate the results of the morphological changes taking place in the focus of ischemic Stroke and in the zone of Penumbra. 4. Analysis of the results obtained in our experiments with taking into account the data available in the literature and getting the generalized conclusions. Studies were conducted on groups of white rats by means of two experimental models: a non-invasive induction of focal ischemic stroke by photochemical method and local hyperthermia, therapeutic method that is used in the treatment of tumors. It was found that in both experimental models: 1. In the process of formation of ischemic stroke greatest complication causes the thrombosis of cerebral vessels due to changes in blood rheological properties. 2. Reduction of the scale and severity of damages in brain tissue (according to the received morphological changes in it) is possible by using antioxidants and free radical scavenger (dimethyl sulfoxide - DMSO) and / or a change in the relationship between the concentrations of Nitric Oxide and Oxygen Radicals (for favors of the first). We believe that these results at least will clarify the general principles of hyperthermic therapy, and its use in cancer clinic, both in terms of tolerance of brain tissue to hyperthermia and incorporating the role of free radicals, nitric oxide, and rheological properties of blood in the process of development and/or prevention of further damages having place especially in penumbra zone.

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Poster

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Topic: C.08.Stroke

Support: The National Basic Research Program of China (973 Program)

Title: Xueshuantong Injection effectively relieves inflammatory reaction induced by ischemic stroke through regulating NF-kappa B signaling pathway

Authors: *X. LIANG

UCSF, San Francisco, CA

Abstract: Inflammatory reaction plays a key role in progression of ischemic stroke. Xueshuantong Injection(XST) is a traditional Chinese medicine which used to treat ischemic stroke widely in China. Objectives: XST can improve the degree of the ischemic stroke patient's neurological impairment through regulating the expression of IκB α, nuclear factor (NF)-κB p65 and some related inflammatory cytokines. Method: In clinic research, we measured the serum levels of hsCRP,MMP-9,TIMP-1,IL-6,IL-18,ICAM-1,IP-10,MIP-1,GFAP and S100B in patients with acute stroke (patients should be admitted into observation within the first 72 h after stroke onset) using the method of enzyme-linked immuno sorbent assay (ELISA). Neurological impairment was scored with National Institute of Health Stroke Scale(NIHSS) at the days of the 1st day, 3rd day, 5th day and 14th day after be admitted into hospital.62 patients were divided into two groups randomly, one group was treated with basic treatment (IS+Vehicle group) and another group was treated with basic treatment and was injected with Xueshuantong Injection additionally(IS+XST group). In vivo, we established middle cerebral artery occlusion(MCAO) model in rats. Western blot analysis was performed to analyze expression levels of IkBa and NFκB p65. Results: In clinical research: XST group can improve the NIHSS significantly at the 14th day after stroke onset compared with basic treatment group in the respect of facial paralysis improvement (P<0.05). Xueshuantong Injection can decrease the serum level of TIMP-1, IL-18,ICAM-1 at 3 days after stroke onset significantly compared with basic treatment (t=1.973,-2.685 and 3.167 respectively, P<0.05). Xueshuantong Injection can decrease the serum level of hsCRP, TIMP-1, ICAM-1 and S100B at 5 days after stroke onset significantly compared with basic treatment (t=-2.241, 2.410, 2.397 and 2.102 respectively,P<0.05). Xueshuantong Injection can decrease the serum level of MMP9, TIMP-1, IL-6, ICAM-1 and GFAP at 14 days after stroke onset significantly compared with basic treatment (t=-2.404,4.260,3.475,5.561 and 1.992 respectively,P<0.05). In vivo research: Xueshuantong Injection can decrease the protein

expression of IkBa and NF-k B p65 compared with model

group(P<0.05).Conclusions:Xueshuantong Injection can improve the neurological impairment through regulating the NF-κB p65 signaling pathway and some related inflammatory cytokines.

Disclosures: X. Liang: None.

Poster

307. Stroke: Neuroprotection

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Support: AHA 17GRNT33450010

NIH 1R21NS095166

Title: Role of the haptoglobin-CD163 scavenging system in intracerebral hemorrhage

Authors: *C. M. LI¹, J. L. LECLERC¹, S. DORÉ²

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Abstract: Hemoglobin (Hb) released by red blood cell lysis following intracerebral hemorrhage (ICH) causes neuroinflammation and cell death, inflicting irreversible brain damage. Haptoglobin (Hp) and CD163 form a primary scavenging system for Hb and protect tissues from its cytotoxic effects. Hp directly binds to Hb, and this complex is endocytosed by macrophages/microglia expressing the membrane receptor CD163. Currently, there is no effective treatment against ICH, and limited research exists on the respective functions of Hp and CD163 after ICH. This study aims to investigate the individual effects that Hp and CD163 have on outcomes after ICH and, furthermore, we also investigate how these individual effects change when both proteins are affected simultaneously in transgenic animals. ICH was induced in wildtype (WT) control, CD163 knockout (CD163-/-), Hp knockout (Hp-/-), and Hp plus CD163 double knockout (Hp-/-xCD163-/-) mice. Various functional and anatomical outcomes were assessed at 3d post-ICH. Lesion volume, hematoma volume and tissue injury in CD163-/- mice was 27.5+/-5.8% (p<0.0001), 30.6+/-6.4% and 23.0+/-4.1% (p=0.0041) smaller compared to WT mice, respectively. Lesion volume and tissue injury in Hp-/- mice was 28.2+/-7.2% (p=0.0006) and 29.5+/-2.5% (p=0.0011) smaller compared to WT mice, respectively. No significant differences in these measurements were detected between the double Hp-/-xCD163-/- and WT mice; thus, CD163-/- and Hp-/- mice had significantly smaller lesion volumes and tissue injuries compared to this double knockout group as well. Preliminary data for additional measures are as follow: Hp-/- mice had smaller hemispheric enlargement (an indicator of brain swelling/edema)

and blood-brain barrier breakdown compared to WT mice, less iron and astrogliosis compared to WT and CD163-/- mice, and less induction of heme oxygenase compared to all other groups. The CD163-/- mice had less total Hb in brain tissue and less blood-brain barrier breakdown compared to WT mice. The Hp-/-xCD163-/- mice had less blood-brain barrier breakdown compared to WT mice. These initial findings demonstrate the differences in recovery following ICH. The outcomes of this preclinical study help in the design of future clinical trials to limit devastating brain damage following ICH.

Disclosures: C.M. Li: None. J.L. Leclerc: None. S. Doré: None.

Poster

307. Stroke: Neuroprotection

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 307.23/AA18

Topic: C.08.Stroke

Support: CAPES/ PROEX

Program of Post Graduation in Psychobiology

Title: Parawixin2, a gaba uptake inhibitor molecule isolated from parawixia bistriata spider venom is neuroprotective against experimental ischemic stroke damage in wistar rats

Authors: *T. BRONHARA¹, J. L. LIBERATO², W. F. SANTOS² ²Biol., ¹Faculdade De Filosofia Ciências E Letras RP FFCLRP, Ribeirao Preto, Brazil

Abstract: Acute ischemic stroke (AIS) is a major cause of adult disability with very few therapeutic options. GABA receptor agonists (GABAr) present neuroprotective effects (NP), which save the brain of AIS damage. However, the undesired effects of GABAr have limited their wider application in patients. So compounds that block GABA uptake represent potential tools for providing an efficient protection against AIS injury. Parawixin2 (Pwx2) presents a potent activity in decrease GABA uptake *in vitro* essays. This study evaluated the NP effect of Pwx2 on striatum (S), motor (M) and somatosensorial (C) cortices, as well as the neurological deficits (ND) after treatment in a model of AIS. Wistar rats under anesthesia were injected with endothelin1 (Et1; 600 pmol, in 6 uL) near to Middle Cerebral Artery (MCA) to induce AIS. Pwx2 (2 ug/uL; 1 uL) or Saline 0.9% (1 uL) were delivered into lateral ventricle and carry out 30 min before from AIS. ND were evaluated 24 hs before and after AIS by Open Field Test (OF) and forepaw touch test (FT). Cell death in S, C, and M infarcted areas was evaluated by Fluoro-Jade C (FJC) methods. Treatment with Pwx2 improved neurological function in all parameters tested (OF and FT). More remarkable, Pwx2 reduced FJC+ cells in all areas analyzed (S =81.6%; M=91.7%; C=82.8%). Considering that Pwx2 is one of very few compounds that block GABA

transporters, our findings suggest that their neuroprotective potential might represent a promissory drug to stroke therapies. Financial Support: CAPES and FAPESP

Disclosures: T. Bronhara: Other; University of Sao Paulo. **J.L. Liberato:** A. Employment/Salary (full or part-time):; CAPES/PNPD. **W.F. Santos:** A. Employment/Salary (full or part-time):; University of Sao Paulo.

Poster

307. Stroke: Neuroprotection

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 307.24/AA19

Topic: C.08.Stroke

Support: DFG Grant FOR 1738

Title: Advances in vasospasm research: Vasoactive impact of heme degradation products (HDPs) on mouse cerebral arterioles *In vivo* and *In vitro*

Authors: A. JOERK¹, N. LANGGUTH¹, M. RITTER², R. A. SEIDEL³, M. GUENTHER¹, G. POHNERT², M. WESTERHAUSEN², K. HOLTHOFF⁴, *O. W. WITTE⁵ ¹Hans Berger Dept. of Neurol., ²Inst. of Inorganic and Analytical Chem., ³Dept. of Anaesthesiology and Intensive Care Medicine, Ctr. of Sepsis Control and Care, Friedrich Schiller Univ. Jena, Jena, Germany; ⁴Friedrich-Schiller-University Jena, Jena, Germany; ⁵Hans Berger Dept. of Neurol., Friedrich Schiller Univ. Jena, Jena, Germany

Abstract: BACKGROUND: Delayed vasospasm of cerebral arteries is alleged to be the most common determinant of mortality and unfavorable prognosis in patients who suffer from subarachnoid hemorrhage (SAH). Recent guidelines concerning vasospasm treatment recommend the application of the calcium channel antagonist nimodipine, hemodynamic interventions like induced hypertension or cerebral angioplasty. All therapeutic options may associate with serious side effects and fail to improve the clinical outcome. Previous studies have demonstrated that heme degradation products (HDPs) like bilirubin oxidation end products (BOXes), originating from the intracranial hematoma surrounding the ruptured aneurysm, are involved in inhibiting large conductance BK_{Ca} potassium channels in vascular smooth muscle cells. It is commonly accepted knowledge that blocked BK_{Ca} channels fail to hyperpolarize cell membrane and increase the myogenic tone. METHODS: Using two-photon laser scanning microscopy we analyzed changes in diameter and perfusion of pial and intraparenchymal arterioles in adult wildtype and BK channel deficient mice *in vivo*. DIC video microscopy was the preferred optical technique to study diameter changes of intracortical arterioles in acute brain slices of mice. RESULTS: We demonstrated that the subarachnoid applications of the

propentdyopents (PDPs) or synthetic Z-BOX isomers cause a long-lasting and sustainable diameter decrease of pial arterioles in living mice. This vasoconstrictive effect depends on BK_{Ca} channel activity, because it was absent in mice, expressing dysfunctional BK_{Ca} channels through a conventional knockout of the Slo1 gene. These data are consistent with our investigations on acute brain slices suggesting that isolated intermediates and end products of oxidative bilirubin breakdown are able to induce arteriolar vasoconstriction in mice with functional BK_{Ca} channels. CONCLUSION: Our findings confirm the contribution of heme degradation products in developing diameter dysregulation of cerebral vasculature after SAH and highlight the involvement of microvessels in arterial vasospasm.

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Poster

307. Stroke: Neuroprotection

Location: Halls A-C

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Program#/Poster#: 307.25/AA20

Topic: C.08.Stroke

Support: NIH grant NS094896

Title: Biodistribution study of human red blood cell microparticles (RMPs) in rat

Authors: *V. SHUKLA¹, A. K. REHNI¹, C. BIDOT, Jr², H. NAVARRO², Y. S. AHN², W. JY², K. R. DAVE¹ ¹NEUROLOGY, ²The Wallace H. Coulter Platelet Laboratory, Div. of Hematology/Oncology, Dept. of Med., MILLER SCHOOL OF MEDICINE, UNIVERSITY OF MIAMI, Miami, FL

Abstract: Spontaneous intracerebral hemorrhage (sICH) is the severe form of stroke and remains a significant cause of morbidity and mortality throughout the world. No proven treatment is available so far. Limiting hematoma expansion in sICH has been attractive therapeutic target. RMPs have hemostatic properties. Thus, RMP can be used as potential therapeutics for preventing hematoma growth. The goal of this experiment was to determine the biodistribution of RMPs in blood and organ/tissues to predict any potential side-effects when injected to rats. RMPs were labeled with Alexa Fluor-488 TFP ester (ThermoFisher Scientific), unconjugated dye was removed using microdialysis membrane (Spectra/Por) and dialyzed against saline overnight with frequent saline changes. Dialyzed labeled RMPs were collected and filtered before use. Labeled RMPs were injected by single bolus injection of the total dosage $(6.07 \times 10^{10} \text{ particles/kg})$ delivered over 20 seconds. Rats were perfused at 15 min or 24 h post-RMPs injection. Blood was collected before perfusing rats. Brain, lung, heart, liver, spleen,

kidney, bone marrow (BM), lymph node (LN), and muscles were harvested and frozen at -80°C until further use. Tissues homogenates were made on ice in RIPA buffer, sonicated and fluorescence was measured in resultant tissue homogenates as an indicator of presence of RMPs in the tissue by measuring fluorescence at Ex: 490/EM: 520. Results are expressed as mean fluorescence (MF)/g tissue or MF/ml blood. MF for RMPs injected was $926,432 \pm 72,643$ AFU/kg body weight and $800,310 \pm 25,554$ AFU/kg body weight for 15 min and 24 h groups, respectively. Major fluorescence was detected in liver (71,360 \pm 6,791, n = 6), spleen (24,518 \pm 2,344, n = 6); moderate fluorescence in heart $(2,395 \pm 455, n = 6)$, kidney $(5,268 \pm 781, n = 5)$, lung $(6,160 \pm 2,088, n = 6)$, and LN $(8,468 \pm 2,700, n = 5)$ while minor fluorescence in brain $(661 \pm 229, n = 6)$, muscle $(670 \pm 157, n = 6)$, and BM $(529 \pm 70, BM$ harvested from femur bone obtained from both the hind limbs, n = 6) at 15 min post-RMP injection. Fluorescence in 24 h post-RMP injection group was significantly lower for liver $(31,515 \pm 1,018, n = 6, p < 0.001)$, spleen (16,943 \pm 1,176, n = 6, p<0.05), and BM (218 \pm 51, n = 5, p<0.01); while non-significant increase was observed in kidney $(74,326 \pm 53,257 \text{ AFU} / \text{g}, n = 6)$, muscle $(1,010 \pm 391, n = 6)$ and LN (9,595 \pm 2,721, n = 5) compared to 15 min time point . We observed MF of 37 \pm 23 and 287 ± 105 for blood at 15 min and 24 h post-RMP injection, respectively. Our results indicate that very small fraction of injected RMPs is retained in the body at both 15 min and 24 h post-RMP injection. Thus, RMP can be safely used for treatment following sICH.

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Poster

307. Stroke: Neuroprotection

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Topic: C.08.Stroke

Support: SanBio, Inc.

Sunovion Pharmaceuticals, Inc., a wholly owned subsidiary of Sumitomo Dainippon Pharma Co., Lrd.

Title: Transplanted modificed bone marrow-derived mesenchymal stem cells, SB623, ameliorate chronic behavioral and pathological deficits in stroke rats

Authors: *E. MORADI¹, ***E. MORADI**¹, Z. WARRAICH¹, A. SATO¹, M. RAZAVIAN¹, M. PERNETT^{1,2}, M. POLEWSKI^{2,3}, K. RAVINA³, M. DUGICH-DJORDJEVIC², M. SHAMLOO³, D. BATES¹, Y. ANDREWS-ZWILLING¹ ¹Sanbio, Inc., Mountain View, CA; ²Wester Univ. of Hlth. Sci., Pomona, CA; ³Stanford Univ. Sch. of Med., Stanford, CA **Abstract:** Stroke is the leading cause of long-term disability in adults. We recently completed a Phase 1/2a clinical trial with intracranial implantation of SB623, which appear to be safe and were associated with improvements in a majority of the clinical outcome end points. In this nonclinical study, we investigated the mechanism of action of SB623, which leads to amelioration of chronic behavioral deficits in rats. The present study assessed the efficacy of SB623 cells implanted intracranially into rats at day 28 following transient Middle Cerebral Artery occlusion. Treatment efficacy was assessed by sensorimotor behavioral tests: Cylinder and Paw Whisker Test. If untreated, the ischemic injury showed stable behavioral deficits for at least 72 days following lesion. Histopathological end-points were correlated with functional endpoints to determine the mechanism of action of SB623. Compared to post-stroke/pretransplantation levels, significant improvements in sensorimotor function were detected in stroke rats that received SB623 vs. vehicle. At 72 days post-transplantation, histopathological analysis indicated no difference in infarct size in both experimental groups, but significantly reduced levels of reactive GFAP-positive astrocytes, activated immune cells and type III collagen in the striatal peri-infarct area of SB623-treated rats. Furthermore, there was a strong negative correlation between improvement of neurological sensorimotor function and GFAP and type III collagen levels. These data demonstrate that SB623 cells ameliorate chronic inflammation, reduce levels of collagen deposits and reactive astrocytes, and this reduction is strongly associated with improvement in recovery in a rodent model of chronic stroke

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Poster

307. Stroke: Neuroprotection

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Program#/Poster#: 307.27/AA22

Topic: C.08.Stroke

Support: NSF Grant 25-21-RC287-568

Title: Neural stem cell derived extracellular vesicle therapy in a porcine model of stroke

Authors: *S. SPELLICY^{1,2,3,6}, B. JURGIELEWICZ^{4,3}, E. KAISER^{4,3}, R. L. WEBB⁷, S. PLATT⁵, F. WEST^{4,3}, S. L. STICE^{4,7,3}

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Abstract: Stroke is currently the 5th leading cause of death in adults in the United States, and has accounted for approximately 14% of total health care spending in recent years¹. Despite thousands of ongoing clinical trials for potential stroke therapies, currently only one FDA approved small molecule therapy, tPA, exists to date. We proposed to use neural stem cell extracellular vesicles, a type of cell derived nanovesicle, and their potential neuroprotective properties as a novel therapeutic to combat behavioral and motor impairments following middle cerebral artery occlusion (MCAO) in a porcine model of stroke. For this study, we used 16 land raised male castrated pigs which were divided into either an extracellular vesicle treatment group or a control saline group. First, we isolated the extracellular vesicles from neural stem cell spent media using ultracentrifugation and filtration, and then characterized their size, morphology, and concentration using nanoparticle tracking analysis. We then IV administered the extracellular vesicles, or a control sham saline solution, at 2, 14, and 24 hours post MCAO. We then performed open field and novel object recognition behavioral tests at 24 hours post, 3 days post, 1 week post, 3 weeks post and 12 weeks post MCAO, and used behavior tracking software to quantify differences between pigs in the extracellular vesicle treated and saline treated groups. During open field testing, we found that by 1 week post MCAO, control pigs had statistically significant decreases in velocity and distance traveled in the testing arena, while the extracellular vesicle treatment group did not. We also saw during open field testing, the total distance traveled by extracellular vesicle treated pigs at 12 weeks was significantly greater than the distance traveled by sham pigs. We did not find any statistically significant differences in the novel object recognition test between the sham and extracellular vesicle treated pigs at any of the 5 tested time points. Overall, we found that neural stem cell derived extracellular vesicle therapy following a right sided MCAO in pigs aided in preventing a significant decrease in velocity and total distance traveled during open field testing as well as had a significant positive influence on these parameters 12 weeks post MCAO compared to control. 1. Mozzafarian D, Benjamin EJ, Go AS, et al. on behalf of the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics-2016 update: a report from the American Heart Association. Circulation. 2016;133:e38-e360.

Disclosures: S. Spellicy: None. B. Jurgielewicz: None. E. Kaiser: None. R.L. Webb: A. Employment/Salary (full or part-time):; ArunA Biomedical. S. Platt: None. F. West: None. S.L. Stice: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ArunA Biomedical.

Poster

307. Stroke: Neuroprotection

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Topic: C.08.Stroke

Support: NIH R01AG007218

AHA Predoctoral Fellowship

Title: Sex differences in stroke neuroprotection via stimulation of FAO

Authors: *M. M. SIFUENTES¹, J. LECHLEITER²

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Abstract: Stroke is a leading cause of death and disability in the United States, yet few options exist for afflicted patients. Our previous research demonstrated that thyroid hormone stimulation of astrocyte fatty acid oxidation (FAO) reduced lesion volume after stroke. However, the mechanism for this neuroprotection is not yet known. Here we show that thyroid hormone increases astrocyte spare respiratory capacity under nutrient deprivation, increasing survival under mitochondrial stress. Accordingly, thyroid hormone rescues the decreased maximal respiration of starved astrocytes in a dose-dependent manner. In vivo experiments using a photothrombotic model of stroke found that thyroid hormone neuroprotection differs based on sex, with males being responsive at lower doses. We show that a 25 ug/kg dose of thyroid hormone is protective up to three hours after stroke, although higher concentrations at longer timepoints have yet to be tested. Overall, our research suggest that thyroid hormone treatment presents as a possible therapeutic approach to reduce injury after stroke.

Disclosures: M.M. Sifuentes: None. J. Lechleiter: None.

Poster

307. Stroke: Neuroprotection

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 307.29/AA24

Topic: C.08.Stroke

Support: Pulse Therapeutics, Inc.

Title: In vitro stroke model for evaluating SPION translation and clot lysis

Authors: *H. H. ENGELHARD¹, A. LEVIN¹, S. PERNAL¹, Z. GAERTNER¹, M. SABO², F. CREIGHTON²

¹Univ. of Illinois, Chicago, IL; ²Pulse Therapeutics, Inc., St. Louis, MO

Abstract: <u>Background</u>: Stroke continues to be a leading cause of death and disability, worldwide. Superparamagnetic iron oxide nanoparticles (SPIONs) hold great promise as vehicles for enhancing drug delivery for stroke, and other CNS diseases. Magnetically-induced rotary

traction (MIRT) offers a means to improve SPION targeting at clinically-relevant distances. Here, we describe a novel, inexpensive in vitro system for evaluating the potential of SPIONdrug combinations which are intended for use in intracranial clot lysis. Methods: Our model system includes three components: 1) a patented rotating magnet (mini-MED), 2) standardized magnetic microbeads (the SPIONs), and 3) a novel tissue culture tray (MIRT tray). The mini-MED contains a neodymium-boron-iron permanent magnet, which is rapidly rotated (3 Hz), causing SPIONs to counter-rotate (like meshed gears) in the MIRT tray, moving by means of surface traction. Rabbit endothelial cells were seeded into the lanes of the tray (1/8th inch wide), to which pre-formed blood clots were added. Movement of SPIONs in the MIRT tray was measured at various distances (and relative positions) using video photography. Results: SPIONs moved readily in the MIRT tray through even 100% serum, at distances 7.5 - 30 cm from the magnet. SPION velocity varied according to position with speeds of 0.26 +/- 0.05 cm/sec maintaining the integrity of the endothelial cell monolayer. SPIONS combined with t-PA showed the greatest potential for clot lysis. Conclusions: SPIONs are easily rotated and translated at physiologic distances, even through 100% serum, by means of surface traction generated by the Pulse mini-MED system. The MIRT tray is a convenient device which allows SPION translation over live cells to be studied. Clot lysis was demonstrated with SPION - enhanced delivery of t-PA. A larger version of the Pulse system is currently being evaluated in clinical trials for stroke.



Disclosures: H.H. Engelhard: 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.; Pulse Therapeutics, Inc.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-

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Poster

307. Stroke: Neuroprotection

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Topic: C.08.Stroke

Support: AHA (16SDG31170008)

NIH (P20 GM109098)

NIH (P01 AG027956)

NIH (U54 GM104942)

Title: Mitochondria in cerebral vascular endothelial cells play a key role in blood-brain barrier opening

Authors: *H. HU, I. FAROOQI, K. GRASMICK, S. RELLICK, X. REN Physiolgoy & Pharmacol., West Virginia University, Ctr. For Basic and Translational Stroke Res., Morgantown, WV

Abstract: The blood-brain barrier (BBB) is a dynamic and specialized vascular endothelial cell (VEC) interface that maintains cerebral homeostasis from peripheral attacks. The BBB is disrupted in acute ischemic stroke, and inflammatory components penetrate into the brain causing cerebral edema. Our previous studies have revealed that compromised mitochondria in VECs opens BBB *in vitro* and *in vivo*. To further elucidate the role of mitochondria in BBB opening, we performed transient middle cerebral artery occlusion (tMCAO) in rats, purified cerebral VECs derived from ischemic and non-ischemic hemispheres, and evaluated mitochondrial function in VECs. Interestingly, we observed that oxidative phosphorylation was

significantly inhibited in VEC mitochondria from the ischemic hemispheres compared to the contralateral hemispheres while simultaneously opening the BBB. Further, using a murine tMCAO model, we demonstrated that rotenone (an inhibitor for mitochondrial respiratory chain complex I) exacerbated stroke infarction, worsened neurological deficits, and increased post-stroke mortality. Consistently, rotenone significantly increased BBB permeability in post-stroke mice demonstrated by Evan's blue extravasation assay. These novel findings provide primary evidence that mitochondria in VECs play a key role in BBB opening and suggest a potential new therapeutic approach for neurological disorders by manipulating VEC mitochondria.

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Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: NIH Grant R01NS092876

NIH Gran R01NS097846

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Shriners Grant SHC-85220

Shriners Grant SHC-84293

Title: PTP σ knockdown promotes Akt phosphorylation after spinal cord transection in the lamprey

Authors: *W. RODEMER¹, K. G. ZHANG¹, J. HU¹, M. SELZER^{1,2} ¹Shriners Hosp. Pediatric Res. Ctr., ²Dept. of Neurol., Lewis Katz Sch. of Med. at Temple Univ., Philadelphia, PA

Abstract: Traumatic spinal cord injury (SCI) results in long lasting functional deficits due to the lack of axon regeneration within the mammalian CNS. After SCI, chondroitin sulfate proteoglycans (CSPGs) contribute to a growth non-permissive extracellular environment by interacting with the receptor protein tyrosine phosphatases, PTP σ and LAR, on axons to activate the small GTPase, RhoA, and antagonize the Akt/mTOR pathway. Unlike mammals, lampreys experience robust functional recovery after complete SCI, despite the presence of CSPGs and other inhibitory molecules. After injury, axons of the lamprey's large identifiable reticulospinal

(RS) neurons regenerate with heterogeneous but predictable probabilities. RS neurons that fail to regenerate undergo a delayed form of apoptotic cell death. Previously, this lab reported that lamprey PTPo and LAR mRNAs are preferentially expressed in "bad regenerator" RS neurons before SCI-induced caspase activation. The present study examines the effect of $PTP\sigma$ knockdown (KD) on caspase activation, axon regeneration, and downstream signaling in the larval lamprey following complete spinal cord transection (TX). PTPo KD was induced using antisense morpholino oligomers (MOs) that targeted the splice junctions flanking exon 3 of lamprey PTP_o pre-mRNA to create a reading frame shift and premature stop codon, leading to nonsense-mediated decay of the mis-spliced mRNA. In subsequent experiments, fluoresceinconjugated control or PTPo-targeting MOs were applied *in vivo* to the proximal spinal cord stump immediately following TX. At 2 weeks post-TX, lampreys administered PTPσ MOs showed a 62% reduction in the number of RS cells positive for PTP σ mRNA compared to controls, as assessed by *in situ* hybridization with a riboprobe specific for the normal transcript. At 2, 4, 7, and 10 weeks post-TX, no difference was observed between $PTP\sigma$ and control MO groups in the percentage of morphant cells that were positive for activated caspases using FLICA. At 10 weeks post-TX, PTPo KD did not increase axon regeneration as assessed by retrograde labeling. Nevertheless, PTPo KD significantly increased Akt phosphorylation (T308) in combined brain/spinal cord homogenates at 2 weeks post-TX. This suggests the PTP σ downstream pathway is conserved between mammals and lampreys. It is possible that compensation from other CSPG receptors attenuates the pro-regenerative effects of PTPo KD alone. Ongoing efforts will assess the effects of LAR KD by itself and in combination with PTPσ.

Disclosures: W. Rodemer: None. K.G. Zhang: None. J. Hu: None. M. Selzer: None.

Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

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Topic: C.09. Brain Injury and Trauma

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NIH grant R01NS97846

Shriners grant SHC-85400

Shriners grant SHC-85220

Shriners grant SHC-84293

Title: The effect of axon resealing time on retrograde neuronal death after spinal cord injury

Authors: *K. G. ZHANG¹, W. RODEMER¹, T. LEE¹, J. HU¹, M. E. SELZER^{1,2} ¹Shriners Hosp. Pediatric Res. Ctr., Lewis Katz Sch. of Medicine, Temple Univ., Philadelphia, PA; ²Dept. of Neurol., Lewis Katz Sch. of Med. at Temple Univ., Philadelphia, PA

Abstract: Spinal cord injury (SCI) leads to permanent disability in mammals because injured axons do not regenerate across the lesion to reconnect their previous targets. The failure of axon regeneration is due to both extrinsic inhibitory factors, e.g., the chondroitin sulfate proteoglycans (CSPGs) or the myelin-associated growth inhibitors (MAIs), and to neuron-intrinsic factors, such as the expression of receptors for the extrinsic inhibitory factors. Evidence for the importance of neuron-intrinsic factors is illustrated by the heterogeneity in the regenerative abilities of the axons belonging to the 18 pairs of individually identified reticulospinal neurons (RNs) in the lamprey, some being very good regenerators and others bad. After transection (TX), the bad regenerators tend to upregulate several chemorepulsive receptors (UNC5, DCC, Neogenin, PlexinA and EphB), as well as α-synuclein. These same neurons undergo very delayed apoptosis post-TX, and we have noticed that most (but not all) of them are among the largest RNs. One possible reason for this is that large neurons have large-diameter axons, and these reseal more slowly than small axons. Thus we tested the hypothesis that delayed axon sealing contributes to retrograde neuronal death by introducing retrograde tracers at different times post-TX. In lamprey, axons belonging to the small neurons of the medial inferior reticulospinal nucleus resealed within 15 min post-transection, whereas axons of the larger neurons, e.g., the Mauthner neuron (Mth), I1, M3, and B1 could take more that 20 hours to reseal. We found a significant inverse correlation between an RN's volume and the probability that its axon would regenerate (r = -0.81), or that the neuron would undergo delayed apoptosis, as indicated by fluorescentlylabeled activator of caspases (FLICA; r = 0.73). Next we determined the effect of polyethylene glycol (PEG), which is commonly used to fuse cell membranes and seal damaged axon membranes, on resealing of lamprey RN axons, retrograde neuronal death and axon regeneration. Compared to normal Ringer-treated controls, applying 40% PEG in lamprey Ringer to the cut axon tips for 5 min. speeded axon resealing, reduced initial axon retraction during the first 2 weeks post-TX, enhanced axon regeneration investigated at 4 weeks, and reduced retrograde neuronal death by 57.8%. Since the relationship between neuron size and retrograde death was not absolute, other factors must play a role in determining regenerative ability and long-term survival post-TX.

Disclosures: K.G. Zhang: None. W. Rodemer: None. T. Lee: None. J. Hu: None. M.E. Selzer: None.

Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: US Department of Veterans Affairs Rehabilitation Research and Development Service Merit Review Award I01 RX001497

Title: Correlation of diffusion tensor indices and axonal injury in rat spinal cord distal from injury

Authors: *O. MOTOVYLYAK¹, M. D. BUDDE², S. N. KURPAD², B. D. SCHMIT^{4,3} ¹Biomed. Engin., Marquette Univ., Milwaukee, WI; ²Dept. of Neurosurg., ³Med. Col. of Wisconsin, Milwaukee, WI; ⁴Dept. of Biomed. Engin., Marquette Univ. Dept. of Biomed. Engin., Milwaukee, WI

Abstract: Introduction: The overall objective of this study was to correlate MRI diffusion measures with histological changes in the cervical cord after a contusion injury to the thoracic region in rats. Diffusion Tensor Imaging (DTI) is a non-invasive method of studying central nervous system structure. Despite its widespread use in research, DTI has not been clinically relevant in spinal cord injury, in part due to stabilization hardware at the site of injury which causes imaging artifacts. Furthermore, secondary injury factors such as inflammation, edema, and hemorrhage make prognostication based on imaging biomarkers more difficult. Previous research has documented diffusion changes in the spinal cord both caudal and cranial to the injury epicenter; however, pathological underpinnings of those changes are not clear. Methods and Materials: Female Sprague-Dawley rats (n=40) underwent a controlled T8 contusion injury at one of four severities (mild, moderate, severe, or sham) determined by the drop height of a weight. The rats underwent *in vivo* DTI scans at 2, 30, and 90 days post injury using a 9.4T Bruker horizontal bore scanner and were assessed on the Basso, Beattie, and Bresnahan (BBB) scale to determine their functional recovery and locomotion. Diffusion weighted images were acquired with a 4-shot echo-planar spin-echo sequence (TE=27 ms; TR>1800 ms) and custom routines were used to reconstruct and register DTI maps. Animals were euthanized and C5 segment was excised and fixed in formalin. Sections were stained with primary antibodies for non-phosphorylated neurofilaments (SMI32), which is a marker of injured axons. Digital images were acquired using a confocal microscope (Leica Microsystems), thresholded, and the number of stained objects was counted using a custom-developed script. Two-way ANOVA and linear regression were used to determine significant differences between time points and injury severities for imaging and histological measures.

Results and Discussion: The group averages of stained axons were correlated with BBB scores at early time points. At 2 and 30 days many of the axons in white matter are injured, thus SMI32 staining was abundant. By 90 days, however, most axons were removed by inflammatory cells. Additionally, axial diffusivity (AD) was well correlated with BBB chronically. This suggests that while AD is a good predictor of axonal injury, secondary injury, such as edema and inflammation, also have a strong influence on diffusion at early time points.

Conclusion: Chronically, AD correlates well with injury severity, but the influence of secondary injury factors acutely needs to be explored further.

Disclosures: O. Motovylyak: None. M.D. Budde: None. S.N. Kurpad: None. B.D. Schmit: None.

Poster

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Support: NIH Grant NS079631

Shriners Hospitals for the Children

Craig H. Neilsen Foundation 280274

Title: Synapse formation of NG2 glia prevents re-entry of sensory axons at the CNS-PNS border

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Abstract: In the first *in vivo* imaging analysis (Di Maio et al., J. Neurosci, 2011), we previously found that dorsal root (DR) axons rapidly terminate regeneration at the dorsal root entry zone (DREZ), where they form numerous axon swellings with the features of presynaptic boutons. These observations suggested that the regeneration failure might be due to aberrant synaptogenesis with incorrect targets, which causes growth to cease prematurely at the DREZ. Here, we report that the unknown postsynaptic cells in contact with presynaptic boutons are NG2 glia (i.e., oligodendrocyte precursor cells (OPCs)). We found that NG2 glia are abundant in the CNS territory of the DREZ, that they rapidly proliferate in response to distant DR injury, and that they form an intense 'cellular net' where DR axons usually terminate regeneration. Almost all of the axon tips and shafts intensely labeled with synapse markers are either co-localized or in close apposition with processes of NG2 glia. ImmunoEM analysis revealed that the gold particlelabeled processes and cell bodies of NG2 glia contact pre-synaptic terminals of DRG neurons. We have also cultured purified NG2 glia from adult mice and co-cultured them with adult DRG neurons. Electrophysiological analysis revealed functional synapses between NG2 glia and DRG neurons. Lastly, we investigated if NG2 glia ablation affects axon regeneration across the DREZ. We used an inducible line, Rosa26-iDTa;PDGFRα-CreERTM, and obtained successful ablation of >90% NG2 glia in spinal cord following DR crush. At 2 weeks after crushing DR axons, we observed modest but significantly enhanced penetration of DR axons across the DREZ. These findings show that DR axons fail to regenerate into the spinal cord, at least in part, because they

form aberrant synapses with NG2 glia at the DREZ. These results support the notion that NG2 glia are a previously unappreciated barrier to CNS axon regeneration.

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Poster

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Topic: C.09. Brain Injury and Trauma

Support: US Department of Defense

Canadian Institutes of Health Research

Title: The effect of duraplasty after a traumatic spinal cord injury on behavioural recovery, spinal cord morphology, and tissue sparing, in a porcine model of SCI

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Abstract: Severe swelling of the human spinal cord is typically observed after a traumatic spinal cord injury (SCI). Intrinsic cord swelling within the pia mater, along with the swollen cord filling the subarachnoid space and pushing against the relatively stiff dura mater, may result in increased intraspinal pressure. Such cord constriction can lead to reduced blood flow, hypoxia and ischemia, leading to secondary damage. Using our porcine model of SCI, we examine the effects of an expansile duraplasty surgery on behaviour, spinal cord morphology, and tissue sparing following an acute SCI.

Female Yucatan mini-pigs received a T10 contusion SCI followed by 5-minutes of compression and were randomized into two groups: 1) SCI without dural expansion surgery (control group) or 2) SCI with dural expansion duraplasty with an artificial graft (Durepair, Medtronic; 1-cm width, 10-cm length) centered around the impact site (duraplasty group). Behavioural recovery was monitored weekly for 12 weeks and animals were scored using the Porcine Thoracic Injury Behaviour Scale (PTIBS). Ultrasound imaging at 7 days post-SCI was used to compare spinal cord morphology (dorsal-ventral height of the spinal cord and subarachnoid space) at various level of the spine between the two groups. At 12 weeks post-SCI, animals were euthanized and histological analysis was done on their spinal cords to quantify both grey and white matter sparing.

At 7 days post-SCI, ultrasound analysis showed an enlargement of the subarachnoid space at the T9-T11 for the duraplasty group (T9-T11: 2.0 ± 0.4 mm; 2.1 ± 0.3 mm; 2.4 ± 0.2 mm) compared to the controls (T9-T11: 1.1 ± 0.4 mm; 0.5 ± 0.3 mm; 0.5 ± 0.2 mm) with an increased spinal cord height (duraplasty: 7.4 ± 0.2 mm; controls: 6.2 ± 0.2 mm). Throughout the 12-week recovery period, most control animals were only capable of hind limb dragging (PTIBS score of 1-3) after SCI, while most duraplasty animals showed weight-supported stepping (PTIBS ≥ 4). Histological analysis of the spinal cords demonstrated more spared white matter at the epicenter in the duraplasty group compared to the controls. No difference in the spare grey matter was demonstrated.

In summary, our results demonstrated that, 7 days after SCI the swollen cord almost fills the subarachnoid space. Expansive duraplasty surgery in a porcine model of SCI, enlarged the subarachnoid space for days after SCI, and allowed for non-restrictive swelling of the spinal cord. This might have lead to improved hind limb function and increased spared tissue at the site of injury as observed at 12 weeks post-SCI. This data supports the potential of improving neurologic outcome with expansile duraplasty.

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Poster

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Topic: C.09. Brain Injury and Trauma

Support: Brain Canada

Rick Hansen Institute

MITACS

VGH & UBC Hospital Foundation

Title: Proteomic biomarkers of acute traumatic spinal cord injury in human cerebrospinal fluid

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Abstract: Introduction: Development and translation of effective therapies for acute traumatic spinal cord injury (SCI) are hindered by both the paucity of information regarding the biology of human SCI, as well as reliance on functional tests of motor function and sensation to grade injury severity. In the acute SCI setting, comorbid substance use and severe injuries often prevent valid baseline examination of SCI severity. In turn, individuals become ineligible for clinical trial enrollment, because they cannot be appropriately stratified into studies within required timelines; this enrollment challenge contributes to the high cost and difficulty of clinical trials for potential therapies, and scarcity of information regarding human SCI biology. Beyond offering the promise of more objective diagnostics, exploratory investigations seeking to identify biomarkers of SCI severity present the opportunity to increase our understanding of the biology of human SCI.

Objective: This study seeks to quantify the abundance of 496 peptides within the cerebrospinal fluid (CSF) of human SCI patients and characterize the changes in related protein loads during the five days immediately following SCI, in order to enhance understanding of human SCI, as well as to broaden the scope of potential protein biomarkers of acute SCI severity.

Methods: Parallel reaction monitoring (PRM), a mass spectrometry approach for targeted protein identification and quantification, is used to analyze the presence of 496 peptides in CSF from AIS A (n = 55), AIS B (n = 12), and AIS C (n = 12) SCI patients obtained through indwelling intrathecal catheters at 24, 48, 72, 96, and 120 hours post-injury, as well as in CSF from non-SCI patients (n = 19). The 496 target panel represents 396 unique proteins and includes a number of CSF contents known to be readily detectable via mass spectrometry, as well as peptides which the literature links to processes of SCI and other neurological conditions, such as: Alzheimer's, Parkinson's disease, stroke, and traumatic brain injury, the literature suggests them to be potential CSF biomarkers.

Results: This project establishes a targeted experimental workflow which defines m/z ranges and retention time windows at which each peptide can optimally be detected. We are in the process of preparing the CSF samples for PRM evaluation.

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Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

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Program#/Poster#: 308.07/AA32

Topic: C.09. Brain Injury and Trauma

Support: Rick Hansen Institute

Title: Serum inflammatory cytokines and biomarkers of injury severity in acute human spinal cord injury

Authors: *N. MANOUCHEHRI¹, K. DONG¹, F. STREIJGER¹, L. BELANGER², L. RITCHIE², S. PAQUETTE³, J. STREET^{1,3}, T. AILON³, M. BOYD³, C. G. FISHER³, M. F. DVORAK^{1,3}, J.-M. MAC-THIONG⁴, S. PARENT⁵, C. BAILEY⁶, S. CHRISTIE⁷, K. SO¹, B. K. KWON^{1,3}

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Abstract: Rapid, accurate assessment of the severity of spinal cord injury (SCI) is critical in determining a course of treatment. Currently the 'gold standard' is the American Spinal Injury Association Impairment Scale (AIS), which uses a functional assessment to determine injury severity. However, it has its drawbacks: it requires verbal information from patients who may not be able to respond, and relies on potentially subjective self-reporting. Biological markers that objectively classify injury severity and predict outcome would circumvent these drawbacks and greatly facilitate efforts to evaluate acute SCI therapies.

In an attempt to identify blood protein biomarkers we previously analyzed serum samples of a small set of AISA A, B, and C patients (n=9) collected 17-23 hours post-injury. Here, we extend our findings by reporting on the analysis of serum from 115 acute SCI patients. Specifically, the purpose of this study is to analyze serum biomarkers over a 5 day period post-SCI to determine if they are able to distinguish between baseline AIS grades, and predict AIS conversion. Patients classified as AIS A, B, or C were recruited as part of an ongoing, prospective multicenter study (Canadian Multicenter CSF Pressure and Biomarker Study, CAMPER). Blood samples were collected every 8h for 5 days in order to determine serum profiles following injury. Additionally, non-SCI control serum samples were collected from patients undergoing hip or knee surgery during their spinal anaesthetics. Blood samples were kept at room temperature for 20 min and then centrifuged at 10,000 rcf for 5 min to obtain serum. Serum concentrations of IL-6, IL-8, TNF-R1, MCP-1, IP-10, S100B, GFAP, and NSE were evaluated using custom multiplex kits. Serum levels of TAU were determined using commercially available kits. Plates were read on the Meso Scale Discovery electrochemiluminescent system.

Initial data confirms that the concentrations of serum proteins such as IL-6, IL-8, MCP-1, Tau, S100b, GFAP and NSE are many orders of magnitude lower in the blood than cerebrospinal fluid (CSF). While our data show clear differences in CSF levels of IL-6, Tau, S100b, and GFAP between the different baseline AIS grades, with the exception of Tau such a clear distinction between baseline AIS grades is not observed in the serum samples. Further analysis is currently being conducted.

By characterizing the acute pathophysiologic responses to traumatic SCI in humans and

establishing serum biomarkers for better injury stratification and improved prognostication of neurologic recovery, we hope to provide the field with tools that can be utilized in clinical trials to facilitate the evaluation of novel therapies.

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Poster

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Topic: C.09. Brain Injury and Trauma

Support: Rick Hansen Institute

Title: Development of a high-thoracic pig model of SCI reveals robust and rapid impairments in cardiac and hemodynamic function

Authors: *K. SHORTT¹, N. MANOUCHEHRI¹, K. SO¹, Z. K. SARAFIS^{1,2}, M.-S. POORMASJEDI-MEIBOD^{1,2}, F. STREIJGER¹, B. K. KWON^{1,3}, C. R. WEST^{1,2} ¹ICORD (International Collaboration on Repair Discoveries), ²Sch. of Kinesiology, Univ. of British Columbia, Vancouver, BC, Canada; ³Dept. of Orthopaedics, Vancouver Spine Surgery Inst., Vancouver, BC, Canada

Abstract: Purpose: 1) To develop a large animal model of SCI that exhibits cardiovascular dysregulation resembling that which is observed in individuals with high lesion SCI, and 2) to examine the acute cardiac and hemodynamic response to SCI. **Methods:** Ten Yorkshire pigs (25Kg, approx. 6 months of age) were instrumented with a triple lumen central venous catheter, femoral artery catheter, and left-ventricular pressure-volume conductance catheter. The left-ventricular catheter was placed via a right carotid artery approach under fluoroscopic guidance. Pigs were subjected to a 20cm weight drop (50g) contusion SCI at T2 or T10 followed by 5 min of compression. Beat-by-beat systolic blood pressure (SBP), mean arterial pressure (MAP), heart rate (HR), left-ventricular pressure (LVP), and rates of contraction (dP/dt_{max}) were continuously monitored until 4 hours post-SCI. **Results:** In response to T2 SCI, there was an immediate pressor response that peaked at 3 min post-SCI, whereby MAP increased by 35mmHg (± 5.5 mmHg), and dP/dt_{max} (1100 ± 342 mmHg/s). For T10 SCI, there was no significant increase in any cardiac or hemodynamic indices (p>0.122). Following decompression at 5min, MAP, LVP, dP/dt_{max} and dP/dt_{min} were significantly reduced relative to baseline in both T2 and

T10 SCI (all p<0.025). Between 30 and 60 minutes following decompression, there was a gradual (albeit insignificant) increase in all hemodynamic indices in both T2 and T10 SCI. From 1 hour post-SCI onwards, MAP and LVP_{max} gradually declined and were consistently lower than pre-SCI at 3hr post-SCI in both T2 and T10 SCI (all p<0.06). Only T2 SCI exhibited a significant reduction in dP/dt_{max} from 3 hours post-SCI onwards (p=0.017). **Conclusions:** High-thoracic SCI is characterized by an immediate pressor response, followed by a rapid and sustained impairment in cardiac and hemodynamic function. Low-thoracic SCI is characterized by a rapid and sustained reduction in blood pressure but not cardiac function.

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Poster

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Topic: C.09. Brain Injury and Trauma

Title: Microrna and cell-free dna biomarkers for injury severity in acute human traumatic spinal cord injury

Authors: *S. S. TIGCHELAAR¹, F. STREIJGER¹, S. SINHA², S. FLIBOTTE³, M. RIZZUTO¹, J. STREET⁴, S. PAQUETTE⁴, M. BOYD⁴, T. AILON⁴, C. FISHER⁴, M. DVORAK⁴, J.-M. MAC-THIONG⁵, S. PARENT⁶, C. BAILEY⁷, S. CHRISTIE⁸, K. VAN KEUREN-JENSEN⁹, C. NISLOW³, B. K. KWON¹⁰

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Abstract: INTRODUCTION

With limited treatment options currently available to clinicians, there is an urgent need for noninvasive biomarkers to aid in the scientific development and clinical validation of novel therapies for acute spinal cord injury (SCI). MicroRNAs are small regulatory noncoding RNA molecules that mediate gene expression. The current body of literature suggests that miRNAs orchestrate a wide range of biological processes such as inflammation, synaptic plasticity and apoptosis. Many miRNAs are highly enriched in the nervous system and are also directly implicated in the pathogenesis of various neurodegenerative diseases including traumatic TBI and SCI. In addition to miRNA, Cell-Free DNA is released from tissues following cell death and is emerging as a diagnostic tool for monitoring pathology. Specific tissues contain DNA fragments with altered methylation, therefore when they are detected in fluids, it is possible to determine their tissue origin and quantify the magnitude of damage in the affected tissue.

METHODS

In this study, we compared the cell-free expression profiles of microRNA (miRNA) and cell-free DNA (cfDNA) in serum and cerebrospinal fluid (CSF) collected from human patients with SCI. Patients classified as AIS A, B, or C were recruited as part of an ongoing, multi-center study called CAMPER. CSF and blood samples were collected every 8h for 5 days in order to compare CSF and serum miRNA profiles following injury. Additionally, control "non-injury" CSF samples were collected via a single lumbar puncture from patients undergoing hip or knee surgery during their spinal anesthetics.

Next-generation sequencing was used to compare effects of injury severity on miRNA and cfDNA levels. Extracellular miRNA and cfDNA were isolated and sequenced using the Illumina HiSeq 2500 system. Generated data was processed using the Mayo Clinic's Comprehensive Analysis Pipeline for miRNA Sequencing Data (CAP-miRSeq), and aligned reads were used to compare differential expression.

RESULTS AND CONCLUSIONS

Here, we present miRNA and cfDNA profiles in CSF and serum clinical samples during acute stages after SCI. This analysis was done in parallel to the investigation of miRNA in the serum of pigs following SCI. This characterization is important to establish whether biomarkers of SCI found in pigs can be transferred to humans and vice-versa.

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Poster

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Topic: C.09. Brain Injury and Trauma

Title: Dendritic cells and their immunomodulatory role after SCI

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Authors: *S. SALIMI ELIZEI<sup>1</sup>, *S. SALIMI ELIZEI<sup>1</sup>, *S. SALIMI ELIZEI<sup>2</sup>, K. DONG<sup>1,2</sup>, M.-S. POORMASJEDI-MEIBOD<sup>1,3</sup>, S. S. TIGCHELAAR<sup>1,2</sup>, N. MANOUCHEHRI<sup>1,2</sup>, K. SO<sup>1,2</sup>, K. SHORTT<sup>1,2</sup>, Y. J. KURTZKE<sup>1,2</sup>, F. STREIJGER<sup>1,2</sup>, B. K. KWON<sup>1,2</sup>
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Abstract: Effective restraint of secondary injury plays a fundamental role in minimizing neurodegeneration and significantly improves functional recovery after spinal cord injury (SCI). Although the complex mechanisms underlying secondary damage after SCI are still not fully defined, the local immune response to SCI has been identified as an important mediator of secondary damage. Following injury, marked changes in cellular inflammation have been observed in spinal cord lesion. Different innate (neutrophils, monocytes, dendritic cells, etc.) and adaptive (T and B lymphocytes) immune cells are activated and recruited to the injured area. Dendritic cells (DCs) are professional antigen presenting cells (APC), with a key role in promoting and modulating immune responses. When these cells mature, they link innate and adaptive immunity by presenting processed antigens and providing costimulatory signals to activate specific T cells. While the presence of different DC subsets in the CSF of patients with some inflammatory neurological diseases, such as lupus erythematosus (SLE), multiple sclerosis (MS), experimental autoimmune encephalomyelitis (EAE), has been reported, despite their recognized importance in regulating immune responses, very little is known about DC subsets and their role in SCI and many fundamental questions, such as the onset and duration of infiltrated DC subsets in acute SCI is remain unanswered. DCs comprise two major classes: plasmacytoid DCs (pDCs) and conventional or classical DCs (cDCs). pDCs rapidly produce type 1 interferon (IFN) following activation through nucleic acid-sensing TLRs, such as TLR7 and TLR9. cDCs are dedicated APCs that have a characteristic dendritic morphology and express high levels of MHC class II molecules. In this study, we have evaluated the frequency and cytokine production profile of infiltrating DC subsets in blood, CSF and spinal cord lesion of rat and porcine models of SCI during acute and chronic phase of spinal cord injury. Pigs and rats were undergone the contusive SCI using a weight-drop apparatus. CSF and blood samples were drawn pre-injury, 4, 8, 12, & 24 hours and then daily until day 7 post-injury. The percentages of pDC and cDC in blood and CSF were evaluated in vitro by flow cytometry. Preliminary data from our rat and pig models of SCI have revealed that there are differences in the pre- and post-SCI pDCs and cDCs frequency in CSF and blood. The data generated from this study, will improve our knowledge of local and systemic immune response mechanisms triggered after SCI. Moreover, it may pave the way for novel cell therapies in SCI, aiming at modulating specific pathogenic cell type.

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Poster

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Wings for Life Spinal Cord Research Foundation

Canadian Institutes of Health Research

UBC Orthopaedics Research Excellence Fund

Title: The acute effects of vasopressor administration timing on perfusion, oxygenation and metabolic responses in a porcine model of traumatic spinal cord injury

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Abstract: Traumatic Spinal cord injury (SCI) can lead to severe irreversible consequences, having a devastating impact on patients, families and society. A decrease in mean arterial pressure (MAP) is often observed after SCI, resulting in decreased cord perfusion and subsequent ischemia, both of which contributes to secondary damage. Early intervention strategies remain limited, with vasopressor support of MAP to improve spinal cord perfusion being one of the few treatment options available. Previous work from our lab has shown that norepinephrine, over phenylephrine, was the more effective vasopressor for restoring blood flow and oxygenation. However, the effect of intervention timing, particularly in the acute setting, on perfusion, oxygenation, and metabolic responses warrants consideration. Indiscriminate augmentation of MAP in the traumatically injured spinal cord with impaired vascular autoregulation could do more harm than good. In this study, we sought to determine the effects of MAP support intervention on the acute hemodynamic and metabolic responses during the compressed and decompressed states of injury.

Using our porcine model of SCI, female Yucatan miniature pigs received a T10 contusion injury followed by 2-hours of sustained compression. After SCI, norepinephrine was used to elevate MAP by 20 mmHg for a period of 1.5 to 3.0 hours while the cord was compressed, after the cord was decompressed, or during both injury states. A control group, which received an SCI,

received no vasopressor treatment. Laser Doppler flowmetry/oxygenation, fibre optic pressure, and microdialysis probes were inserted into the cord at 2mm and 22mm caudal from injury to measure spinal cord blood flow (SCBF), oxygenation (PO2), spinal cord pressure (SCP), and from which microdialysis samples were collected to analyze for lactate, pyruvate, glucose, glutamate and glycerol. Preliminary data show that MAP support during cord compression only mildly improved SCBF and PO2 and did not reduce L/P ratios back to baseline levels. However, MAP support after decompression improved SCBF and PO2 more effectively, restoring both parameters to pre-injury levels and decreased L/P ratio back to baseline levels. Combined, our preliminary results suggest that MAP support is more effective at improving cord perfusion and preventing ischemia after decompression than during the compression state of injury. Histological analysis for haemorrhagic damage resulting from vasopressor treatment will be examined and further work to consolidate our current findings is being carried out.

Disclosures: E.B. Okon: None. K. So: None. F. Streijger: None. N. Manouchehri: None. K. Shortt: None. M. Strawford: None. D.E. Griesdale: None. M. Sekhon: None. B.K. Kwon: None.

Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 308.12/BB1

Topic: C.09. Brain Injury and Trauma

Support: KAKENHI

Title: Gene and cellular localizations of chemokines and their receptors after spinal cord injury(SCI) in mice

Authors: K. YAGURA^{1,3}, H. OHTAKI¹, T. TSUMURAYA³, A. SATO³, J. WATANABE⁵, K. MIYAMOTO⁴, *S. TANAKA^{6,7}, Y. HIRAIZUMI², K. KANZAKI³, K. HONDA¹ ¹Anat., ²Orthopedic Surgery, Showa Univ. Sch. of Med., Tokyo, Japan; ³Orthopedic Surgery, ⁴Emergency & Clin. Care Med., Showa Univ. Fujigaoka Hosp., Yokohama, Japan; ⁵Ctr. for Biotech., Showa Univ., Tokyo, Japan; ⁶Dept. of Pharmacology, Toxicology and Therapeut., ⁷Pharmaceut. Educ., Showa Univ. Sch. of Pharm., Tokyo, Japan

Abstract: Controlling inflammatory responses is important for functional recovery after spinal cord injury (SCI). Infiltrating inflammatory cells such as neutrophils and monocyte/macrophages are suggested to deteriorate the tissue damage, and to support for tissue recovery. Although the cell migration into the injury site is regulated by several chemokines, expression patterns and distributions of the chemokines and the receptors have not been determined in detail. Male young adult C57/BL6 mice were subjected to spinal cord transection by razor at level between

T9 and T10 intervertebral spinal cord. The spinal cord from T8 to T11 was collected on shamoperated control mice (day 0), and on post-operative day 1, 3, 7 and 14. The tissues were quantified gene expressions of monocyte/macrophage-related CC- and CXC-chemokines, and the receptors by SYBR-Green based qPCR. Moreover, the frozen sagittal sections of spinal cord were immunostained against CCL2, CCL5 and the receptor antibodies on day 0, 1 and 14. There were two patterns of increases the chemokines. Increases of the gene expression within 3 days after SCI were including such as CCL2 and CXCL1. The increase gene expressions of the receptors also consisted with the chemokines. The gene expressions of these chemokines and receptors were gradually decreased at day 7. On the other hand, the CCL5 gene expression gradually increased after SCI and reached a peak on day 14. A receptor of CCL5, CCR5 increased and reached a peak on day3 and remained elevated until day 14. Immunoreactivities of CCL2, and CCL5 on spinal cord were merged with NeuN-positive cells in the peri-injury sites at day 1. Although immunoreactivity of CCL2 was diminished, CCL5 immunoreactivity was recognized in Iba1-positive and GFAP-positive cells, suggesting microglia/macrophage and astroglia in peri-injury site at day 14. These results were suggested diverse chemokines were increased in spinal cord after injury and could regulate an increase of inflammation and the resolution communicated with glial cells. (No COI)

Disclosures: K. Yagura: None. H. Ohtaki: None. T. Tsumuraya: None. A. Sato: None. J. Watanabe: None. K. Miyamoto: None. S. Tanaka: None. Y. Hiraizumi: None. K. Kanzaki: None. K. Honda: None.

Poster

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ETH 'Eat to learn to move' network

Christopher and Dana Reeve Foundation

Title: The spinal transcriptome after large unilateral cortical stroke and its impact on sprouting and 'side-switch' of contralateral corticospinal fibers

Authors: *J. KAISER¹, I. SALPETER¹, V. BARBOSA C. DE SOUZA², M. D. ROBINSON², M. E. SCHWAB¹ ¹Brain Res. Inst., Zürich, Switzerland; ²Inst. of Mol. Life Sci., Zürich, Switzerland

Abstract: Functional recovery of skilled forelimb movements after a large insult to the primary motor and premotor cortex involves activation of the contralateral hemisphere with the contralesional cortex growing new collaterals crossing the midline into the denervated hemi-cord on cervical levels of the spinal cord. The underlying molecular mechanisms of growth induction in these adult, intact, fully wired and functional corticospinal tract (CST) fibers, midline crossing and target innervation are yet unclear. Such mechanisms could be of high importance for future therapies. Cervical sprouting following a large photothrombotic insult to the motor cortex in adult C57bl/6 mice induced pronounced sprouting of fibers of the intact CST across the spinal cord midline into the denervated hemi-cord. These sprouts were most numerous at cervical levels C5-C6 and targeted mostly the intermediate laminae (Lam 5-7). Total RNA of this target area was prepared at different time points after stroke corresponding to previously identified key plastic events: initiation phase of fiber growth (7dpi), maintenance phase (14dpi), period of highest density of sprouts (28d) and anatomical rearrangement and pruning phase (42dpi). RNA-Seq (Illumina Hi-Seq) reads were mapped to the mouse transcriptome and quantified for differential expression analysis. Genes were ranked by their fold change to controls, and top differentially regulated genes for each time point were identified. This unbiased approach allows for evaluation of the spinal transcriptome in response to de- and re-innervation and the defined key steps of this process. Further analyses include gene ontology (GO) analysis as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis to establish functional relevant processes at all time points.

Disclosures: J. Kaiser: None. I. Salpeter: None. V. Barbosa C. de Souza: None. M.D. Robinson: None. M.E. Schwab: None.

Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: Swiss National Science Foundation

ERC advanced grant

Christopher and Dana Reeve Foundation

Title: Transcriptional screen in the target region of sprouting hindlimb corticospinal fibers after thoracic spinal cord injury in rats

Authors: *N. RUSSI, A.-S. HOFER, A. K. ENGMANN, S. IMOBERSTEG, J. M. HELDNER, M. E. SCHWAB

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Abstract: Depending on the severity of a CNS injury, in humans as well as in experimental animal models certain degrees of functional recovery are observed. Rodent studies have provided much evidence for underlying anatomical changes involving neurite outgrowth of both spared and axotomized fiber tracts, also referred to as sprouting.

Following a thoracic spinal cord injury in adult rats, axotomized fibers of the hindlimb corticospinal tract (CST) were found to sprout into the cervical spinal cord at different rostrocaudal levels. Anterograde tracings showed that these new collaterals of motor cortical hindlimb fibers project into the intermediate laminae of the cervical grey matter, particularly pronounced at the level of cervical segment five (C5). The molecular cues which induce the growth and guide these sprouts of hindlimb CST fibers are unknown. We hypothesize that sectreted factors and guidance and adhesion molecules are specifically expressed in the intermediate laminae of the cervical spinal cord to direct and target the ingrowth of the sprouting hindlimb CST fibers.

To test our hypothesis a transcriptional screen in adult rats with a thoracic dorsal hemisection injury is currently being performed. At different time points post injury the intermediate laminae of C5 were dissected. Total RNA was extracted and subjected to deep sequencing. Data are being analysed, and differentially expressed genes will be presented. The results will allow us to determine the role of the cervical spinal cord (target region) for sprouting of hindlimb CST fibers.

Disclosures: N. Russi: None. A. Hofer: None. A.K. Engmann: None. S. Imobersteg: None. J.M. Heldner: None. M.E. Schwab: None.

Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

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Topic: C.09. Brain Injury and Trauma

Title: Emergence of epigenetic mechanisms by metalloproteinases and purinergic receptors modulation in the maladaptive plasticity in the rat spinal cord

Authors: A. VIRTUOSO¹, C. DE LUCA¹, G. CIRILLO¹, L. ALBERGHINA², A. COLANGELO², *M. PAPA¹

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Abstract: A significant reshaping of the spinal neuroglial circuitry follows peripheral nerve injury (PNI), it perturbs spinal homeostasis and induces a condition defined maladaptive synaptic plasticity. Metalloproteinases (MMPs) activity and the purinergic system "in vivo" both, play a key role in determining maladaptive changes in the central nervous system (CNS) following nerve injury. The maladaptive plasticity is an enduring process, producing long-lasting changes; therefore it claims a genomic remodeling. The aim of this study was to investigate the mechanism through which, MMPs inhibition and/or purinergic receptors (P2X) blockage may produce transcriptional and post-transcriptional regulation, having a neuroprotective role. We study inflammatory and epigenetic markers in the spared nerve injury (SNI) of the sciatic nerve rat model. Rats were divided into 5 groups: 1) sham, 2) SNI, 3) SNI-treated with MMP inhibitor (GM6001), 4) purinergic receptors inhibitor [oxidized ATP (oxATP)], or 5) both GM6001 and oxATP. Inflammatory and epigenetic factors were evaluated by western blot and immunocytochemical analysis at day 3 and 8 after surgery. Histone deacetylase (HDAC)1, HDAC2 and the Recombination signal Binding Protein for immunoglobulin kappa J region (RBPJ) expression were analyzed in the lumbar spinal cord. The inhibition of MMPs activity and the P2Xrs were effective to reduce reactive gliosis and neuroinflammation markers, chiefly 8 days after the injury. Moreover, investigating the neurotrophic pathways, both treatments decreased TrkA receptor expression; the oxATP modified pro-Nerve Growth Factor (pro-NGF) and p75 expression. MMPs inhibition modulates HDAC1 protein expression while combined therapy affected HDAC2 levels, thus suggesting a key role in epigenetic modifications. Taken together, these data suggest that MMPs and P2Xrs play a dual role as transcriptional and posttranscriptional regulators, moreover confirmed the therapeutic potential of GM6001 and/or oxATP in reducing the maladaptive plasticity changes in the CNS, in response to injury.

Disclosures: A. Virtuoso: None. C. De Luca: None. G. Cirillo: None. L. Alberghina: None. A. Colangelo: None. M. Papa: None.

Poster

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Topic: C.09. Brain Injury and Trauma

Support: Swedish Research Council

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NINDS

Hjarnfonden

Title: Abnormal posture and muscle spasms after spinal cord injury: different neuronal circuits but a common neural mechanism

Authors: *C. BELLARDITA¹, O. KIEHN²

¹Karolinska Institutet, Stockholm, Sweden; ²Dept. of Neuroscience, Karolinska, Stockholm, Sweden

Abstract: Spinal cord injury (SCI) results in sustained involuntary muscle contractions that may generate abnormal and chronic posture or sudden and temporally-limited muscle spasms. The neuronal mechanisms for these aberrant motor responses are not well understood. Here we used a mouse model of chronic SCI with a sacral lesion that exhibits an abnormal posture of the tail, characterized by sustained activity in small sized motor units (MUs), and spontaneous muscle spasms, characterized by a sudden activation of large sized MUs. After SCI optogenetic activation of excitatory spinal interneurons triggered and maintained spasms with temporallydefined recruitment of large MUs. Further in vitro calcium imaging in lesioned mice revealed a persistent neural activity in excitatory spinal interneurons during spasms. On the contrary, stimulation of excitatory neurons did not evoke a chronic tail posture and the concurrent sustained change in activity of the small MUs, indicating different circuits generating the two responses. However complete silencing of the calcium channels Ca_V 1.3 in all spinal neurons reduced both spasms and the abnormal posture. However when the Cav 1.3 channels were silenced in excitatory spinal neurons the mice exhibited a decrease in spasms even though they developed dystonia, similar to the wild-type, but a. In conclusion abnormal posture and spasms may emerge from small and large MUs whose activity is driven by different pre-motor neuronal circuits. However the two motor dysfunctions are characterized by sustained activity of the MUs thus the Cav 1.3 channels may represent a shared neural mechanism, allowing output amplification.

Disclosures: C. Bellardita: None. O. Kiehn: None.

Poster

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Topic: C.09. Brain Injury and Trauma

Title: Identification of a novel binding domain for heparin in RPTP σ , but not LAR or RPTP δ : Implications for proteoglycan signaling Authors: H. KATAGIRI¹, S. HIGASHI¹, A. A. MORGAN^{3,1}, N. J. BANGAYAN¹, R. JUNKA¹, *P. YU⁴, H. M. GELLER² ²Office of Educ., ¹NIH, Bethesda, MD; ³Columbia Univ., New York, NY; ⁴GHMICR, Jinan Univ., Guangzhou, China

Abstract: Type IIa Receptor protein tyrosine phosphatases (RPTPs) have been shown to modulate neural regeneration and development. All members of this family (RPTP σ , RPTP δ , and LAR) have a cell adhesion molecule-like extracellular domain that includes three N-terminal Ig domains and four to nine fibronectin type III (FNIII) domains, as well as tandem intracellular tyrosine phosphatase domains. Compelling evidence suggests that both heparan sulfate (HS) and chondroitin sulfate (CS) are ligands for RPTPo and LAR with similar nanomolar dissociation constants, and the Lys-loop located in the first Ig domain is responsible for ligand binding. However, the reason for the different functional outputs of HS and CS binding to RPTPo (respectively, promotion and inhibition of axonal growth) remains a mystery. Although attributed to the differential oligomeric state of RPTP σ upon binding to HS, but not to CS, our data indicate that both HS and CS can cause RPTP_o clustering. Furthermore, we demonstrate a differential contribution of FNIII domains required for high affinity binding to glycosaminoglycans (GAGs). In particular, binding of $PTP\sigma$ to heparin was not completely abolished by disruption of the Lys-loop. This, along with a decrease in binding with deletion of the fourth FNIII-containing domain, leads us to hypothesize that there is a greater contribution of the FNIII domain in binding to GAGs than previously believed. To support this notion, we found the fourth FNIII-containing domain alone binds to heparin independently of the three Ig domains and does not bind to CS-E or DS. Intriguingly, while addition of heparin to 293 cells expressing wild-type RPTP σ (full length) altered tyrosine phosphorylation transiently, disruption of the Lysloop in RPTPo (full length) did not affect this transient response induced by heparin. Thus, our findings clearly indicate that this novel HS-specific binding domain on RPTPo plays an important role in controlling the different biological actions of HS and CS.

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Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: Craig Neilsen Foundation

Title: Impact of spinal cord injury on synaptic transmission in neurons of mouse major pelvic ganglia

Authors: *C. W. KYI¹, V. B. GARCIA², D. J. SCHULZ³

¹Biol. Sci., ²Div. of Biol. Sci., Univ. of Missouri, Columbia, MO; ³Div. of Biol. Sci., Univ. of Missouri-Columbia, Columbia, MO

Abstract: Lower urinary tract function is one of the autonomic functions impaired after spinal cord injury (SCI). Acute SCI results in areflexic bladder and complete urinary retention while patients with chronic SCI suffer from hyperreflexic bladder, incontinence and inefficient voiding. The major pelvic ganglia (MPG) of the mouse contain neurons that innervate the urinary bladder, genitals and distal colon. MPG is a mixed ganglion that primarily receives parasympathetic input from the preganglionic neurons located in the sacral cord through pelvic nerve and sympathetic input from those in the lumbosacral cord through hypogastric nerve. Nicotinic cholinergic transmission is the major neurotransmitter system involved in pre-to postganglionic communication at the MPG. The goal of this project is to understand the effects of spinal cord injury on synaptic transmission at the MPG neurons involved in micturition function. The spinal cord was completely transected at thoracic 8 (T8) vertebral level of transgenic mice (B6.Cg-Tg (RP23-268L19-EGFP) 2Mik/J) containing cholinergic neurons labelled with green florescent protein. We used both male and female mice that were 8 to 12 weeks old. We had four experimental groups- acute SCI (3days post-surgery), chronic SCI (28 days post-injury), and sham-surgical control groups for each time point. We quantified mRNA abundance of receptor genes using quantitative PCR as well as measured functional properties of the neurons via intracellular recording. We measured the excitability, passive properties, and firing patterns of the neurons in response to current injection, and also measured excitatory postsynaptic potentials (EPSPs) and excitatory postsynaptic currents (EPSCs) in response to pelvic nerve stimulation, and pressure ejection of 1mM acetylcholine (ACh). We further characterized the major subunits involved in the neurotransmission by measuring current responses before and after application of subtype-specific blockers. We measured the mRNA abundance of $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 6$, $\alpha 7$, $\beta 2$ and β4 nicotinic receptor subunits in whole MPGs. Our preliminary data indicate that after 3 days post-injury, the mRNA for a3 subunit was significantly decreased compared to those of sham controls. However, after 28 days, the mRNA levels were no longer significantly different from those of sham controls. These results suggest that acute SCI results in downregulation of one of the major receptor subunits involved in synaptic transmission at the MPG, and this may contribute to impairment of target organ function following SCI.

Disclosures: C.W. Kyi: None. V.B. Garcia: None. D.J. Schulz: None.

Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: WFL-US-004/12

I01BX007080

Title: Reciprocal interaction between mesenchymal stem cells and macrophages support the formation and maturation of blood vessels. An *In vitro* study

Authors: *I. MALDONADO-LASUNCION^{1,2}, M. OUDEGA³, J. VERHAAGEN⁴ ¹The Miami Project to Cure Paralysis, Univ. of Miami, Miami, FL; ²Vrije Univ. Amsterdam, Amsterdam, Netherlands; ³Dept. of Neurolog. Surgery, The Miami Project to Cure Paralysis, Univ. of Miami Dept. of Neurolog. Surgery, Miami, FL; ⁴Netherlands Inst. for Neurosci., Amsterdam, Netherlands

Abstract: Mesenchymal stem cell (MSC) transplants support spinal cord nervous tissue repair, in part, by mediating the formation of new blood vessels (angiogenesis) resulting in tissue sparing associated with improved functional outcome. Usually, at the time of transplantation, the injury site is populated by inflammatory cells, including many macrophages presenting the inflammatory phenotype (M1). From previous studies using different types of tissues we know that MSCs and macrophages engage in a molecular cross communication supporting the establishment of a reparative environment. At present, the interactions between transplanted MSCs and endogenous inflammatory macrophages in the injured spinal cord are poorly understood. We have employed an *in vitro* system to start understanding the presence and molecular mechanisms of the crosstalk between transplanted MSCs and endogenous macrophages, with special emphasis on angiogenesis and immunomodulation. We showed that the secretion of vascular endothelial growth factor (VEGF) by MSCs was dramatically increased after exposure to inflammatory macrophage-conditioned medium. VEGF plays an important role in the formation of new blood vessels. Interestingly, when MSCs were exposed to the conditioned medium of reparative macrophage (M2)-conditioned medium, they significantly increased the secretion of platelet-derived growth factor (PDGF) as well as that of brain-derived neurotrophic factor (BDNF) and insulin growth factor-1 (IGF-1). Within angiogenesis, PDGF is known for its role in stabilizing newly formed blood vessels. We hypothesized that VEGF in an autocrine loop enhances the production and secretion of anti-inflammatory cytokines by MSCs, which in turn mediate the shift in macrophage phenotype from M1 to M2. Such reciprocal crosstalk would orchestrate the creation of a reparative milieu in the injury site. Revealing MSCmacrophage interactions may help identifying molecular targets for enhancing MSC-mediated nervous tissue repair.

Disclosures: I. Maldonado-Lasuncion: None. M. Oudega: None. J. Verhaagen: None.

Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: Craig H. Neilsen Foundation

Title: Role of local neurogenesis in functional recovery following spinal cord injury in larval zebrafish

Authors: *D. VASUDEVAN

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Abstract: Successful treatment for human spinal cord injury (SCI) is currently an insurmountable goal. The biggest obstacle is the inability of severed axons and neurons to regenerate. Adult mammals have a conspicuous inability to regenerate post SCI, but zebrafish show full functional recovery throughout life due to axon regrowth, rewiring of existing circuits and/ or local neurogenesis. Our aim is to understand the role of local neurogenesis in functional recovery. Studies from our lab have shown that resident radial glia undergo a change in morphology, and actively make neurons post injury. We have generated a unique combinatorial approach to genetically label newly-born neurons from a radial glial lineage after injury, using the Cre-lox recombination system. To test the hypothesis that neurons born after SCI integrate into existing circuits and are required for recovery of sensorimotor behavior, we are using two approaches. In the first approach, we are genetically ablating neurons that arise from a radial glial lineage post injury to evaluate their specific requirement in swimming behavior. The second approach is to express a calcium reporter specifically in neurons arising from radial glia after injury, to determine whether they exhibit physiological activity during a defined escape behavior. We are also tracing the lineage of radial glia to test whether specific neuronal identities with known physiological properties are restored. Testing swim and escape behaviors following recovery will tell us about the relative contributions of axon regrowth and neurogenesis to the reestablishment of local circuitry. These studies will establish the significance of neuronal regeneration as a potential therapeutic response to SCI.

Disclosures: D. Vasudevan: None.

Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: NIH Grant NS055976

Title: Acute immune response to spinal cord injury in the dorsal root ganglia as a predictor of pain development

Authors: *S. J. CHHAYA, A. ONG, J. D. HOULE, M. R. DETLOFF Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Spinal cord injury (SCI) damages sensory systems and causes chronic, intractable neuropathic pain. Selective upregulation of pro-inflammatory mediators such as the chemokine CCL2 by specific phenotypes of myeloid cells causes secondary tissue damage, influences neuronal plasticity, alters nociceptive processing by nociceptors in the dorsal root ganglia (DRG) and may contribute to the development of chronic pain. There is a gap in our understanding of chemokine signaling events that transpire in the DRG after SCI that could induce pathological changes in the nociceptor and drive pain. This study aimed to determine whether myeloid cell recruitment after SCI is essential to pain development and explore whether the phenotype and cytokine release profile of macrophages in the DRG dictates pain development. We used a previously established C5 unilateral contusion model of spinal cord injury-induced neuropathic pain in female Sprague-Dawley rats, where 40% of rats develop pain. Rats were sacrificed early after SCI (12, 24, 72 hours and 5 days) as well as at 14 days post-injury (dpi) when pain was assessed using von Frey, Hargreaves' and mechanical conflict avoidance tests. qPCR was used to measure pro-inflammatory cytokine expression and expression of myeloid cell phenotype markers in whole C7-8 ipsilesional DRG lysates. Preliminary analysis revealed that rats are distributed into two distinct clusters based on cytokine gene expression-those with a large foldchange in cytokine mRNA expression and those with near-normal expression (n=6/timepoint). ED-1 immunohistochemistry was used to identify infiltrating myeloid cells in the DRGs at these time points. Infiltration began as early as 12 hours post-injury, and rats with pain at 14 dpi had an increased number of ED1+ myeloid cells than rats without pain. We are conducting experiments to block myeloid cell infiltration using a CCR2 inhibitor (INCB3344) early (0-72h) post-SCI to prevent CCL2-mediated recruitment, and will assess the necessity of myeloid cell recruitment in the development of pain, as well as correlations between pain development and macrophage phenotype and inflammatory environment in the DRG. Studying the acute immune response after SCI in the DRG could reveal key mechanisms that initiate maladaptive processes that lead to nociceptor dysfunction and prolonged pain.

Disclosures: S.J. Chhaya: None. A. Ong: None. J.D. Houle: None. M.R. Detloff: None.

Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: Pennsylvania Department of Health

Department of Biomedical Sciences, PCOM and Health Research Grant

Title: AAV9-driven neuron-specific transgene expression: A tool to study the role of IMP2 in axon regeneration

Authors: *S. BLIZARD, D. PARK, N. O'TOOLE, S. NOOROZ, M. DELA TORRE, S. AUSTIN, J. HARMAN, S. HARASZTI, M. XU Philadelphia Col. of Osteo. Med., Philadelphia, PA

Abstract: Insulin-like growth factor-II (IGF-II) mRNA-binding protein-2 (IMP2) is one of the three homologues (IMP1-3) that play an important role in the post-transcriptional regulation of gene expression during development. Its alternative splice product aberrantly expresses in human hepatocellular carcinoma, and is therefore identified as HCC. Several lines of studies have demonstrated that IMP1/ZBP1 (zipcode binding protein) is critical in axon guidance and regeneration by regulating localization and translation of specific mRNAs. However, the role of IMP2 in the nervous system is largely unknown. Previously, we used transfection to overexpress IMP2 and HCC in the cultured dorsal root ganglion (DRG) neurons to study their roles during axon regeneration in vitro. We noted significant toxicity. We also tried to overexpress IMP2 and HCC by rabies-G pseudotyped lentiviral vector in the severed sciatic nerve, and found the expressions predominantly in neurons and but occasionally in non-neuronal cells as well. In this study, we used the synapsin promoter-driven adeno-associated viral (AAV) 9 constructs to specifically express YFP-IMP2 and -HCC in neurons both in vitro and in vivo. We applied AAV9.hSyn.YFP, AAV9.hSyn.YFP-hIMP2 and AAV9.hSyn.YFP-hHCC in the DRG neuron culture. We also injected the viral vectors into the crushed sciatic nerve. These viral vectors have proven to be effective to introduce neuron-specific transgene expression of IMP2 and HCC. In addition, our data shows that overexpression of HCC promotes axon outgrowth of the cultured DRG neurons. Ongoing experiments are aimed at studying the role of IMP2 and HCC in the nerve crush model and understanding the underlying mechanisms.

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Poster

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Topic: C.09. Brain Injury and Trauma

Support: CSIC I+D 2014-327

ANII MOV_CA_2016_1_1284

Title: Genomic basis of spinal cord regeneration in the freshwater turtle Trachemys scripta elegans

Authors: ***A. VALENTIN**¹, G. LIBISCH², O. TRUJILLO - CENÓZ¹, C. ROBELLO², R. E. RUSSO¹, F. ALVAREZ-VALIN³

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Abstract: Spinal cord injury in humans generates devastating conditions leading to the permanent loss of sensory-motor and autonomic functions. This is because the injured cord is unable to self-repair as in some non-mammalian vertebrates. Remarkably, freshwater turtles are able to heal the injured spinal cord and partially regain lost functions even during adulthood. The importance of this relies on the fact that they are the only amniotes exhibiting this feature. Gene expression analysis at the sub-acute phase of the response (4 days after injury) shows that several genes involved in diverse functions are activated : reaction to ischemic insult, extracellular matrix re-organization, immune response, and inflammation and axonal growth. Interestingly we found that almost all of these genes are present in mammals too. Additionally, we failed to find genes exclusively belonging to regenerating taxa. Indeed, the comparison of expression patterns among species shows that the response to spinal cord injury in the turtle is more similar to that of mice and non-regenerative Xenopus than to Xenopus during its regenerative stage. This observation, along with current accepted phylogenetic placement of turtles (sister group of crocodilians and birds), indicates that that turtles developed this capability from a nonregenerative ancestor, that was achieved by re-organizing gene expression patterns. In this work we extend our previous studies by characterizing the gene expression profile using RNASeq analysis during the whole process of regeneration after injury from the acute phase (1 day after injury) till the moment in which functional recovery is observed (at least 30 days after injury). We speculate our findings may provide useful clues to develop new therapies for the treatment of spinal lesions.

Disclosures: A. Valentin: None. G. Libisch: None. O. Trujillo - Cenóz: None. C. Robello: None. R.E. Russo: None. F. Alvarez-Valin: None.

Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 308.24/BB13

Topic: C.09. Brain Injury and Trauma

Support: NC3R (NC/1001063/1)

BBSRC (BB/L021498/1) to CGB, TB

Wellcome Trust (108906/Z/15/Z) to CGB

German Research Council (DFG WE5736/1-1) to DW

Title: Macrophages promote axonal regeneration after spinal cord injury in zebrafish larvae

Authors: *T. TSAROUCHAS, D. WEHNER, T. BARRETT, T. CARR, L. CAVONE, T. BECKER, C. G. BECKER

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Abstract: Zebrafish, in contrast to mammals, regenerate lost neurons and functional axonal connections after spinal cord lesion. We find that the immune response, occurring after injury, is essential for neuronal regeneration in larvae (Ohnmacht et al., 2016, Development 143, 1464-74), but its contribution to axonal regrowth across a non-neural lesion site is less clear. Here we characterize the role of the innate immune response for axonal regeneration by assessing axonal regrowth, debris clearance and cytokine levels in wildtype larvae and macrophage-deficient irf8 mutants. We find that at 2 days after a lesion of 3-day-old larvae, 81% of wildtype larvae showed axonal continuity between rostral and caudal spinal cord, whereas in irf8 mutants only 37% showed axonal bridging. Recovery of swimming function was also impaired in the mutants compared to wildtype fish, which regain their swimming capacity within 48 hours post-injury. Debris clearance was strongly inhibited in the irf8 mutants compared to lesioned age-matched wildtype larvae. Cytokine levels were increased in wildtype animals after injury and higher than in irf8 mutants. A notable exception was the pro-inflammatory cytokine *il1-beta*, which was increased in expression in the mutant at 2 days post-lesion. At that time point, *il1-beta* was already down-regulated to basal levels in wildtypes. Currently, we are testing the relevance of *ill-beta* upregulation in the mutant. These results suggest important functions of macrophages for debris clearance and resolution of inflammation in the spinal lesion environment, which in turn promotes axonal regrowth in successful spinal cord regeneration in zebrafish.

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Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: Halls A-C

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Program#/Poster#: 308.25/BB14

Topic: C.09. Brain Injury and Trauma

Title: miR-21 deletion reduces inflammation and promotes locomotor recovery in spinal cord injury

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Abstract: MicroRNAs (miRs) are known to regulate the development and maintenance of the CNS. Specifically, miR-21 is among the most robustly upregulated genes following spinal cord injury (SCI). However, the impact of miR-21 on SCI pathobiology is not fully understood. Predictive algorithms suggest that miR-21 targets multiple genes in the Interleukin-6 pathway (IL6ST, IL6RA, and STAT3). The IL-6/STAT3 pathway is known to influence CNS inflammation and has been implicated in SCI; therefore, we hypothesized that knockout of miR-21 will alter the activation of microglia, the primary inflammatory cells of the CNS, in a model of non-traumatic SCI. Wild-type and miR-21 Knockout mice were randomly assigned to either sham or injury groups (n=7/group). For injury groups, a biomaterial that facilitates bone deposition was inserted under the C5-C6 laminae, causing progressive compression of the cervical spinal cord. Injured mice were assessed for motor deficits using the rotarod test. Standard immunohistochemistry for Iba1+ microglia was performed followed by cell counting using unbiased stereology. To determine effects of miR-21 on target genes during inflammation, primary microglia were isolated from Day 2 pups and stimulated with E.coli Lipopolysaccharide (LPS). RNA was isolated and analyzed by RT-qPCR. Deletion of miR-21 improved locomotor performance on the rotarod by 70% compared to wild type injured mice (p<0.01). Active Iba1+ microglia were reduced by 45% in the compressed region of miR-21 knockout mice compared to wild-type spinal cords (p<0.001). Isolated miR-21 knockout microglia also expressed 4-10 times more IL6 signal transducer (IL6ST) RNA than wild-type after stimulation with LPS (p<0.01). In summary, knockout of miR-21 reduced inflammation at the injury site and resulted in significant motor preservation relative to wild-type animals. These effects on microglia activation were

associated with differential expression of predicted miR-21 target genes in miR-21 knockout mice. Given these relationships, miR-21 and its downstream genes may provide interesting therapeutic targets for future SCI research.

Disclosures: A.M. Laliberte: None. S.K. Karadimas: None. P. Vidal Vera: None. K. Satkunendrarajah: None. M.G. Fehlings: None.

Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

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Topic: C.09. Brain Injury and Trauma

Support: Paralyzed veterans of America

AOSNA

Title: Stimulation of cervical excitatory cells restores breathing after cervical SCI

Authors: *K. SATKUNENDRARAJAH¹, S. K. KARADIMAS², A. M. LALIBERTE³, S. SIVAKUMARAN⁴, M. G. FEHLINGS⁵

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Abstract: Progressive cervical spinal cord injury (cSCI) results in subclinical and mild respiratory dysfunction in contrast to the acute and traumatic cSCI, despite the significant disruption of the cervical neural network. Using a mouse model of progressive cSCI, we found a significant loss of phrenic motoneurons (PMNs) that innervate the main inspiratory muscle, without severe respiratory insufficiency similar to what is observed in humans with this disease. Observation of progressively increased Vglut2 positive boutons on the preserved PMNs indicate that despite the significant PMNs loss the respiratory motor output is maintained via glutamatergic synaptic plasticity onto preserved PMNs. Retrograde tracing from the diaphragm using pseudorabies technology in *vglut2::cre; tdtomato* mice demonstrated that this increased input derives from cervical glutamatergic interneurons. To confirm the role of the cervical glutamatergic synaptic input in promoting respiratory plasticity and ventilation in progressive cSCI, we injected *AAV-FLEX-PSAML141F–GlyR-IRES-eGFP* in the ventromedial area of C3-7 spinal levels of *Vglut2::cre* mice two week prior to the induction of cSCI. Subsequently, PSEM-mediated silencing of these neurons disrupted ventilation in CSM mice. Further, pharmacogentic stimulation of cervical glutamatergic neurons following acute and traumatic cSCI promoted

significant respiratory recovery compared to control mice after injury. In conclusion, this study provides novel insights into mechanisms of respiratory plasticity that can be exploited to promote respiratory recovery following traumatic SCI.

Disclosures: K. Satkunendrarajah: None. S.K. Karadimas: None. A.M. Laliberte: None. S. Sivakumaran: None. M.G. Fehlings: None.

Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 308.27/BB16

Topic: C.09. Brain Injury and Trauma

Title: Stimulation of lumbar excitatory cells preserves function after cervical spinal cord injury

Authors: *S. K. KARADIMAS¹, K. SATKUNENDRARAJAH², A. M. LALIBERTE³, S. SIVAKUMARAN⁴, S. GOSGNACH⁵, M. G. FEHLINGS⁶

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Abstract: Spinal cord injury (SCI) is devastating, causing significant locomotor deficits and potential paralysis with no optimal treatment available to date. Many approaches for restoring locomotor function have focused on reestablishing the necessary excitation by either coaxing axons across the lesion to their synaptic partners, or directly activating the CPG using electrical stimulation based on the assumption that the lumbar networks remain intact after trauma. However, the extent to which the intrinsic anatomical properties of these distal circuits remain intact following rostral SCI has been conspicuously overlooked. Using mice and human with cervical SCI (cSCI), first we demonstrate significant neuronal loss in the ventromedial regions of the distal lumbar spinal cord following cSCI. Ablation of the lumbar projecting cervical neurons in naïve mice reproduces this disintegration lumbar locomotor network. Anatomical and spatial analysis of the lumbar spinal cords of transgenic mice demonstrated that cSCI induces significant loss of excitatory cells in the rhythmogenic core of the locomotor CPG, ventromedial area of the upper lumbar spinal cord, while there was no change in the inhitory population. Moreover, we found a significant loss of motoneurons within the lumbar enlargement of cSCI compared to sham ChAT-GFP mice. With the preserved motoneurons displaying altered morphological characteristics such as decreased dendritic arborizations and soma size. These findings dramatically alter the way in which we approach the development of treatments to restore walking in cSCI patients. Indeed, targeted and exogenous stimulation of lumbar excitatory

neurons immediately after cSCI was able to prevent this degeneration distal locomotor network and promote functional outcome.

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Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 308.28/BB17

Topic: C.09. Brain Injury and Trauma

Support: ICL start up

Title: Epigenomic signatures

Authors: I. PALMISANO¹, M. C. DANZI², A. HERVERA¹, E. MACLACHLAN¹, L. LEVI⁴, E. ELLIOTT⁴, V. LEMMON³, J. L. BIXBY⁵, *S. DI GIOVANNI¹ ¹Imperial Col. London, London, United Kingdom; ³Neurolog. Surgery, ²Univ. of Miami, Miami, FL; ⁴Bar Ilan Univ., Bar Ilan University, Israel; ⁵Miami Proj to Cure Paralysis, Univ. Miami, Miller Sch. Med., Miami, FL

Abstract: Following injury, peripheral nervous system (PNS) axons mount a regenerative response while axons in the central nervous system (CNS) fail to regenerate. The dorsal root ganglia (DRG) bipolar neurons are a well-suited model to investigate the molecular mechanisms of this differential regenerative ability: a cell body extends one regenerative competent axon in the peripheral nerves and one regenerative incompetent in the CNS spinal cord. A sciatic nerve lesion elicits gene expression changes, including in regenerative pathways, which are radically distinct from those that follow a spinal cord lesion. While recent evidence suggests that epigenetic modifications can underlie this differential response a systematic study of epigenetic states upon regenerative vs non-regenerative axonal injury is still missing. To this end, we have performed combined high throughput studies including RNAseq, ChIPseq for both active (H3K27ac and H3K9ac) and repressive (H3K27me3) histone modifications, and ATACseq (Assay for Transposase-Accessible Chromatin sequencing) that explores chromatin accessibility, from L4-L6 DRG in response to a peripheral (sciatic nerve axotomy- SNA,) vs a central spinal (dorsal column axotomy- DCA) axonal injury. The combined genome wide analysis showed that: 1) SNA leads to a more robust transcriptional response than DCA, with upregulated genes involved in regulation of transcription and regenerative signalling cascades, and downregulated genes involved in ion transport, axonogenesis and synapse formation; 2) transcriptional response to SNA occurs via increased H3K27ac/H3K9ac occupancy at promoters of genes involved in transcription; 3) in contrast, following DCA, genes related to transcription are enriched for

H3K27me3; 4) H3K9/K27ac enriched genes upon SNA lie in genomic region characterized by a differential chromatin accessibility with respect to DCA; 5) injury-dependent genes are enriched in binding sites to CTCF, a well -known chromatin folding organizer and gene expression regulator, involved in neuronal function. Together, these data suggest that a looser chromatin conformation characterized by active histone marks represents the epigenomic signature discriminating regenerative versus non-regenerative states following peripheral or spinal axonal injury respectively. As proof of principle that proper chromatin folding is required for axonal regeneration, CTCF null mice failed to respond to conditioning-dependent increase in DRG outgrowth and showed drastically reduced axonal regeneration after sciatic nerve injury.

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Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

Location: Halls A-C

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH/NINDS R21NS096670 (AGR)

NRSA F31NS093904 (JLG)

NIH/NINDS 2P30NS051220

Title: Transplantation of mitochondria following spinal trauma in rats

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Abstract: We sought to test whether transplanting purified mitochondria into contused rat spinal cords results in 1) incorporation into various host cell types, 2) preservation of acute host cell bioenergetics, and 3) long-term functional neuroprotection. For visualization of transplanted mitochondria, we used transgenically modified PC-12 cells in which mitochondria were labeled with tGFP. Alternatively, and for clinical relevance, we also transplanted mitochondria isolated from syngeneic rat soleus muscle. Freshly isolated tGFP (50, 100 or 150 μ g total) or muscle (50 or 100 μ g total) mitochondria were microinjected into the penumbra of severely contused spinal cords within 1 hr after injury at L1/L2 (250 kdyn, IH Impactor) in adult female Sprague-Dawley

rats. Depending on outcome measures, they survived 24 hr, 48 hr, 7 days or up to 6 weeks. Results showed that transplantation of either tGFP (n=6-7/group) or muscle (n=3-4/group) mitochondria significantly (p<0.05) maintained bioenergetics of injured spinal cord tissues 24 hr after injury, with maximum effects at the 100ug dosage. Confocal imaging showed prominent rostro-caudal spread of exogenous tGFP mitochondria from injection sites after 24-48 hr that dissipated by 7 days (n=4/time point). At the earlier time points, tGFP mitochondria co-localized conspicuously with microglia/macrophages and endothelial cells, with less incidences in astrocytes and oligodendrocytes, and none in neurons. Assessments of hindlimb functional recovery (BBB-LRS) and paw withdrawal latencies (Von Frey hair) over 6 weeks after transplanting 100ug tGFP or muscle mitochondria (n=8-11/group) showed no significant differences in hind limb locomotion or mechanical hypersensitivity compared to vehicle-injected injured groups. Morphometric analyses further showed no differences in grey or white matter tissue sparing. In summary, while intraspinal injections of mitochondria after spinal trauma improved cellular bioenergetics acutely, such maintenance of respiration did not translate into improved long-term functional neuroprotection. Ongoing studies are more fully characterizing the cell-type incorporation propensities and investigating the comparative effects of delayed grafting times.

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Poster

308. Spinal Cord Injury: Cellular and Molecular Mechanisms

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Wings for Life

International Spinal Research Trust

Henry Smith Charity

Miami Project to Cure Paralysis

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Title: Environmental enrichment induces a lasting increase in axon regeneration potential via activity-dependent epigenetic reprogramming

Authors: *T. H. HUTSON¹, L. ZHOU², I. PALMISANO¹, A. HERVERA¹, F. DE VIRGILIIS¹, E. MCLACHLAN¹, G. KONG³, C. KATHE⁴, K. BARTHOLDI⁵, M. C. DANZI⁶, A. MEDRANO-FERNANDEZ⁸, J. LOPEZ-ATALAYA⁸, A.-L. BOUTILLIER⁹, L. D. MOON¹⁰, T. K. KUNDU¹¹, J. L. BIXBY¹², V. LEMMON⁷, A. BARCO⁸, G. COURTINE⁵, S. DI GIOVANNI¹

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Abstract: Injury to the adult mammalian central nervous system (CNS) leads to permanent deficits in sensory and motor function. Dorsal root ganglion (DRG) neurons are essential for sensorimotor function as they receive and convey sensory information from the environment to motor circuits in the spinal cord and ultimately, the brain. Here, we show that exposing mice to environmental enrichment (EE) prior to an injury induces a long-lasting increase in the regenerative potential of DRG neurons, priming them for robust axon regeneration after injury in both the peripheral and central nervous systems. The EE-mediated increase in axon regeneration is dependent on proprioceptive afferent feedback and increased neuronal activity leading to epigenetic reprogramming via CREB Binding Protein (CBP)-dependent histone acetylation. This results in gene expression changes in proprioceptive DRG neurons, increasing their intrinsic growth capacity. These findings highlight prior exposure to EE as a robust physiological means of priming sensory neurons for enhanced axon regeneration. Finally, we were able to mimic exposure to EE by delivering a small-molecule activator of the acetyltransferase CBP/p300 after spinal cord injury that promoted axonal regeneration and functional recovery.

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Poster

309. Opioids and the Treatment of Pain

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: D.03. Somatosensation: Pain

Support: University of Arizona Start-Up Fund

Title: Phosphatidylethanolamine-binding protein reduces mu opioid receptor induced βarrestin2 recruitment and promotes opioid mediated antinociception

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Abstract: There is currently an opioid abuse epidemic occurring in the United States, due to many factors including over-prescribing and the lack of effective, side-effect null opioids. Current efforts employ multiple strategies to increase the therapeutic index of analgesics. These strategies include restricting opioid drugs to the site of injury, creating functionally selective compounds to elicit only therapeutic effects, and developing non-opioid drugs as analgesics. Unfortunately these methods have not fulfilled their promise or are slow to develop. Part of the reason is due to a lack of understanding of the signaling cascades and regulators involved in opioid receptor signaling. There are three opioid receptors, mu (MOP), delta (DOP), and kappa (KOP), with the majority of clinical opioid analgesic effects being mediated through the MOP. A proteomic screen performed after chronic morphine treatment has shown an upregulation of phosphatidylethanolamine-binding protein (PEBP) in the brain. PEBP is a novel kinase inhibitory protein which in its monomeric form binds and inhibits Raf-1 signaling; however after phosphorylation it dimerizes and binds g-protein receptor kinase 2 (GRK2). It has not been previously implicated in MOP signaling, however its interactions with Raf-1 and GRK2 suggest the potential. Here we utilize a combination of target knockdown using siRNA and pharmacological inhibition to show that PEBP decreases ßarrestin2 recruitment and increases morphine-mediated anti-nociception in vitro and in vivo. PEBP knockdown in vitro causes an increase in Barrestin2 recruitment to MOP by 30.2% without altering downstream Raf-1 to MAP kinase signaling. Disruption of the interaction between PEBP and its binding partners, Raf-1 and GRK2, via a covalent PEBP inhibitor locostatin causes a 41.7% decrease in the effectiveness of morphine in the tail flick assay, without altering performance in the rotarod test. Taken together these results suggest a role for PEBP whereby phosphorylation and subsequent dimerization can inhibit GRK2 and Barrestin2 recruitment, therefore enhancing opioid receptor signaling and antinociception. Investigation of the molecular mechanisms by which PEBP regulates MOP signaling and anti-nociception are currently under investigation. Eventually, PEBP may be

exploited for the creation of novel drug screening and development strategies, based on the elucidated function and mechanism we show here.

Disclosures: J.E. LaVigne: None. K.A. Edwards: None. J.M. Streicher: None.

Poster

309. Opioids and the Treatment of Pain

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 309.02/BB21

Topic: D.03. Somatosensation: Pain

Title: Modulation of alcohol reward by a truncated variant of the mu opioid receptor gene OPRM1

Authors: T. G. BROWN¹, S. MARTINEZ², J. XU⁴, G. W. PASTERNAK⁵, Y. PAN⁴, *G. C. ROSSI³

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Abstract: Alcohol use remains a global problem with approximately 10-20% of chronic alcohol users becoming alcoholics. Despite severe consequences, alcohol continues to be a rewarding and highly addictive substance as other drugs of abuse. The opioid system has long been hypothesized to involve alcohol reward. Recent work in our laboratories has led to the generation of a knockout (KO) mouse model in which exon 11 (E11) of the mu opioid receptor gene (OPRM1) was disrupted. In E11-KO mice, analgesia for several mu opioids, including M6G, fentanyl and heroin, was significantly reduced, while analgesic action for morphine and methadone was intact. Moreover, the analgesic action for a novel class of opioids, such as IBNtxA, that fails to produce the typical side effects associated with traditional opiates, was completely lost in E11-KO mice. To investigate the role of E11-associated variants in alcohol reward, we evaluated chronic alcohol self-administration in wild-type (WT) and E11 knockout (E11-KO) mice using a two bottle choice paradigm. Total consumption was measured over a 30day period with escalating concentrations of alcohol (2-10%). Results showed E11-KO mice drank significantly higher amounts of alcohol as compared to WT control mice, suggesting involvement of E11-associated variants in the rewarding effects of alcohol. Although alcohol is presumed to have a very different mechanism of action than heroin, the physiological properties of reward might use a common E11 genetic map.

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Poster

309. Opioids and the Treatment of Pain

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: D.03. Somatosensation: Pain

Title: The nitric oxide-cGMP-ATP-sensitive K^+ channel pathway participates in the peripheral antinociceptive effect of nalbuphine, but not of buprenorphine on the rat formalin test

Authors: *M. I. ORTIZ¹, R. CARIÑO-CORTÉS¹, H. A. PONCE-MONTER¹, E. FERNÁNDEZ-MARTÍNEZ¹, G. CASTAÑEDA-HERNÁNDEZ² ¹Área Académica De Medicina, ICSA, UAEH, Pachuca de Soto, Mexico; ²Dept. de Farmacología, CINVESTAV. IPN., Ciudad de México, Mexico

Abstract: The aim of this study was to examine if the peripheral antinociceptive effects of the opioid agonist/antagonist nalbuphine and the opioid partial agonist buprenorphine involve the sequential participation of nitric oxide (NO) and cyclic guanosine monophosphate (cGMP) synthesis followed by potassium channel opening. We evaluated the participation of this pathway in the formalin test. Female Wistar rats (180-220 g) were injected in the dorsal surface of the right hind paw with 50 microliter of formalin (1%). Nociception was quantified as the number of flinches of the injected paw during 1 hour, whereas a reduction of the number of flinches was considered antinociception. Rats received a subcutaneous (s.c.) injection (50 microliter) into the dorsal surface of the right hind paw of vehicles or increasing doses of nalbuphine (50-200 µg/paw), buprenorphine (1-5 µg/paw) or pinacidil (a potassium channel opener; 25-100 µg/paw) 20 min before formalin injection into the ipsilateral paw. All compounds produced significant local peripheral antinociception (p<0.05). Nalbuphine effect was reverted by the s.c. injection into the ipsilateral paw of NG-L-nitro-arginine methyl ester (L-NAME, an inhibitor of NO synthesis; 10-100 µg/paw), by methylene blue (a non-selective inhibitor of guanylyl cyclase; 100-500 µg/paw), by glibenclamide or glipizide (ATP-sensitive K⁺ channel inhibitors; 10-100 µg/paw) and by 4-aminopyridine and tetraethylammonium (voltagedependent K⁺ channel inhibitors; 10-100 µg/paw). Pinacidil effect was blocked by glibenclamide and glipizide. The antinociceptive effect produced by buprenorphine was blocked by the s.c. injection of 4-aminopyridine (10-100 µg/paw) and tetraethylammonium (50-200 µg/paw), but not by glibenclamide (100 µg/paw), glipizide (100 µg/paw), L-NAME (100 µg/paw) or methylene blue (500 µg/paw). The present results suggest that the NO-cGMP-ATP-sensitive K⁺ channel pathway is involved in the peripheral antinociceptive effect of nalbuphine, but not of buprenorphine, and thus provide evidence for differences in peripheral mechanisms of action among these opioid drugs.

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Poster

309. Opioids and the Treatment of Pain

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Support: NIH/NIGMS RO1 GM 106035

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Title: Targeting delta opioid receptor- kappa opioid receptor (DOR-KOR) heteromers in peripheral pain sensing neurons: A strategy for peripheral analgesic drug development

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Abstract: Activation of DOR-KOR heteromers expressed on rat pain-sensing neurons (nociceptors) produces robust, peripherally-mediated, antinociception (Berg et al., 2012). Here we show that the ligand 6'-guanidinonaltrindole (6'-GNTI) is a selective agonist for DOR-KOR heteromers in peripheral nociceptors. Further, we provide evidence that DOR-KOR heteromers may be viable targets for peripheral analgesic drug development. Opioid-mediated inhibition of PGE2-stimulated adenylyl cyclase (AC) activity was measured in primary cultures of adult rat peripheral sensory neurons (ex vivo model). The efficacy of 6'-GNTI to inhibit AC was lost after siRNA knockdown of either DOR or KOR expression. Further, under these conditions, 6'-GNTI acted as an antagonist at both KOR and DOR suggesting that 6'-GNTI binds both DOR and KOR but agonist activity requires simultaneous occupancy of both receptors. To further validate that 6'-GNTI activates DOR-KOR heteromers in peripheral nociceptors, we tested effects of a TAT- peptide corresponding to the TM1 domain of DOR on a rat behavioral model of thermal nociception in response to DPDPE (DOR agonist), U50488 (KOR agonist) or 6'GNTI. Intraplantar injection of the DOR TM1-TAT peptide had no effect on DPDPE- or U50488-mediated reduction of PGE₂-evoked thermal allodynia, however, the anti-allodynic response to 6'-GNTI was lost completely. By contrast, a control TAT peptide had no effect on the antinociceptive response to 6'-GNTI. We next determined the ability of 6'-GNTI to reduce thermal nociception following induction of inflammation in the rat hind paw with carrageenan. Even though 6'-GNTI, as well as DPDPE and U50488 each inhibited carrageenan-induced nociceptive responses completely when injected 15 min after carrageenan, only 6'-GNTI inhibited nociception when tested 3h or 24h after carrageenan administration.

These results suggest that, unlike DOR and KOR, the DOR-KOR heteromer remains responsive for a prolonged period of time following inflammation induced by carrageenan. Thus, DOR-KOR heteromers expressed in peripheral nociceptors may be attractive targets for the development of drugs for improved treatment of pain.

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Poster

309. Opioids and the Treatment of Pain

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: D.03. Somatosensation: Pain

Support: NIH Grant DA038645

Title: Functional selectivity profiles of U50,488 analogues at kappa opioid receptors (KOR) expressed in peripheral nociceptors

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Abstract: Functional selectivity describes the ability of agonists to differentially regulate individual signaling pathways coupled to a given receptor. By fine-tuning the structure of a ligand, the activity of therapeutically efficacious signaling can be enhanced whereas signaling that leads to adverse effects can be minimized. We have found that inhibition of peripheral painsensing neurons by activation of peripheral KOR has profound antinociceptive effects in rats. However, in addition to activation of Gai signaling leading to antinociceptive efficacy. The goal of this study was to determine if replacement of either the diamino group (analogues SJ-1-147 and SJ-1-163) or the phenacyl group (the analogue SJ-1-171) of U50488 altered KOR-mediated signaling and peripheral antinociceptive responses.

In primary cultures of adult rat peripheral sensory neurons (ex vivo model), all three analogues inhibited PGE2-mediated cAMP accumulation with similar potencies and efficacies as U50488. However, unlike U50488, all three analogues did not increase ERK activity. Ex vivo, U50488 inhibited release of the neuropeptide, CGRP, however, the concentration response curve (CRC) was U-shaped but rendered monotonic in the presence of the ERK inhibitor, U0126. By contrast, the CRCs for inhibition of CGRP release by SJ-1-147 and -163 were monotonic (not U-shaped) which is consistent with the loss of ERK signaling due to the structural modifications.

Interestingly, the CRC for SJ-1-171-mediated inhibition of CGRP release was U-shaped despite the loss of ERK signaling. In a rodent model of thermal nociception, intraplantar (ipl) injection of peripherally-restricted doses of U50488 produced antinociception with an inverted U-shaped dose response curve (DRC). By comparison, ipl injections of SJ-1-147 and -163 reduced PGE2-evoked thermal allodynia with monotonic DRCs. Consistent with the U-shaped CRC for inhibition of CGRP release, SJ-1-171 produced antinociception with an inverted U-shaped DRC. Overall, our data suggest that structural modifications of a KOR ligand can selectively alter its efficacy for individual signaling cascades, which may lead to improved therapeutic outcomes for peripherally-mediated analgesia.

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Poster

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Support: Institutional Support, University of Arizona

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Arizona Area Health Education Centers Research Grant

Title: siRNA screening of Regulator of G Protein Signaling (RGS) and adenylyl cyclase (AC) genes to determine the mechanism of endomorphin cAMP biased signaling

Authors: A. KERESZTES, J. LAVIGNE, D. CHIEM, K. OLSON, *J. M. STREICHER Pharmacol., Univ. of Arizona, Tucson, AZ

Abstract: The opioid receptor family consists of three G-protein coupled receptors, the mu, delta, and kappa (MOR, DOR, KOR). These receptors are responsible for a constellation of overlapping (anti-nociception) and non-overlapping (reward, sexual behavior, etc.) physiological roles, and are often expressed together in the same synaptic regions. These receptors can be endogenously activated by opioid peptides such as endorphins, enkephalins, endomorphins, and dynorphins. However, we performed *in vitro* studies using human opioid receptors expressed in CHO and N2a cells, which revealed that these ligands possess similar potencies and efficacies in ³⁵S-GTP_γS coupling at MOR and DOR (except that endomorphins were selective for MOR, while only dynorphins activated the KOR). This creates a problem for how endogenous opioid peptides can selectively signal to different opioid receptors present in the same physical location. We hypothesized that biased agonism could be a potential mechanism to explain endogenous

ligand selectivity. We thus screened Dynorphin A and B, Endomorphin 1 and 2, Leu-enkephalin, and Met-enkephalin, along with reference ligand controls, in ³⁵S-GTP_YS coupling, βarrestin2 recruitment, and cAMP inhibition in CHO and N2a cells expressing the human opioid receptors. We found a significant bias of both Endomorphin 1 and 2 for cAMP signaling (vs. ³⁵S-GTP_YS coupling) at the MOR in both CHO and N2a cells. Seeking a mechanism for these changes, we reasoned that differential RGS protein regulation or differential AC isoform coupling could explain the bias of Endomorphin for cAMP signaling. By performing the cAMP assay in permeabilized cells in the presence of $GTP\gamma S$, we were able to equalize the potency of Leuenkephalin with the endomorphins, supporting our hypothesis and suggesting the involvement of RGS proteins in endomorphin biased signaling. However, the RGS4 inhibitor CCG-50014 had no effect on Leu-enkephalin vs. Endomorphin potencies, suggesting that this isoform is not involved. Since no other RGS inhibitors are commercially available, we designed and optimized an siRNA screen to sequentially knock down each of the ~22 RGS genes and 10 AC genes in MOR-N2a cells, to comprehensively test which of the genes is responsible for Endomorphin cAMP biased signaling. We report here our progress to date in carrying out this screen. Once identified, we will further test the molecular mechanism of the identified gene in Endomorphin biased agonism, and further confirm the role of the gene in vivo. This study thus promises to identify an important molecular mechanism of biased signaling, which could explain in part how endogenous peptides selectively signal in vivo.

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Poster

309. Opioids and the Treatment of Pain

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Topic: D.03. Somatosensation: Pain

Support: NIH Grant DA15353

Title: Characterization of DALDA peptides following bolus intrathecal delivery (1)

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Abstract: The present studies characterized the potency and side effect profile of a series of small opioid peptides with high affinity for the mu opioid receptor. Male Sprague Dawley rats were prepared with chronic intrathecal catheters, assessed on hind paw thermal escape and

evaluated for side effects including Straub tail, truncal rigidity, pinnae and corneal reflex. In this work, TICP ([Dmt1]DALDA-(CH2)2-NH-TICP[psi]; MW=1519), DMT-DALDA (H-Dmt-D-Arg-Phe-Lys-NH2 MW=981), H-Dmt (H-Dmt-Cit-Phe-Lys-NH2 MW=868), Nle (H-Dmt-D-Cit-Phe-Nle-NH2 MW=739), and TAPP (H-Tyr-D-Ala-Phe-Phe-NH2 MW=659) were examined. 1) All agents resulted in a dose-dependent reversible effect upon motor function (Straub Tail > Truncal rigidity). 2) The ordering of analgesic activity (%MPE) at the highest dose (pmol) lacking reliable motor signs after bolus delivery was: DMT-DALDA (80%±6/3 pmol); H-DMT (75%±8 /1 pmol); Nle (84%±10 /300 pmoL); TAPP (56%±12 /10 nmol); TICP $(52\% \pm 27/300 \text{ pmol})$. 3) All analysic effects of all drugs were reversed by naltrexone (1mg/kg). Naltrindole (3 mg/kg) had minimal effect upon the maximum usable dose. Antagonists failed to reverse effects upon motor function. 4) Tolerance and cross-tolerance development after daily repeated bolus of DMT-DALDA (3 pmoL) and morphine (30 nmoL) revealed both agents displayed progressive decline over 5 days. Cross-tolerance assessed on day 5 revealed a significant reduction in the response to morphine in the DMT-DALDA treated animal but not DMT-DALDA in the morphine treated animal indicating an asymmetric cross- tolerance. These results emphasize the potent mu agonist properties of the DALDA structure series and their propensity to produce tolerance. The asymmetric cross-tolerance between equiactive doses may reflect upon the relative intrinsic activity of morphine and DMT-DALDA. (Supported by NIH R01-DA015353)

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Poster

309. Opioids and the Treatment of Pain

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Topic: D.03. Somatosensation: Pain

Support: NIDA grant R01DA03531

T32 training grant T32 OD010993-14

Title: Sustained release buprenorphine induces acute analgesic tolerance

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²Neurosci., ¹Univ. of Minnesota, Minneapolis, MN; ³Dept Neurosci, Pharmacol, Dermatol, Univ. Minnesota Med. Sch., Minneapolis, MN; ⁴Depts Pharmaceut, Pharmacol & Neurosci, Univ. Minnesota, Minneapolis, MN **Abstract: Background:** A sustained-release formulation of buprenorphine is a widely used formulation in animal care and veterinary medicine. The manufacturer states serum levels are sustained for 72 hours following a single injection. Dose recommendations range from 0.5-1 mg/kg but veterinary recommendations range up to 2 mg/kg. These doses have not been comprehensively assessed for analgesic tolerance or opioid-induced hyperalgesia. We report that SR-Bupe 1 mg/kg induces analgesic tolerance within hours of delivery and high dose (2 mg/kg) induce responses suggestive of opioid-induced hyperlageisa.

Methods: Male ICR mice were injected with Complete Freund's Adjuvant in the hind paw and allowed to induce inflammatory hyperalgesia for 3 days prior to further testing. 0.1, 0.3, 0.5, 1, and 2 mg/kg doses (varied by volume of injectate) of sustained-release buprenorphine were delivered and paw von Frey thresholds were monitored for 1, 2, 3, and 24 hours post-injection. **Results:** The 1 mg/kg dose provided a 60% reversal in vF threshold responses for 1 and 2 hours which was greatly diminished by 3 hours and gone by 24 hours post-injection. Lower doses resulted in small analgesic effects. A high dose of 2 mg/kg showed no analgesia and some evidence of hypersensitivity.

Conclusion: Sustained-release buprenorphine induces rapid analgesic tolerance and high doses may evoke opioid-induced hyperalgesia.

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Poster

309. Opioids and the Treatment of Pain

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Topic: D.03. Somatosensation: Pain

Support: NINDS NS093894

AHA 13SDG14590005

Title: Optogenetic dissection of the descending pain modulatory system

Authors: *M.-H. LI, Q. CHEN, K. L. SUCHLAND, Y. ZHANG, M. M. HEINRICHER, S. L. INGRAM

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Abstract: The descending pain modulatory system regulates nociceptive processing via projections from the brainstem to the spinal cord dorsal horn. Cortical and midbrain modulation

of pain is integrated by the periaqueductal gray (PAG) and rostral ventromedial medulla (RVM). RVM output neurons to the spinal cord can both facilitate and suppress nociception. This system also contributes to abnormal pain in animal models. We have used optogenetics to further understand the "bottom-up" inputs to RVM neurons, relays from spinal cord through the parabrachial area (PB), as well as "top-down" inputs from the PAG to RVM. Channelrhodopsin (ChR2) AAV viral vectors (AAV9.hSyn.hChR2(H134R)-EYFP.WPRE.hGH, Penn Vector Core) were microinjected into the PB complex and whole-cell patch-clamp recordings from in vitro brain slices containing the RVM were done 2-4 weeks later. Light stimulation of ChR2expressing terminals from PB to RVM elicited synaptic currents in 29 neurons. 21/29 of the neurons had synaptic currents that were blocked by glutamate antagonists but were unaffected by a GABA_A receptor antagonist bicuculline (BIC) indicating a direct glutamate projection. The remaining 8/29 RVM neurons had light-evoked synaptic currents that were blocked by BIC but were unaffected by glutamate antagonists indicating that they were direct GABAergic inputs. These data provide evidence that direct PB inputs to RVM neurons release either glutamate or GABA but not both onto individual RVM neurons. Microinjections of ChR2 into the PAG elicited a different profile of inputs to the RVM. Many RVM neurons receive both direct glutamate and GABA inputs. There is also evidence of local recurrent synaptic activity following light stimulation of ChR2-expressing terminals from PAG. Examination of opioid and cannabinoid modulation of inputs from these specific circuits demonstrates differential sensitivity to these modulators. One striking result is that the mu opioid receptor agonist, metenkephalin, strongly inhibits glutamate inputs from both PB ($80 \pm 5\%$, n = 6) and PAG ($56 \pm$ 8%, n = 11), in addition to the canonical inhibition of GABA inputs from the PAG ($43 \pm 9\%$, n = 6). In contrast, the cannabinoid agonist WIN 55,212 inhibits only PAG GABA inputs to RVM neurons $(39 \pm 7\%, n = 8)$ with little effect on glutamate inputs $(9 \pm 3\%, n = 12)$. We have previously demonstrated plasticity in the endocannabinoid system in persistent inflammation. These results indicate that opioids and cannabinoids may target separate circuits, providing a cellular substrate for new pain therapies via selective targeting of specific descending circuits that mediate pain inhibition versus pain facilitation.

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Poster

309. Opioids and the Treatment of Pain

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Program#/Poster#: 309.10/CC2

Topic: D.03. Somatosensation: Pain

Support: NIH - NIDA 5R01DA036680-04

Title: Imatinib prevents morphine tolerance without inducing MOR endocytosis

Authors: *S. PUIG, P. M. WHITE, S. R. SZOTT, H. B. GUTSTEIN Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Analgesic tolerance limits the use of opioids for the treatment of chronic pain. It was previously hypothesized that sustained signaling by the mu opioid receptor (MOR) could cause morphine tolerance, which could be prevented by promoting MOR endocytosis. However, some studies have challenged this theory. We have shown that Imatinib, by inhibiting PDGFR- β (platelet-derived growth factor receptor-beta), selectively prevents morphine tolerance. In this study, we determine whether inhibition of morphine analgesic tolerance with Imatinib involves endocytosis of the MOR. Rats were injected i.t. with either vehicle, 0.6 nM morphine, 10 µg imatinib or morphine + imatinib. Analgesia was measured daily using tail flick latency (TFL). After either one or five days of treatment, spinal cords were extracted and processed for immunohistochemistry with anti-NeuN (Millipore) and anti-MOR (Abcam) antibodies. Images of the substantia gelatinosa (SG) were analyzed using confocal microscopy. The average number of internalized vesicles containing MOR in neurons was determined utilizing an automated, unbiased software algorithm (Imaris, Bitplane). Co-administration of Imatinib with morphine prevented tolerance without altering morphine's analgesic effect. Interestingly, the levels of MOR internalization in SG neurons were not different between groups. Our results show that imatinib did not prevent tolerance by promoting MOR endocytosis, challenging the hypothesis that the lack of MOR internalization could be the cause of morphine tolerance.

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Poster

309. Opioids and the Treatment of Pain

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Topic: D.03. Somatosensation: Pain

Support: The Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

Medtronic PLC

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

Title: Mast cell degranulation: In vivo and Ex vivo screening of spinal analgesics

Authors: *T. L. YAKSH¹, Z. WANG⁴, S. A. MALKMUS², P. W. SCHILLER^{5,6}, K. HILDEBRAND⁷, L. PAGE⁷, E. S. RONDON^{2,8}, A. DI NARDO⁴, S. KOKUBU³ ¹Dept. of Anesthesiology, UCSD, LA Jolla, CA; ²Dept. of Anesthesiology, UCSD, La Jolla, CA; ³Anesthesiol., Dept. of Anesthesiology, UCSD, San Diego, CA; ⁴Dept. of Dermatology, UCSD, La Jolla, CA; ⁵Montreal Clin. Res. Inst., Montreal, QC, Canada; ⁶Pharmacol. and Physiol., Univ. of Montreal, Montreal, QC, Canada; ⁷Medtronic, PLC., Minneapolis, MN; ⁸Dept. de Medicina Veterinária, Univ. Federal de Mato Grosso do Sul, Campo Grande, Brazil

Abstract: Background: Spinal opioids yield potent naloxone-reversible analgesia. Continuous infusion produces masses (granulomas) composed of meningeally-derived fibroblasts in a collagen matrix. The initiating step may be degranulation of meningeal mast cells through a nonopioid-receptor-mediated mechanism (*Anesthesiology*.118:664,2013). We examined spinal opioid analgesic molecules for their ability to degranulate mast cells in vivo (mast-cell-dependent flare after intradermal injection in dog) and in vitro (degranulation of primary human mast cell (hMC) cultures).

Methods: The area of skin flares produced by intradermal (ID, 50µL) injections in anesthetized male Beagle dogs were measured 30 minutes after injection. Degranulation of primary hMC was determined using ß-hexosaminidase concentrations in culture supernatant. Drugs studied included nonpeptide and peptide opioid agonists and an N-type Ca channel blocker (ziconotide). **Results:** Ordering of flare area after ID delivery of nonpeptidic opioids (0.1-10mg/mL) was: (morphine, hydromorphone, methadone, morphine-3- glucuronide, morphine-6- glucuronide) >> (alfentanil, fentanyl) = 0. For peptides, rank ordering was ziconotide > (DPDPE, TICP, DMT-DALDA, [Dmt¹,Nle⁴]DALDA) >0 (see AAPSJ. 14:E560-5, 2005 for structures). Flares were reduced by cromolyn (10mg/kg, IM), a mast cell stabilizer, but not naltrexone (3mg/kg). In hMCs, ordering of degranulation potency covaried with cromolyn-sensitive flare activity. Conclusions: Agents producing intrathecal masses in dogs and/or humans showed a high correlation with the ability to produce a cromolyn-sensitive flare and hMC degranulation (morphine, hydromorphone, methadone, DPDPE) whereas those that did not yield a flare have not been observed to yield a granuloma (alfentanil, fentanyl). Ziconotide is a potent mast cell degranulator, but is not associated with granulomas, presumably because concentrations required to produce analgesia are lower than those required to degranulate mast cells. Accordingly, the propensity to produce a cromolyn-sensitive flare increases the likelihood of a granuloma if the therapeutic concentration required approaches those producing degranulation. (Funded by NIH DA015353; Medtronic, PLC; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq))

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Poster

309. Opioids and the Treatment of Pain

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Topic: D.03. Somatosensation: Pain

Support: National Research Foundation (NRF) of Korea (NRF-2017R1A2B3005753).

Title: Inhibition of fatty acid amide hydrolase in the insular cortex produces analgesic effects in neuropathic rats

Authors: *K. KIM^{1,2}, M. KIM^{1,2}, B. LEE^{1,2}

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Abstract: The insular cortex (IC) is one of important brain regions involved in the processing of pain information and emotions. According to recent studies, lesion of the IC induced pain asymbolia and reversed neuropathic pain. Previously, anodynes such as anti-inflammatory drugs, anticonvulsants, and opioids were used to treat neuropathic pain despite the lack of efficacy or unexpected side effects. Cannabinoids have been used for treating diverse illnesses including pain for centuries. However, cannabinoids have several unpredictable outcomes in animals and humans. Particularly, plant-derived cannabinoids have critical side effects such as euphoria, hyperphasia and hyperphagia. These physiological actions are produced via an endogenous cannabinoid neurotransmission system that involves mainly the G-protein coupled receptors named cannabinoid 1 Receptor (CB1R) and cannabinoid 2 receptor (CB2R). Increasing the endocannabinoid anandamide by blocking fatty acid amide hydrolase (FAAH) has shown analgesic effects in neuropathic pain models. However, analgesic effects via FAAH inhibition in the IC have yet to be determined in neuropathic pain rats. Therefore, the present study was conducted to reveal the analgesic effect of the selective FAAH inhibitor URB597. Under pentobarbital anesthesia, male Sprague-Dawley rats were subjected to nerve injury. Behavioral test with electronic von Frey was performed. Nerve-injured rats were treated with microinjections of or vehicle in the IC. In addition, the FAAH signaling pathway-related proteins were extracted and quantitatively analyzed from the IC tissue. Neuropathic pain was developed indicative of decreased level of hind paw withdrawal thresholds 14 days following nerve injury, In addition, the expression of CB1R increased significantly in neuropathic pain group compared to the sham-operated control group. In the behavioral test followed by microinjections, the mechanical threshold significantly decreased in the group treated with 2 nM and 4 nM URB597. As a result, the level of NAPE-PLD increased in the group injected with 2nM and 4nM URB597. These results suggest that the microinjection of FAAH inhibitor into the IC exhibits an analgesic effect, indicating the increase in NAPE-PLD, a synthetic enzyme of AEA in the IC. This work

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Poster

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Topic: D.03. Somatosensation: Pain

Support: Baptist Health Foundation

Nathan Shock Center of Excellence AG013319 and R21 AG 047514

Title: Reduced antinociceptive responses to mu but not kappa opioid agonists in aged rats

Authors: *E. JENNINGS, H. SMITH, L. SULLIVAN, R. JAMSHIDI, K. A. BERG, W. P. CLARKE

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Abstract: Moderate to severe pain can significantly increase risk of disability and reduce quality of life in the elderly. Therefore improved pain management in the aged population is a major concern for both health care providers and researchers. Opioid drugs that target mu opioid receptors (MOR) continue to be the gold standard for treatment of pain. Unfortunately there are serious adverse effects that limit their use particularly in the elderly. Peripherally restricted opioids that inhibit the function of pain-sensing neurons (nociceptors) and do not enter the CNS would be safer and better tolerated drugs to relieve suffering in older adults. However little is known regarding the effects of aging on nociceptor responsiveness in the periphery and there is some evidence that aging is associated with reduced peripheral sensitivity to MOR agonists. We examined the effects of MOR agonists morphine (100 µg, 150 µg) and DAMGO (20 µg, 40 µg) and the kappa opioid receptor (KOR) agonist, Salvinorin A (0.1 µg), in young (4-months-old) and aged (26-months-old) Fisher x Brown Norway rats. Behavioral responses to noxious heat were measured following intraplantar injections of opioid agonists. In addition, we measured mRNA expression of MOR and KOR in the L4-L6 dorsal root ganglia (DRG) of aged and young rats using RT-qPCR. Intraplantar administration of Salvinorin A produced significant antinociceptive responses in both aged and young rats. However morphine and DAMGO failed to attenuate nociceptive behavior in aged rats while producing a robust antinociceptive effect in young rats. Additionally we found that MOR expression in the DRG was 7 times higher in aged rats compared to young, suggesting compensatory mRNA overexpression. By contrast, there was
no difference in KOR expression. Overall these data suggest that peripherally restricted kappa agonists may be more effective analgesics than mu agonists to treat pain in the elderly.

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Poster

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Support: CIHR Grant MOP-123399

Quebec Pain Research Network

Title: DOPr displays constitutive internalization and sorting to the rapid recycling pathway

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Abstract: The delta opioid receptor (DOPr) is currently among the most promising targets for the development of new analgesic therapies. Despite a low efficacy under normal conditions, we have previously demonstrated that chronic inflammation or a treatment with morphine can improve DOPr-mediated antinociception. Interestingly, this effect is paralleled by an increased density of DOPr at the neuronal plasma membrane, but the underlying mechanism explaining this increase remains elusive. In the current study, we investigated constitutive internalization as a potential mechanism controlling the level of DOPr expressed at the cell surface. In HEK293 cells stably expressing Flag-tagged DOPr, we first confirmed that DOPr internalizes constitutively, *i.e.* in the absence of agonist. Immunofluorescence experiments revealed that internalized DOPr colocalizes with Rab5-positive early endosomes. When a constitutively active Rab5 mutant (Rab5 QL) was expressed, we found that internalized DOPr remains trapped in early endosomes. Conversely, an inactive form of Rab5 (Rab5 SN) decreases internalization of the receptor. The absence of DOPr colocalization in Rab7-positive late endosomes suggests that constitutively internalized DOPr is not directed to lysosomes for degradation. The presence of DOPr in Rab4- and Rab11-positive endosomes, which are respectively involved in rapid and late recycling pathways, was also studied. Within these compartments, we observed that DOPr mainly colocalizes with Rab4-positive endosomes. Altogether, these results suggest that upon constitutive internalization through a Rab5-dependent pathway, DOPr is rapidly recycled to the

cell surface via Rab4-positive endosomes. Undergoing experiments aim at determining whether this process can be modulated by morphine under chronic pain conditions. Understanding the mechanisms regulating this process may lead to the identification of a new pharmacological target for pain management by DOPr activation.

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Poster

309. Opioids and the Treatment of Pain

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Topic: D.03. Somatosensation: Pain

Support: NIDA Grant 5T32DA007234-29

NIDA R01 DA030316

Title: MMG22: A bivalent ligand that contains a MOR agonist and an mGluR₅ antagonist exhibits potent antinociception without adverse effects

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Abstract: Opioids are the most effective pharmacological tools to treat moderate to severe pain; however, the use of opioids for the treatment of chronic non-cancer pain is controversial due to concerns about tolerance, abuse, and addiction. MPEP, an mGluR₅ antagonist, has been shown to increase the analgesic efficacy of opioids while simultaneously decreasing the development of analgesic tolerance and addiction. MMG22 is a novel bivalent ligand made of two pharmacophores derived from oxymorphone, a MOR agonist, and M-MPEP, an allosteric mGluR₅ antagonist, linked via a 22-atom spacer. Previous studies have shown intrathecal administration of MMG22 to be incredibly potent at decreasing inflammatory pain without tolerance or respiratory depression. In the present studies, we sought to characterize the ability of MMG22 to modulate neuropathic pain, evaluate its abuse potential and elucidate its site of action. These studies used the spared nerve injury model of neuropathic pain in male C57/B6 mice. Hyperalgesia was quantified by determining the frequency of withdrawal responses evoked by a von Frey monofilament with a bending force of 3.5 mN applied to the plantar surface of the paw. To evaluate the antinociceptive effects of MMG22 or morphine, paw withdrawal frequency

(PWF) was determined before and after subcutaneous (sc) administration of each drug at doses ranging from 0.001mg/kg to 1mg/kg. We also assessed the rewarding properties of MMG22 as compared to morphine in naïve mice using a conditioned place preference assay. Expression and localization of mGluR₅ and MOR in the mouse spinal cord superficial dorsal horn were assessed using immunohistochemistry and fluorescence microscopy. Preliminary results show that while MMG22 is effective in producing antinociception, supra-threshold doses do not produce conditioned place preference. Both MOR and mGluR₅ are expressed in the superficial dorsal horn of the spinal cord of Naïve mice, suggesting the spinal cord as a potential site of action. MMG22 has the potential to be an effective therapeutic for the treatment of neuropathic pain that may lack the addictive properties of traditional opioid analgesics.

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Poster

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Support: CIHR Grant MOP-123399

FRQS

QPRN

Title: A conditional knockin mice to study the delta opioid receptor in pain pathways

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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 relieve 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. A mouse line expressing a Flag-tagged DOPr in place of the endogenous receptor (Flag-DOPr knockin) was then generated by breeding the STOP::Flag-DOPr mice with Zp3-Cre mice. A thorough characterization of these mice reveals that the Flagged receptor is expressed at similar levels and in the same areas as its endogenous counterpart. In these mice, we found that DOPr agonists produce similar behavioral effects than in wildtype animals, suggesting that the Flag-DOPr knockin mice display similar pharmacological and behavioral properties than mice expressing endogenous DOPr. These appear as a useful complement to already available genetic mouse models (ex. DOPr-KO and cKO-, DOPr-eGFP knockin) to study the role and the regulation of DOPr, *in vivo*. In particular, one could use these mice to identify the role of DOPr in a specific brain region/subpopulation of neurons or to identify new protein partners in a natural environment.

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Poster

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Title: NR2B subunit mRNA is upregulated in the spinal cord following development of analgesic tolerance to intrathecal opioids and inflammation

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Abstract: Background: The NMDA receptor is known to contribute to the development of analgesic tolerance to spinal administration of opioids as well as development of chronic pain. It is thought that the NR2B receptor subunits are expressed in a site-specific manner that could

may offer an advantage in therapeutic targeting. Little is known about the expression of specific subunits of the NMDA receptor in spinal cord dorsal horn under conditions of chronic opioid exposure and pain. We evaluated mRNA expression of NR2B and NR2A specific subunits following chronic spinal morphine and CFA exposure.

Methods: ICR male mice were injected intrathecally twice daily for up to 4 days with 10 nmol of morphine or saline (5 μ L). Thermal sensory responses to tail flick (52.5°C) were monitored daily 10 min. following the second daily injection. In a second set of animals, CFA was injected to induce inflammation and von Frey thresholds monitored. At conclusion of the tolerance induction schedule or the CFA inflammation time course, mice were anesthetized and spinal cords, DRGs, were extracted and prepared for mRNA analysis. Total RNA was isolated by TRIzol method and the mRNA abundance for NR2A, NR2B & 18S was determined by one-step RT-qPCR. The fold change in expression levels of NR2A & NR2B in morphine group v/s saline group were calculated by relative quantitation method.

Results: Thermal sensory responses diminished daily with increasing numbers of injections of morphine as expected. Analgesic tolerance was induced. The mRNA expression was increased 9-28 fold for NR2B in mice exposed to morphine, but not saline. In mice exposed to CFA, mRNA expression was increased 15-fold on day 3 and 2-fold on day 7 for NR2B. For NR2A, mRNA levels were not changed in any group.

Conclusion: This study supports the hypothesis that the NR2B subunit is upregulated under conditions of neuroplasticity, including opioid induced tolerance.

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Poster

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Topic: D.03. Somatosensation: Pain

Support: CIHR

Louie and Alan Edwards Foundation

Brain Canada

Title: Decreased morphine analgesia in t-cell deficient mice

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Abstract: It is now known that neurons are not the only cell type involved in pain processing, which involves cell of the immune system, such as T-cells. Many pain researchers have adopted the use of T-cell deficient mice in their experimental methods to elucidate the role of T-cells in neuropathic pain (Yu et al., 2004; Costigan et al., 2009), and T cells have been shown to release endogenous opioids (Bouè et al., 2011). While it is well known that opioids have varying effects on T-cell populations (Wang et al., 2003), very little attention has been given to how T-cells may affect opioid regulation. Here, we observe that T-cell deficient mice (CD-1 nude and Rag1 null mutant) exhibit pronounced deficiencies in morphine analgesia, measured using the tail withdrawal or formalin test. In addition, T-cell deficient mice do not exhibit stress-induced analgesia after restraint. The adoptive transfer of CD4+ T-cells into nude mice rescues the morphine analgesia. Furthermore, there are reported sex differences in morphine analgesia, with females requiring 2-3 times more morphine than males to produce equal analgesia. We observe an equivalent sex difference in CD1 mice, however T-cell deficient mice do not exhibit a sex difference in morphine analgesia. The sex difference in morphine analgesia is restored in nude male and female mice receiving CD4+ T-cells from their respective sex. These results suggest that CD4+ T-cells play a role in opioid analgesia, and may be a driver of the observed sex differences in morphine analgesia.

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Poster

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Topic: D.03. Somatosensation: Pain

Support: NIH DA015353

Title: Opiod activation of fibroblast

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Abstract: Background: Continuous intrathecal infusion of morphine can produce a potent analgesia and in several species including humans produce a space-occupying mass composed of

meningeal fibroblasts in a collagen matrix. This effect is not prevented by naltrexone (Anesthesiology, 118:664-678,2013). We addressed the effects of opiates on the migration and proliferation of mouse embryonic fibroblasts (MEFs) and meningeal fibroblasts. **Methods:** MEFs (ATCC #CRL-2214) were cultured in media consisting of 1x DMEM supplemented by 10% non-heat inactivated fetal bovine serum, 1% Pen Strep, 1% sodium pyruvate (100mM), 1% Glutamax 100x and 1% MEM non-essential amino acids. Cells were Incubated at 37°C in a humidified 5% CO2 environment and grown to 70---90% confluence. For cell migration assay, cells were plated onto the top of trans-well plates (50K/well/200 ul) precoated with collagen (1µg/mL) and incubated for 4 hrs. Cells were counted after removing the top layer and staining. To assess proliferation MEFs were seeded (2x 10⁴ cells/chamber) in 12 well plates and incubated for 24 hrs, followed by 48 hrs incubation with test material. In both assays, cells were exposed to concentration of morphine or fentanyl and then with naloxone at the optimal concentration. We also measured collagen I mRNA in rat meningeal fibroblasts following morphine treatment.

Results: In the migration assay, morphine and fentanyl treatment on MEF cells induced significant migration at doses 2-20 μ M and 0.2-2 μ M, respectively. At higher doses, suppression of migration was observed. Naloxone (20 μ M) did not reverse migration induced by both morphine and fentanyl. In the proliferation assay, morphine enhanced proliferation of MEF cells in a dose-dependent manner with maximum effect observed at 0.3 μ M. At higher doses, a suppression of proliferation was observed. Fentanyl, however, did not show similar effects on proliferation as morphine. Morphine at 10 μ M dose showed 1.73-fold significant increase in collagen type I mRNA in rat meningeal fibroblasts.

Conclusions: These results show that morphine increased both migration and proliferation of MEF's, whereas fentanyl increased migration but not proliferation. The effects of morphine and fentanyl on migration occurred in a naloxone-independent fashion. Further, increase in collagen type I mRNA suggests that morphine enhances the synthesis of collagen type I in MEF's that forms the matrix in granuloma formation. These results suggest that these two opiates enhance fibroblast function independent of the opiate receptors.

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Poster

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Topic: D.03. Somatosensation: Pain

Support: Arthritis Research UK Pain Centre grant 20777

MRC studentship

Title: An investigation of the influence of endogenous anxiety on opioid sensitivity in a model of osteoarthritis pain

Authors: *A. LILLYWHITE¹, J. BURSTON¹, G. J. HATHWAY², V. CHAPMAN¹ ¹Sch. of Life Sciences/ Arthritis Res. UK Pain Ctr., ²Sch. of Life Sci., Univ. of Nottingham, Nottingham, United Kingdom

Abstract: Introduction

Osteoarthritis (OA) is often associated with joint pain that can be difficult to treat. Patients with OA and co-morbid anxiety or depression experience more pain, take more medication and experience worse outcomes than patients without these co-morbidities. In particular, opioid use to provide analgesia is associated with catastrophizing after total joint replacement and may be a predictor of risk for opioid misuse in chronic pain patients. The aim of this study was to use an animal model to investigate the mechanisms underlying the relationship between anxiety, knee pain and opioid receptor function in a model of OA pain. Methods

The Wistar-Kyoto (WKY) strain of rats, which is more vulnerable to anxiety, was used with Wistar rats as normal anxiety controls. Male adult rats (125-150g) were anaesthetised (3% isoflurane) and received an intra-articular injection of 1mg monosodium iodoacetate or vehicle into the left knee. Sensory processing was measured using weight bearing asymmetry (WB) and mechanical hindpaw withdrawal thresholds (PWTs) up to day 21 post injection. The open plus maze was used to measure anxiety prior to intra-articular injection (day 0) and 21 days later. At this timepoint, 3 doses of morphine (0.5, 2.0 and 3.5mg/kg, cumulative dose 6 mg/kg) were injected subcutaneously (hourly) in MIA-treated WKY and Wistar rats, WB and PWTs were measured. In separate groups of rats 21 days following intra-articular injection, the opioid receptor antagonist naloxone was administered subcutaneously (0.1, 0.3 and 1mg/kg, hourly) and PWTs were measured. Data were analysed with a 2-way ANOVA with GraphPad Prism 7.01. Results

MIA-treated Wistar rats and WKY rats exhibited significant pain behaviour at day 21. Only the WKY rats showed an anxiety-like phenotype at baseline and this was increased at day 21, irrespective of the treatment received. 0.5 mg/kg of morphine significantly inhibited pain behaviour (WB: 29.89g versus 54.57g, p=<0.0001; PWTs: 22.2g versus 15.7g, p= 0.035) in the MIA-treated Wistar rats, whereas only 6mg/kg inhibited pain behaviour in MIA-treated WKY rats, weight bearing asymmetry (p= 0.0023) and PWTs (p = 0.0024). Naloxone (0.3 and 1 mg/kg) lowered ipsilateral PWTs, irrespective of whether they had received intra-articular injection of MIA or vehicle, in WKY rats but not in Wistar rats. These data suggest there are changes in opioid receptor function / endogenous tone in WKY rats which may lead to the reduced opioid sensitivity of pain behaviour in the MIA model of joint pain.

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Poster

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Support: NINDS R01NS086444-01A1

Title: An essential role of RGSz1 in signal transduction events underlying opioid tolerance and addiction-related behaviors

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Abstract: Regulator of G protein signaling z1 (RGSz1) is a member of the RGS family of proteins, known to modulate the amplitude and direction of signal transduction of several G protein coupled receptors (GPCR) by binding to activated Galpha subunits. RGSz1 is expressed in moderate amounts throughout the brain, and is present in several regions expressing mu opioid receptor (MOPR). Using genetic mouse models for global or brain region-targeted manipulations of RGSz1 expression, we demonstrate that the analgesic efficacy of MOPR agonists such as morphine, fentanyl and methadone is increased by preventing RGSz1 action. Specifically, the downregulation of RGSz1 delays the development of morphine tolerance in male and female mice, while decreasing the sensitivity to rewarding and locomotor activating effects. Using biochemical assays and next-generation RNA sequencing, we identified a key role of RGSz1 in the periaqueductal gray (PAG) in intracellular adaptations associated with morphine tolerance. Our analysis revealed a robust downergulation of the Wnt/beta-catenin signal transduction pathway in the PAG of morphine tolerant mice. Chronic morphine administration promotes RGSz1 activity in the PAG, which in turn modulates transcription mediated by the Wnt/betacatenin signal transduction pathway and promotes analgesic tolerance to morphine. Conversely, prevention of RGSz1 action stabilizes Axin2-Galphaz complexes, and promotes the action of beta-catenin as transcriptional activator. These data show that the regulation of RGS complexes, particularly those involving RGSz1-Galphaz, represents a promising target for optimizing the analgesic actions of opioids without increasing the risk of dependence or addiction.

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Poster

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Topic: D.03. Somatosensation: Pain

Title: Test

Authors: *A. E. KALYUZHNY, R. REED, J. HAGEN, N. HOPP, M. GRAHEK, A. PTAK, J. HUMPHREY, G. DU, P. MURTHA, B. AGGELER Bio-Techne, Minneapolis, MN

Abstract: Mu-opioid receptors encoded by OPRM1 gene mediate the actions of different narcotic analgesic including morphine and fentanyl which are widely used in hospitals all over the world. It has been found that mu-opioid receptors exist as multiple splice variants of the OPRM1 gene mediating different pharmacological activity of opioids. For example, C-terminal splice variants such as MOR1, MOR1A and MOR1B are widely distributed in rat CNS with levels of MOR1 and MOR1A being greater than MOR1B. To study the anatomical distribution of MOR1A expressing neurons among all OPRM1 neurons in rat brain we combined RNAscope® ISH protocol using a MOR1A probe with IHC. For IHC detection we generated rabbit anti-rat mu-opioid receptor monoclonal antibodies directed at the N-terminus. The open reading frame for rat mu-type opioid receptor (OPRM1/MOR1, amino acid 1-398, P33535) was isolated from a rat cDNA library by PCR. After the DNA sequence was confirmed, the construct was cloned into an expression vector with an antibiotic selection marker. The expression plasmid was transfected into HEK293EBNA cells by calcium phosphate precipitation and stable clones were selected. Expression levels were measured by fluorescence intensity using flow cytometry. Using 10 um thick formaldehyde-fixed frozen rat brain tissue sections we developed a 2-color fluorescence detection protocol allowing to identify neurons co-labeled for OPRM1 protein and MOR1A splice variant message. We observed a low number of double-labeled neurons in brain cortex, hippocampus and caudate putamen. A more profound double-labeling was observed in ventral lateral PAG and rostral ventral medulla representing the descending antinociceptive brainstem circuit. All ISH-positive neurons were always IHC-positive indicating that our rabbit monoclonal antibodies are highly specific for rat OPRM1. The number of neurons doublelabeled by IHC and ISH was a fraction of all OPRM1-immunoreactive neurons suggesting that such neurons express splice variants of mu-opioid receptor other than MOR1A. Combining ISH and IHC can be a useful tool to study the ratio of neurons expressing different OPRM1 splice variants in different brain regions.

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Poster

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Support: GW: R01 DA015438

GW: Committee for Pharmaceutical Development

GW: MN-REACH

GW: RW Goltz Professorship in Dermatology

DB: T32 DA007097

Title: Transdermal administration of Loperamide/Oxymorphindole combination reduces evoked responses of C-fiber nociceptors in Na_v1.8⁺-ChR2 optogenetic mice

Authors: *M. L. UHELSKI¹, D. J. BRUCE², C. A. FAIRBANKS³, G. L. WILCOX⁵, D. A. SIMONE⁴

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Abstract: Concerns over the unwanted side effects of commonly used opioids have increased interest in the development of novel treatments which provide adequate pain relief without the associated risk for addiction. The impact of drugs that target peripheral opioid receptors on the activity of nociceptors has not been fully explored. Loperamide (Lo) is a highly efficacious μ -opioid receptor agonist that does not activate receptors in the central nervous system, and oxymorphindole (OMI) is a δ -opioid receptor agonist that shows a synergistic effect with morphine or loperamide in the spinal cord. Behavioral studies have shown that these drugs have a synergistic analgesic effect when administered peripherally, including by topical application to the hindpaw. To test the impact of these drugs on nociceptor activity, our study used teased-fiber electrophysiology in the tibial nerve of adult *Na*_v*1.8*⁺-*ChR2* mice, which co-express the voltage-gated sodium channel Na_v*1.8* along with the light-sensitive channelrhodopsin2. In C-fiber nociceptors (*n* = 4) that were responsive to blue light stimulation, transdermal application of a

series of Lo/OMI concentrations (0.5 μ M, 5.0 μ M, and 50.0 μ M) reduced the number of impulses in response to a 5 s suprathreshold blue light stimulus 15 min after application. The degree of attenuation increased with dose, consistent with behavioral studies. Ongoing studies aim to determine how Lo/OMI affects nociceptor sensitization during ongoing inflammatory pain.

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Poster

309. Opioids and the Treatment of Pain

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Committee for Pharmaceutical Development

MN-REACH

RW Goltz Professorship in Dermatology

T32 DA007097

Title: Peripherally restricted opioid combination therapy synergizes in multiple pain states

Authors: *D. J. BRUCE¹, C. D. PETERSON², K. F. KITTO³, C. HARDING-ROSE⁴, K. D. WICKMAN¹, C. A. FAIRBANKS⁵, G. L. WILCOX⁶

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Abstract: 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 analgesic to date has delivered on the promise of effective pain control without the risk for addiction and diversion. Therefore, we studied the ability of 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, to mediate peripheral analgesia when administered alone, or in combination. We studied this combination in spinal cord slices to determine whether Lo and

OMI synergize spinally to inhibit neurotransmitter release from the central terminals of nociceptors. Our recordings indicate a 100-fold synergism when the two agonists are combined spinally. Subsequently, we investigated the behavioral analgesic profile of the combination in inflammatory, nerve-injured, post-operative, and tumor-induced pain models. Efficacy studies using intraplantar, systemic, or topical applications showed that when combined in a 1:1 dose ratio OMI-Lo produces a robust anti-hyperalgesic synergy. This synergy was blocked by the peripherally restricted opioid antagonist, naloxone methiodide, reinforcing the peripheral localization of the effect. The synergistic interaction is also completely ablated when G proteincoupled, inwardly rectifying potassium channels (GIRKs) are blocked or knocked out. Although supra-therapeutic doses induce tolerance, therapeutic doses do not. Initial self-administration studies also indicate a significantly reduced abuse liability compared to prescription opioids such as oxycodone. We conclude that MOR agonists significantly synergize with DOR agonists at peripheral sites of action, providing strong evidence in support of peripherally restricted combination opioid therapy. The systemic and topical efficacy of the combination, along with loperamide's extremely low abuse liability, suggests that this combination might be therapeutically useful to control multiple pain modalities in the clinic.

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Poster

309. Opioids and the Treatment of Pain

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Topic: D.03. Somatosensation: Pain

Title: Expression of Gi-insensitive adenylyl cyclase isoform AC2 in mouse sensory neurons: Contributions to nociceptive signaling

Authors: *J. J. HAVELIN¹, J. R. YASKO², C. ESANCY¹, R. GEGUCHADZE¹, K. M. BAUMBAUER³, T. E. KING¹, D. C. MOLLIVER¹ ¹Biomed. Sci., Univ. of New England, Biddeford, ME; ²Dept. of Neurosci., Uconn Hlth., Farmington, CT; ³Sch. of Nursing, Univ. of Connecticut, Storrs, CT

Abstract: Gs-coupled metabotropic receptors play a significant role in the sensitization of nociceptors in response to inflammatory insult or injury by activating the adenylyl cyclase (AC)-cAMP-protein kinase A (PKA) pathway. Conversely, ACs are inhibited by Gi/o-coupled receptors, which is one mechanism underlying analgesia provided by opiates. Type II AC isoforms are uniquely regulated in that they can be activated by protein kinase C or Gbetagamma as well as Galpha-s, making them integrators of signaling through multiple second messenger pathways usually considered to be distinct. Coincident activation of Gq/11-coupled receptors

(leading to activation of PKC) and Gs-coupled receptors is likely to occur in response to peripheral injury or insult. Furthermore, Type II ACs are insensitive to inhibition by Gi/ocoupled receptors, suggesting the possibility that these AC isoforms, if expressed in primary afferent nociceptors, could contribute to persistent pain resistant to exogenous opiates or descending inhibitory influences. Therefore, we examined the distribution of Type II ACs ADCY2, ADCY4 and ADCY7 in sensory neurons of the dorsal root ganglion (DRG) from adult male mice. Real-time PCR revealed mRNA for all 3 isoforms in whole DRG (ADCY2 > ADCY7 > ADCY4). Single cell quantitative PCR was performed on acutely isolated cutaneous DRG afferents retrogradely labeled with either IB4 or wheat germ agglutinin (n=40). Widespread expression of AC2 was identified in afferents co-expressing TRPV1 and/or MrgprD, used to identify subsets of putative nociceptors. The functional role of ADCY2 in nociception was examined by evaluating the impact of local or spinal administration of an inhibitor of AC2, SKF 83566 (10 or 30 µM), on acute responses to noxious heat stimulation and the formalin test. Withdrawal latencies to noxious heat were not altered by spinal administration the AC2 inhibitor, suggesting little contribution of AC2 to acute noxious heat responses. In contrast, both spinal and local (intraplantar hindpaw) administration of inhibitor attenuated nocifensive responses in both phase 1 (1-15 min post-injection) and phase 2 (15-45 min post injection) of the formalin test. These results support a role for AC2 in formalin-evoked pain. Ongoing experiments are investigating a role for AC2 in a model of persistent behavioral hypersensitivity known as hyperalgesic priming.

Disclosures: J.J. Havelin: None. **J.R. Yasko:** None. **C. Esancy:** None. **R. Geguchadze:** None. **K.M. Baumbauer:** None. **T.E. King:** None. **D.C. Molliver:** None.

Poster

309. Opioids and the Treatment of Pain

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 309.26/CC18

Topic: D.03. Somatosensation: Pain

Title: Dopamine receptor modulators enhance opioid analgesia in a rodent model of spinal cord injury

Authors: E. EVANS¹, J. JENKINS¹, J. YOW¹, S. CLEMENS², *K. L. BREWER¹ ¹ECU Emergency Med., ²Dept. of Physiol., Brody Sch. Med. East Carolina Univ., Greenville, NC

Abstract: Background: Opiate agonists such as morphine are often ineffective in neuropathic pain related to spinal cord injuries (SCI) and also carry a high risk for addiction and dependence. There is a need to identify other possible analgesic agents that are effective at managing chronic SCI-related pain, without the unwanted side effects of opiates. Purpose: This study examined the

pairing of low-dose morphine with dopamine D3 and D1 receptor modulators to improve analgesia for SCI-related pain, while reducing morphine's rewarding effects. Methods: 55 female, Long-Evans rats were tested for mechanical thresholds prior to receiving a spinal contusion (SCI) or sham surgery. Mechanical pain thresholds were measured 21-27 days postsurgery under the following conditions: Morphine alone (1.0 or 2.5 mg/kg); Morphine (1.0 or 2.5 mg/kg) + 0.1 mg/kg Pramipexole (PRAM; D3R agonist); Morphine (2.5 mg/kg) + 0.1 mg/kg SCH23390 (SCH: D1R antagonist); 0.1 mg/kg Pramipexole alone; 0.1 mg/kg SCH23390 alone. Conditioned Place Preference (CPP) to each drug combination was assessed to determine the addictive potential of that specific combination. Results: In sham animals, the addition of PRAM to 1.0 mg/kg of morphine increased mechanical thresholds over that of baseline and morphine alone. PRAM had no additional effect when added to 2.5 mg/kg morphine. Neither PRAM nor SCH alone provided analgesia in sham animals compared to baseline. In SCI animals, PRAM and SCH increased analgesia over morphine alone when combined with 2.5 mg/kg of morphine. Neither PRAM nor SCH exacerbated CPP to morphine at either dose in sham or SCI animals. Conclusions: Pairing a dopamine D3 receptor agonist with low-dose morphine provides better analgesia than morphine alone without exacerbating the addictive potential of morphine for SCIrelated pain. This combination therapy may represent a potential clinical therapeutic intervention for neuropathic pain.

Disclosures: E. Evans: None. J. Jenkins: None. J. Yow: None. S. Clemens: None. K.L. Brewer: None.

Poster

309. Opioids and the Treatment of Pain

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 309.27/CC19

Topic: D.03. Somatosensation: Pain

Title: Combining neurotensin and opioid receptor agonists to relieve pain

Authors: *E. EISELT, V. BLAIS, J.-M. LONGPRÉ, P. SARRET, L. GENDRON Pharmacol. and Physiol., Sherbrooke Univ., Sherbrooke, QC, Canada

Abstract: Opioid and neurotensin receptors are expressed in both the central and peripheral nervous systems where they modulate nociceptive responses. Nowadays, opioid analgesics like morphine remain the most prescribed drugs for the treatment of moderate to severe pain. However, commonly used opioids, known to activate the mu opioid receptor (MOPr) are also responsible for most unwanted effects (tolerance, constipation or respiratory depression). Previous studies have established that the strong analgesic action of neurotensin (NT) is independent of the opioid system. However, NT acting at the NTS1 receptor site also causes hypothermia and hypotension, thus limiting the development of new analgesic drugs. Here, we

hypothesized that the combined use of NT and opioid compounds would require lower doses to produce significant analgesic effects, hence decreasing the unwanted effects induced by either drug classes. In this study, we used isobologram analyses to determine if the combination of a NT brain-penetrant analog with morphine results in an inhibition, a synergic or an additive analgesic response. We found that intravenous administration of the NT analog reduced by 90% the nocifensive behaviors induced by formalin injection, at the dose of 0.018 mg/kg. Likewise, subcutaneous morphine attenuated the pain behaviors by 90% at the dose of 1.8 mg/kg. Importantly, the use of isobologram analyses revealed that the co-injection of the NT agonist together with morphine induced an additive analgesic effect in the rat formalin test. We finally assessed the effects of these mu opioid and NT compounds on the gastrointestinal tract motility using the charcoal meal test. As opposed to morphine which significantly reduced the intestinal transit time at the analgesic effective dose of 1.8 mg/kg, the NT brain-penetrant analog did not affect the progression of the charcoal meal in the intestine at the dose of 0.018 mg/kg. Interestingly, at the dose providing 90% pain relief in the formalin test, the co-administration of morphine (0.09 mg/kg) with the NT analog (0.009 mg/kg) reduced the constipation profile of morphine. Altogether, these results suggest that the combination of NT derivatives with morphine may improve its analgesic benefit/adverse effect ratio.

Disclosures: E. Eiselt: None. V. Blais: None. J. Longpré: None. P. Sarret: None. L. Gendron: None.

Poster

309. Opioids and the Treatment of Pain

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 309.28/CC20

Topic: D.03. Somatosensation: Pain

Title: Muscarinic allosteric modulation alleviates chronic post-ischemia pain in rats

Authors: V. GOURA, A. VUYYURU, R. KALLEPALLI, P. JAYARAJAN, *S. M. IRAPPANAVAR, R. NIROGI SUVEN LIFE SCIENCES LTD, HYDERABAD, India

Abstract: Patients with chronic complex regional pain syndrome (CRPS-I) shows tissue abnormalities caused by microvascular dysfunction in the blood vessels of skin, muscle and nerve. Chronic post-ischemia pain (CPIP) is a neuropathic-like pain syndrome following prolonged hind paw ischemia and reperfusion, which models CRPS-I. In this study we evaluated the effect of muscarinic allosteric modulator, on microvascular dysfunction and neuropathic pain like syndrome caused by CPIP in rats. Mechanical allodynia was assessed using Von-Frey monofilaments in CPIP rats. Laser doppler flowmetry of plantar blood flow was used to examine the effects of BQCA on CPIP-induced alterations in post-occlusive reactive hyperemia (reflecting microvascular dysfunction). Further, we investigated to translate "bedside-to-bench" outcomes from the human pain phenotype to rodents for the first time in CPIP rats through burrowing behavior (which evaluates non-evoked and unbiased pain response). Muscarinic allosteric modulator significantly attenuated mechanical allodynia and reduced reactive hyperemia. The natural burrowing deficits in CPIP rats were significantly ameliorated. An improvement in microvascular function might account for its analgesic activity in our experimental model of CRPS-I.

Disclosures: V. Goura: A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd., Hyderabad, India. A. Vuyyuru: A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd., Hyderabad, India. R. Kallepalli: A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd., Hyderabad, India. P. Jayarajan: A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd., Hyderabad, India. S.M. Irappanavar: 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. R. Nirogi: 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. R. Nirogi: 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. R. Nirogi: 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

309. Opioids and the Treatment of Pain

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 309.29/CC21

Topic: D.03. Somatosensation: Pain

Support: CNPq Grant 800585/2016-0

Title: Evaluation of Mygalin anti-nociceptive activity, analogue synthesized from natural acylpolyamine of the A. gommesiana (Araneae, Theraphosidae) hemolymph, in a model of neuropathic pain in Wistar rats

Authors: *A. C. MEDEIROS¹, J. L. LIBERATO¹, P. MEDEIROS², R. L. FREITAS², P. I. SILVA, Jr.³, N. C. COIMBRA², W. F. SANTOS¹

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Abstract: Pain is a recurring sensation that affects one third of the world population, associated with this are the unwanted side effects and limited effectiveness of current drugs. In this sense, the study of natural compounds, such as Mygalin (MY), is an alternative. This is a polyamine synthesized from the natural molecule present in the hemolymph of the spider *Acanthoscurria gomesiana*. Polyamines are characteristic of the modulation of *L*-glutamate receptors, which in turn are closely linked to the pathway of pain. In this way MY is a molecule with analgesic potential. The objective of this study was to evaluate the effect of MY on the neuropathic pain

model induced by partial sciatic nerve ligation (PLS). Wistar rats were submitted to PLS surgery and, after 14 days, underwent stereotactic surgery to implant a guide cannula in the dorsal raphe nucleus (ND). After seven days, the behavior of these rats were evaluated in the tests of mechanical allodynia and hyperalgesia, both in heat and cold. The tests adopted were: von Frey (VF), Tail Flick (TF), Hot Plate (HP) and acetone test (AC). These experiments were applied at regular intervals of 15 and 30 minutes after treatment. Three concentrations of MY (0.2, 0.02, $0.002 \mu g/\mu L$) were used. There are also control groups like sham (Sh) and vehicle (Ve). The project was submitted to the Committee of Ethics in the Use of Animals (CEUA) of the University of São Paulo - Campus of Ribeirão Preto under protocol 0134/2017. When compared to Ve, the MY group presented a significant difference in both the VF (p < 0.001) and the TF (p<0.001) and HP (p<0.05) groups, showing antinociceptive effect from the first minutes (5 min). In these same tests the effect lasted until the end of the tests (90 min), with only the last time (120 min) and peak between 60 and 90 min. The treatments with MY of lower concentration showed a more significant effect in relation to the other groups, as well as a more prolonged effect Based on the data obtained so far, the lowest concentration (0,002 μ g/ μ L) of MY had an antinociceptive effect. In view of these results we can conclude that MY acts in other ways besides glutamatergic.

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Poster

309. Opioids and the Treatment of Pain

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 309.30/CC22

Topic: D.03. Somatosensation: Pain

Support: James Battaglia Endowed Chair

NIH 1R01GM111421

Title: Analgesic properties of plant-derived analgesic compounds in infant rats

Authors: C. J. MASCARENHAS¹, R. LIU², *G. A. BARR³

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Abstract: Premature and ill neonates undergo multiple painful but medically necessary procedures while hospitalized. Such procedures are associated with negative neurodevelopmental outcomes such as hindered cognitive and motor development, altered cortical rhythmicity, and

altered cortical thickness. Although opiate drugs are sometimes administered as analgesics, problems associated with their side effects, tolerance, and potential dependence compel research into alternative pain-relieving medication. Here we tested two plant-derived analgesic compounds for analgesia in infant rats: sinomenine, an alkaloid found in the root of Sinomenium acutum; and salvinorin A, isolated from Salvia divinorum, In adult animals both sinomenine and salvinorin A alleviate hyperalgesia, allodynia, and neuropathic pain, but the mechanisms of action of each compound is not understood, and neither has been tested in infants. To assess the effects of sinomenine and salvinorin A on pain during development, we used the formalin and thermal plantar tests in 3-day-old (for salvinorin A only), 7-day-old, and 21-day-old rats to determine how varying systemic doses of each drug affected the behavioral signs of inflammatory pain and acute thermal withdrawal latencies. In addition, brain sections were stained using Fos immunohistochemistry to examine patterns of brain activation in the midbrain periaqueductal gray and the paraventricular nucleus of the hypothalamus. Thermal and formalin test results from salvinorin A groups showed no analgesic drug effect at any age or dose (0.05 -0.45 mg/kg). Sinomenine treated groups showed an analgesic drug effect in both the formalin and thermal tests at 21-days of age. Multiple comparisons of 21-day sinomenine doses (20, 40, 80 mg/kg) showed that the high and middle doses reduced nociceptive responses in the formalin test and all three doses increased thermal withdrawal latencies. At 7-days of age the highest dose was lethal and there was no effect of sinomenin in the formalin test (10, 20, 40 mg/kg). Only the highest dose (40 mg//kg) elevated response latencies in the thermal test at this age. Analysis of Fos expression in the sinomenine treated animals showed no drug effect, in contrast to the behavioral results, although the Fos data are preliminary at this point. Thus, sinomenine might act at different sites or by different mechanisms. The increased effectiveness of sinomenine in older animals and the lack of a salvinorin A drug effect suggest that the compounds act on sites that develop during infancy (sinomenine) or after infancy (salvinorin A).

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Poster

310. Somatosensation: Transduction Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 310.01/CC23

Topic: B.04. Ion Channels

Title: Developmental expression of the mechanically-gated channel Piezo2

Authors: *C. I. FÜRST, G. R. LEWIN, A. HAMMES-LEWIN Max-Delbrueck-Center For Mol. Med., Berlin, Germany

Abstract: Mechanotransduction is the conversion of mechanical stimuli into electrical signals, a process crucial for a variety of many biological functions. On a molecular level, the mammalian

mechanosensitive ion channel Piezo2 has been described to act as a highly sensitive mechanosensor in several cell types. Piezo2 is required for mechanotransduction in the proprioceptive system and also for mechanoreceptors and touch in the somatosensory system. Furthermore it has also been described to be an airway stretch sensor crucial for normal respiration. The importance of mechanosensitive ion channels in the human body was revealed in clinical studies in which patients with mutations in PIEZO2 were described to suffer from Gordon syndrome, Marden-Walker syndrome or Distal Arthrogryposis type 5. Symptoms of these syndromes include congenital contractures of hands and feet, ophthalmoplegia, scoliosis or cleft palate. In addition, patients with loss of function *PIEZO2* mutations display a selective loss of discriminative touch perception and proprioception. Although many functions of Piezo2 have been described in the adult mouse, there are as yet no thorough studies of *piezo2* expression throughout development. To better understand the mechanisms underlying the etiology of PIEZO2 related diseases we aimed at defining the spatial and temporal expression of Piezo2 in the mouse embryo. Using in situ hybridization on embryonic mouse sections we discovered a localized and dynamic expression pattern of the peizo2 mRNA. At embryonic day 14 piezo2 is strongly expressed in the trigeminal ganglion, the dorsal root ganglia and in a restricted cellular population in the spinal cord. As Piezo2 acts as a mechanosensor in dorsal root ganglia its expression early on probably correlates with the early onset of mechanotransduction in these cells. To further elucidate the fate of *piezo2*-positive cells we used a reporter mouse line which expresses the red fluorescent protein tdTomato dependent on Cre recombinase activity driven from the *piezo2* locus. First results with these reporter lines indicate a wide spread expression pattern for *piezo2*. The identity of these *piezo2*-positive cells will be further characterized with co-expression studies using different cell type specific markers. Our results suggest expression of the mechanically-gated ion channel Piezo2 in a variety of different tissue types throughout development.

Disclosures: C.I. Fürst: None. G.R. Lewin: None. A. Hammes-Lewin: None.

Poster

310. Somatosensation: Transduction Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 310.02/CC24

Topic: D.04. Somatosensation: Touch

Support: NIH Grant DE018661

NIH Grant DE023090

Title: Effects of vincristine on Piezo2 channels and tactile responses of whisker hair follicles

Authors: *M. SONEKATSU, W. CHANG, H. KANDA, J. LING, J. GU Dept. of Anesthesiol. and Perioperative Med., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Chemotherapy induced peripheral neuropathy (CIPN) is a dose-limiting factor for treating cancers by a number of important drugs including vincristine. Clinical symptoms of CIPN include numbress, tingling sensation, and neuropathic pain. The pain aspect of CIPN induced by vincristine and other chemotherapy drugs is thought to be due to afferent demyelination and axon degeneration induced by chemotherapy drugs. Although numbress or loss of touch sensitivity is one of the earliest and most common symptoms of CIPN, mechanisms underlying the numbress aspect of CIPN remain poorly understood. The sense of touch is mostly transduced by specialized tactile end organs. Merkel disc is a main type of tactile end organ located in mechanically sensitive spots of the epidermis including whisker hair follicles. A Merkel disc consists of a Merkel cell and an Aβ-afferent fiber. We have recently shown that Merkel cells use Piezo2 channels to transduce mechanical stimulation into electrophysiological tactile responses manifested as slowly adapting type 1 (SA1) impulses. In the present study, we characterized mechanical sensitivity and membrane excitability of mouse Merkel cells. We further investigated effects of vincristine on whisker afferent SA1 responses. We found that treatment of animals with vincristine resulted in the reduction of Piezo2-mediated mechanically activated currents and also the suppression of SA1 impulses of whisker afferents. Our findings show that vincristine affect mechanical transduction of Merkel cells to subsequently compromise the tactile sensitivity of whisker hair follicles, providing a putative mechanism underlying the numbness aspect of CIPN in patients treated with vincristine.

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Poster

310. Somatosensation: Transduction Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 310.03/CC25

Topic: D.04. Somatosensation: Touch

Support: MC-A022-5PB91

WT103784MA

Title: Characterizing the expression and function of TMC proteins in dorsal root ganglia neurons

Authors: *K. M. WEBSTER, R. C. KNABLE, W. R. SCHAFER MRC Lab. of Mol. Biol., Cambridge, United Kingdom **Abstract:** The role of transmembrane channel-like family member proteins in mechanosensation in the inner ear has been well characterized. Although challenges with heterologous expression systems have kept the field from definitively demonstrating that these proteins serve as the poreforming subunit in the receptor complex, it is well accepted that Tmc1 and Tmc2 are required for the transduction of the mechanosensory signal in hearing. However, the role the proteins from TMC sub-family A (Tmc1, 2, & 3) elsewhere in the nervous system have been poorly characterized. Here, we show that sub-family A Tmc proteins are expressed in the peripheral nervous system, particularly in the dorsal root ganglia of mice. Through RT-PCR, we characterized the isoforms of the proteins expressed specifically in peripheral tissue and have identified at least two different isoforms of Tmc3. Additionally, through immunofluorescence, we show that Tmc1 has limited expression in a subset of low-threshold mechanoreceptors and Tmc3 is expressed in two different populations, a subset of low-threshold mechanoreceptors and A-delta fibers, responsible for thermosensation. Ectopic expression of the mammalian Tmc1 & 3 in *C. elegans* neurons confers mechanosensitivity to pairs of neurons that do not usually respond to mechanosensitive stimuli. Combining mechano-clamp and whole-cell electrophysiology, we are able to test whether Tmc1 and Tmc3 contribute to the mechanical currents in specific subsets of known mechano-sensitive neurons in the dorsal root ganglia. We will compare cells from subgroups of interest from dissociated wild type, Tmc1^{-/-}, and Tmc3^{-/-} ganglia in culture. Using a motor-driven glass probe, we will deform the cell body membrane and record whole-cell mechano-sensitive currents. Thus, we will present a characterization of the expression and function of Tmc1 and Tmc3 in DRG neurons.

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Poster

310. Somatosensation: Transduction Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 310.04/CC26

Topic: D.04. Somatosensation: Touch

Support: the National Research Foundation of Korea

the Ministry of Science, ICT, and Future Planning (2011-0018358)

Title: Evidence for mechanosensitive channel activity of tentonin 3/TMEM150C

Authors: *G. HONG, J. WEE, U. OH Korea Inst. of Sci. & Technol., Seoul, Korea, Republic of

Abstract: Mechanosensation is essential for various physiological processes, and it is mediated by mechanotransductionchannels. Recently, we reported that TMEM150C/Tentonin 3 (TTN3)

confers mechanically activated currents with slow inactivation kinetics in several cell types, including dorsal root ganglion neurons(Hong et al., 2016). The accompanying Matters Arising by Dubin, Murthy, and colleagues confirms that naive heterologous cells demonstrate a mechanically activated current, but finds that this response is absent in CRISPR-Cas9 Piezo1 knockout cell lines and suggests that TTN3 is a modulator of Piezo1. We present and discuss evidence based on coexpression of TTN3 and Peizo1 and mutant variants of the pore region of TTN3 to support that TTN3 is a pore-forming unit, not an amplifying adaptor for Piezo1 activity. This Matters Arising Response paper, along with Zhao et al. (2017), addresses the Matters Arising from Dubin et al. (2017), published concurrently in this issue of Neuron.

Disclosures: G. Hong: None. J. Wee: None. U. Oh: None.

Poster

310. Somatosensation: Transduction Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 310.05/CC27

Topic: B.04. Ion Channels

Support: R01NS093073

Title: Polarized initiation of neuronal varicosities

Authors: *C. GU, Y. GU Biol. Chem. and Pharmacol., Ohio State Univ., Columbus, OH

Abstract: Little is known about mechanical regulation of morphological and functional polarity of central neurons. Here we report that mechanical stress specifically induces varicosities in the axons but not the dendrites of central neurons by activating TRPV4, a Ca^{2+}/Na^+ -permeable mechanosensitive channel. This process is unexpectedly rapid and reversible, consistent with the formation of axonal varicosities *in vivo* induced by mechanical impact in a mouse model of mild traumatic brain injury (mTBI). By contrast, prolonged-stimulation of glutamate receptors induces varicosities in dendrites but not axons. We further show that axonal varicosities are induced by persistent Ca^{2+} increase, disassembled microtubules and subsequently reversible disruption of axonal transport, and are regulated by stable tubulin-only polypeptide (STOP), a microtubule-associated protein. Finally, axonal varicosity initiation can trigger action potentials to antidromically propagate to the soma in retrograde signaling. Therefore, our studies demonstrate a new feature of neuronal polarity—axons and dendrites preferentially respond to physical and chemical stresses, respectively.

Disclosures: C. Gu: None. Y. Gu: None.

Poster

310. Somatosensation: Transduction Mechanisms

Location: Halls A-C

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Deutsche Forschungsgemeinschaft (DFG, Exc 257 NeuroCure, FOR 1341, FOR 2143, SFB 665)

Berlin Institute of Health (BIH)

Thyssen Foundation

Helmholtz Society

Title: Sensory afferent coding of non-noxious thermal perception in mice

Authors: *R. PARICIO MONTESINOS^{1,2}, F. SCHWALLER¹, A. UDHAYACHANDRAN^{1,2}, G. R. LEWIN¹, J. F. A. POULET^{1,2}

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Abstract: Alongside an object's texture, its temperature gives important contextual information to help form an accurate somatosensory percept during active tactile exploration. However, little is known about the perceptual thresholds and neural coding of non-noxious thermal stimuli. Here, we trained head-fixed mice to report non-noxious warming and cooling stimuli using a behavioral paradigm that allows spatial and temporal control of a thermal stimulus delivered to the right forepaw and went on to correlate perception with thermal responses of primary sensory afferent neurons using a skin/nerve preparation. Mice took 2-3 days to learn to report a 10°C cooling stimulus (32 to 22°C), using a small diameter Peltier element (3x3mm), but 1-2 weeks to learn to report a warming stimulus of the same amplitude (32 to 42°C). Using a larger diameter Peltier element (8x8mm) improved the speed of learning to detect a warming stimulus to 3-4 days, while cooling was possible to learn in the very first session. Perceptual thresholds reflected the difference in learning times with cooling being detected with lower threshold (0.5°C) than for warming (1°C). Mice trained to report warming stimuli withheld licking to cooling and cool trained mice withheld licking to warm stimuli hinting at segregated neural pathways for warm and cool processing. Thermal responses in afferent neurons were mostly restricted to C-fibres and reflected behavioural data with robust responses to cooling from ~ 15% of all tested neurons, and a weak, but distinct, response to warming in a lower proportion of tested neurons. Our data

indicate that the sparser coding of warming by cutaneous C-fibres correlates with the low fidelity of behaving mice to learn to report skin warming in comparison to skin cooling.

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Poster

310. Somatosensation: Transduction Mechanisms

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Topic: D.04. Somatosensation: Touch

Support: BBSRC Grant BB/L502674/1

BBSRC Grant BB/L002787/1

Title: STIM-Orai channels may detect cold in the somatosensory and sympathetic nervous system

Authors: *T. J. BUIJS, P. A. MCNAUGHTON

Wolfson CARD, King's Col. London, London, United Kingdom

Abstract: Introduction: Cold temperatures are sensed by specific cold-activated nerve fibres in the somatosensory nervous system. In these fibres, non-noxious cool temperatures around 25°C are detected by the menthol-sensitive TRPM8 ion channel, but how lower noxious cold temperatures <15°C are detected remains unclear. Previous evidence for a role of one candidate -TRPA1 - in noxious cold sensation has been disputed, because genetic deletion of TRPA1 has little effect on behavioural sensation of cold¹. The sympathetic nervous system is also coldsensitive, but does not express TRPM8². Similarly, TRPA1 is expressed in less than 4% of sympathetic neurons, which is far fewer than the observed cold sensitivity in >75% of neurons³. We sought to determine the mechanism of extreme cold sensation in the somatosensory and sympathetic nervous systems, and hypothesized that cold sensitivity may be mediated by activation of the STIM-Orai system for calcium entry, which is normally responsible for replenishing intracellular calcium stores. Methods: Adult C57BL/6 mouse sensory (dorsal root) and sympathetic (superior cervical) ganglia were removed, dissociated using enzymes, and cultured on glass coverslips for one day before use. Intracellular calcium levels were measured using ratiometric calcium imaging. During recording, neurons were perfused with a cold ramp to 5°C to test the effect of various Orai channel antagonists on the calcium increase evoked by cold. **Results:** In sympathetic neurons, cold responses were abolished by $Gd^{3+}(1 \mu M)$ which blocks both Ca_V and Orai channels. The selective Orai channel blocker YM58483 (3 µM) caused a 70% reduction in cold-response amplitude. Furthermore, YM58483 (3 μ M) completely abolished cold

sensitivity in the presence of Cav1 L-type calcium channel blocker *verapamil (100 \muM)*. Similarly, in the sensory nervous system *YM58483 (3 \muM)* caused a 50% reduction in coldresponse amplitude in neurons that do not express TRPM8, TRPA1, or TRPC5. **Conclusion:** Both the somatosensory and sympathetic nervous system probably use Orai channels to detect cold <15°C. Activation of peripheral nerves by extreme cold may trigger cold-induced vasodilation, a mechanism responsible for preventing frostbite. *References:* 1. Bautista DM et al. (2007). Nature. 448: 204-208. 2. Smith MP et al. (2004). NeuroReport 15(9): 1399-1403. 3. Munns C et al. (2007). Cell Calcium 41: 331-342.

Disclosures: T.J. Buijs: None. P.A. McNaughton: None.

Poster

310. Somatosensation: Transduction Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 310.08/CC30

Topic: D.04. Somatosensation: Touch

Title: Glucosylsphingosine, an endogenous pruritogen in atopic dermatitis, activates serotonin receptor 2a and 2b

Authors: *W.-S. SHIM¹, A. RAMSHA²

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Abstract: Recent reports claimed that glucosylsphingosine (GS) is highly accumulated and specifically evoking itch-scratch responses in the skins of atopic dermatitis (AD) patients. However, it was unclear how GS can trigger itch-scratch responses, since there were no known molecular singling pathways revealed yet. In the present study, it was verified for the first time that GS can activate mouse serotonin receptor 2a (mHtr2a) and 2b (mHtr2b), but not 2c (mHtr2c) that are expressed in HEK293T cells. Specifically, effects of GS on all mouse serotonin receptor 2 subfamily were evaluated by calcium imaging techniques. The GS-induced intracellular calcium increase was dose-dependent, and antagonists such as ketanserin (Htr2a antagonist) and RS-127445 (Htr2b antagonist) significantly blocked the GS-induced responses. Moreover, the proposed GS-induced responses appear to be mediated by phospholipase C (PLC), since pretreatment of a PLC inhibitor U-73122 abolished the GS-induced responses. Additionally, the GS-induced calcium influx is probably mediated by endogenous TRPC ion channels in HEK293T cells, since pretreatment of SKF-96365, an inhibitor for TRPC, significantly suppressed GS-induced response. In conclusion, the present study revealed for the first time that GS can stimulate mHtr2a and mHtr2b to induce calcium influx, by utilizing PLC-dependent pathway afterwards. Considering that GS is regarded as a pruritogen in AD, the present study implicates a novel GS-induced itch signaling pathway.

Disclosures: W. Shim: None. A. Ramsha: None.

Poster

310. Somatosensation: Transduction Mechanisms

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 310.09/CC31

Topic: D.04. Somatosensation: Touch

Title: Change in pupillary autonomic activity by mandible grinding with different types of simulated dental occlusion

Authors: *H. YOSHIMI^{1,2}, M. SHINOMIYA¹, Y. KOMORIYA¹, Y. ONO²

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Abstract: Key words: Intraoral occlusal conditions, Autonomic nervous activity, Pupillary Light Reflex Irregular condition of dental occlusion has been considered to cause autonomic imbalance and its associated health problem. Generally, good and bad dental occlusions are defined by the canines and the molar teeth contacts dominantly during a grinding motion, respectively. However the definition of 'good dental occlusion' is still in debate. The aim of the current study is therefore to clarify whether the differences in intraoral occlusal conditions affect the autonomic nervous activity. Healthy 8 volunteers took part in the study. All subjects had excellent oral health and the tested teeth were intact and found healthy by means of clinical and radiographic findings. The local ethics committee approved the study. All volunteers gave their written informed consent to participate. We measured pupil diameter and pupil light reflex using Iriscorder Dual C10641 (Hamamatsu Photonics, Shizuoka, Japan) with simultaneous electromyogram (EMG) measurements of the masseter muscle using Multi Channel Telemeter WEB-1000 (Nihon Kohden Co., Ltd., Tokyo, Japan). Subjects performed mandibular grinding for 30 seconds, which was immediately followed by a recording of pupil light reflex. We installed metal overlays into either the canines or the first molars to simulate optimal or nonoptimal occlusal conditions. In addition, subjects performed another run of pupil light reflex after grinding with their natural occlusion as control condition. Subjects performed grinding with all occlusal conditions in a random order. Analysis were carried out observing the initial pupil diameter (D1) and minimum pupil diameter (D2) during the pupillary light reflex. The rectified EMG waveform is smoothed with a 5 Hz low pass filter and normalized with Maximum Voluntary Contraction (MVC). Two subjects who had no change in the masseter muscle activity during grinding were excluded from statistics. We found statistically significant increase in D2 of the left eye with optimal occlusal condition compared to control condition. Parasympathetic

nerve activity may be suppressed during grinding with optimal condition. Our results suggest that the occlusal condition affect autonomic nervous activity.

Disclosures: H. Yoshimi: A. Employment/Salary (full or part-time):; Medical Corporation JIvaka Yoshimi Dental Office. M. Shinomiya: A. Employment/Salary (full or part-time):; Medical Corporation JIvaka Yoshimi Dental Office. Y. Komoriya: A. Employment/Salary (full or part-time):; Medical Corporation JIvaka Yoshimi Dental Office. Y. Ono: A. Employment/Salary (full or part-time):; Department of Electronics and Bioinformatics, School of Science and Technology, Meiji University.

Poster

310. Somatosensation: Transduction Mechanisms

Location: Halls A-C

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Topic: D.04. Somatosensation: Touch

Support: Korea Institute of Oriental Medicine (Y17021)

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Title: Connective tissue does not mediate the therapeutic effect of acupuncture

Authors: *Y. FAN¹, Y.-H. RYU², S. CHANG¹, O.-S. KWON², S. BANG², N. KIM¹, D.-H. KIM¹, H. KIM¹, E. JANG¹, C. YANG¹, H. KIM¹ ¹Col. of Korean Medicine, Daegu Haany Univ., Daegu, Korea, Republic of; ²Acupuncture, Moxibustion& Meridian Res. Center, Div. of Standard Research, Korea Inst. of Oriental Med., Daejeon, Korea, Republic of

Abstract: Acupuncture has been used to treat a variety of disease and symptoms for more than 2500 years. While previous studies have suggested that acupuncture needle stimulates peripheral nerve in acupuncture point to generate the therapeutic effects of acupuncture, some of researchers have insisted that connective tissue during needling would initiate acupuncture signals. To determine which, nerve or connective tissue, mediate acupuncture effects , we developed a twisting acupuncture instrument; TAI which mimicked a twisting-rotating acupuncture needle manipulation by acupuncturists and examined the role of nerve or connective tissues in generation of acupuncture effects, by pharmacologically blocking nerve or connective tissues during twisting acupuncture in cocaine-induced locomotion, hypertension, and colitis models.

When connective tissues were destroyed by by injecting type I collagenase in acupoints, the torque force during needle rotation/twisting was declined but acupuncture effects on cocaine-induced locomotion, hypertension or colitis were not affected, compared to the corresponding control groups. On the other hand, pretreatment of bupivacaine, a local, long-lasting nerve blocker, into completely blocked the acupuncture effects in cocaine-induced hyperactivity, hypertension and colitis rat models.

Our findings suggest that nerve, but not connective tissue is a culprit in generating acupuncture effects.

Disclosures: Y. Fan: None. **Y. Ryu:** None. **S. Chang:** None. **O. Kwon:** None. **S. Bang:** None. **N. Kim:** None. **D. Kim:** None. **H. Kim:** None. **E. Jang:** None. **C. Yang:** None. **H. Kim:** None.

Poster

311. Auditory Processing: Circuits, Synapses, and Neurotransmitters I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 311.01/CC33

Topic: D.06. Audition

Title: Effect of chronic radio frequency exposure in the superior olivary nuclear complex: An immunohistchemical study

Authors: *D. MASKEY¹, M. KIM², H.-G. KIM³

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Abstract: Exponential growth of mobile communication has raised concerns on the possible health risk to human due to radiofrequency (RF) exposure on human brain owing to its close proximity during usage. Alterations in intracellular calcium (Ca²⁺) concentration could trigger aberrant synaptic action or cause neuronal apoptosis, which may exert an influence on the cellular pathology in the central auditory brain stem nucleus such as superior olivary nuclear complex (SOC). Calcium binding proteins (CaBPs) such as calbindin D28-k (CB) and calretinin (CR) are able to bind Ca²⁺ ions with high affinity. Changes in Ca²⁺ ion concentrations via CaBPs can disturb Ca²⁺ homeostasis leading to cell death. Inhibitory molecule such a glycine that is dominantly localized in the auditory brainstem, play a major role in interplay auditory processing along with excitation molecules interaction while the neurotrophic factors like brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF), which is present in the auditory system, are responsible for the maintenance of the auditory neurons. The present study applied RF exposure at SAR 1.6 W/kg (E1.6 group) and sham-control 0 W/kg (SC group) for three months to determine the distribution of CB, CR, GlyR, BDNF, GDNF and GFAP in the mice's SOC through immunohistochemistry. ABR analysis performed revealed significant

elevation of threshold in the E1.6 group, which may implicate auditory dysfunction. Significant loss of CB and CR IRs in the neuropil as well as cells in the different nuclei of SOC of E1.6 was noted. A decrease in the number of GlyR immunoreactive cells was also noted (LSO-36.85%, SPN-24.33%, MSO-23.23%, MNTB-10.15%) in E1.6. Neuropil staining with BDNF and GDNF IRs in the nuclei of SOC were significantly decreased in the E1.6 while GFAP increased significantly when compared with the SC. Decrease in CaBP and GlyR IRs could lead to impairment of Ca²⁺ homeostasis and decrease ability to segregate sounds leading to auditory dysfunction while decrement of neurotrophic factors insinuate detrimental effect. The present study showed susceptibility of the auditory brainstem region to chronic RF exposure with changes in the ABR suggesting a possibility of detrimental effect in the auditory brainstem circuit.

Disclosures: D. Maskey: None. M. Kim: None. H. Kim: None.

Poster

311. Auditory Processing: Circuits, Synapses, and Neurotransmitters I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 311.02/DD1

Topic: D.06. Audition

Support: BRiC INAIL 2016-DiMEILA17

ONR Global N62909-15-1-2002

D1 Funds Università Cattolica

Title: Anodal transcranial direct current stimulation modulates structural plasticity in the rat auditory cortex and counteracts cortical alterations induced by chronic exposure to noise

Authors: *M. V. PODDA¹, F. PACIELLO², R. ROLESI³, S. COCCO¹, A. R. FETONI³, G. PALUDETTI³, C. GRASSI¹, D. TROIANI¹

¹Inst. of Human Physiology, Univ. Cattolica, Rome, Italy; ²Inst. of Cell Biol. and Neurobiology, CNR, Rome, Italy; ³Dept. of Head and Neck Surgery, Univ. Cattolica, Rome, Italy

Abstract: Transcranial direct current stimulation (tDCS) is a non-invasive tool capable of modulating cortical functions by affecting neuronal excitability and synaptic plasticity. Here we investigated the effects of anodal tDCS on structural plasticity of auditory cortex (AC) in healthy (H) rats and following noise-induced hearing loss (NIHL). Rats were exposed to noise (100 dB, 60 min/day for 10 days), subjected to anodal tDCS over the left AC (350 μ A for 20 min for two days) starting from 24 h after the end of NIHL paradigm, and sacrificed 21 days later for morphological analyses by Golgi-Cox staining and electrophysiological field recordings.

In agreement with our previous report (*Fetoni et al., 2013*) NIHL decreased spine density in layer 2/3 and 5-6 AC pyramidal neurons. Interestingly, we found that tDCS counteracted the NIHL-induced spine loss both in apical and basal arborizations. In particular, in NIHL rats spine loss in neurons of layer 2/3 was~30% in apical dendrites and~40% in basal dendrites, while in those exposed to tDCS (NIHL-tDCS) spine density was not significantly different from that of H rats (p=0.82).

Of note, tDCS increased spine density (+20-30%) in apical dendrites of layer 2/3 and 5-6 neurons in H rats with normal auditory function similarly to what reported following auditory environmental enrichment.

Twenty-four hours after tDCS Bdnf levels in the AC increased both in H and NIHL rats (p=0.01 and 0.02, respectively). Similarly, the expression of synaptophysin was enhanced by tDCS in H (+80%, p=0.01) and NIHL rats (+60%, p=0.04).

Field recordings were performed from AC slices to assess tDCS impact on basal synaptic transmission at layer 2/3 horizontal connections. Comparison of the input-output (I-O) curves showed that in NIHL rats the amplitude of field excitatory post-synaptic potentials was significantly smaller than in H rats (p=0.01). Interestingly, responses to current pulses were significantly increased in NIHL-tDCS group compared to sham-stimulated NIHL rats (p=0.003) and I-O curves of NIHL-tDCS rats were similar to those of H rats, suggesting that tDCS counteracted the effects of noise on synaptic function.

Our findings provide novel evidence that anodal tDCS affects structural plasticity in the AC and counteracts the detrimental effects of sensory deafferentation. These results widen the horizons on brain areas whose plasticity is targeted by tDCS and open the way to the possibility to exploit tDCS to treat diseases thought to be related to altered plasticity like tinnitus.

Disclosures: M.V. Podda: None. F. Paciello: None. R. Rolesi: None. S. Cocco: None. A.R. Fetoni: None. G. Paludetti: None. C. Grassi: None. D. Troiani: None.

Poster

311. Auditory Processing: Circuits, Synapses, and Neurotransmitters I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 311.03/DD2

Topic: D.06. Audition

Title: Channelrhodopsin-assisted circuit mapping of ascending and commissural synaptic inputs to VIP neurons in mouse inferior colliculus

Authors: *D. GOYER, P. T. MALINSKI, A. P. GEORGE, M. T. ROBERTS Kresge Hearing Res. Inst., Univ. of Michigan, Ann Arbor, MI

Abstract: The inferior colliculus (IC), the midbrain center of the auditory pathway, is a major hub for nearly all auditory information coming from the brainstem and a critical station in sound

processing. To better understand how neural circuits in the IC process sounds, a robust method for identifying the different neuron types within the IC is necessary. It is also essential to know how identified neuron types function within the local circuitry of the IC and in the ascending auditory pathway. So far, it has proven difficult to delineate specific neuron types with standard anatomical and physiological techniques.

Recently we identified a novel class of stellate cells that are labeled in Vasoactive Intestinal Peptide (VIP)-IRES-Cre mice. We crossed VIP-IRES-Cre mice with Ai14 reporter mice to drive tdTomato expression in Cre-expressing VIP neurons. Fluorescently labeled VIP neurons were distributed throughout the central region of the IC with the highest density in the caudal pole of the IC. We targeted whole cell recordings to fluorescent VIP neurons in the IC in acute brain slices of mice aged 10 to 57 days (median = 28.5 days). VIP neurons had a moderate input resistance of 199 M Ω (± 158.5 M Ω), membrane time constant of 13.4 ms (± 7.7 ms), moderate to no hyperpolarization activated cation current (Ih) and 88% (82/93) exhibited a sustained firing pattern with relatively little spike frequency adaptation (SFA ratio < 2). Post hoc reconstruction of recorded neurons showed that VIP neurons have stellate morphologies and all but one had spiny dendrites (31/32).

We are currently using Channelrhodopsin-assisted circuit mapping to identify ascending and commissural sources of synaptic input to VIP neurons. Specifically, we inject recombinant adeno-associated viruses (rAAV2/5.Syn.Chronos-GFP or rAAV2/1.Syn.Chronos-

GFP.WPRE.bGH) encoding the optogenetic protein Chronos into one hemisphere of the IC or in select auditory brainstem nuclei. After allowing the virus to express for 3-4 weeks, we perform whole cell recordings from VIP neurons while activating Chronos-expressing inputs with flashes of blue light (1 - 10 ms, 3.0 - 14.3 mW/mm²). Activation of commissural inputs elicits a mixture of excitatory and inhibitory postsynaptic potentials in VIP neurons. In addition, activation of ascending input from the dorsal cochlear nucleus evokes excitatory postsynaptic potentials that are often coupled with GABA-mediated feedforward inhibition.

Together, these experiments identify VIP neurons as a novel subclass of IC stellate cells and suggest that individual VIP neurons might integrate input from the dorsal cochlear nucleus and the contralateral IC.

Disclosures: D. Goyer: None. P.T. Malinski: None. A.P. George: None. M.T. Roberts: None.

Poster

311. Auditory Processing: Circuits, Synapses, and Neurotransmitters I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 311.04/DD3

Topic: D.06. Audition

Support: NIH-SC1 grant #1SC1GM118242-01

Title: Characterization of a brainstem-amygdala pathway important for attention processing

Authors: *J. C. CANO¹, K. FENELON²

¹Biol. Sci., Univ. of Texas at El Paso Dept. of Biol. Sci., El Paso, TX; ²Biol. Sci., Univ. of Texas at El Paso, El Paso, TX

Abstract: Sensorimotor gating is a pre-attentive neural filtering process that allows our brain to suppress irrelevant sensory information, and helps us to focus attention. Patients suffering from neuropsychiatric disorders, such as schizophrenia and anxiety, exhibit sensorimotor gating deficits that greatly impact their daily lives. Clinically, sensorimotor gating can be measured using the prepulse inhibition (PPI) of the acoustic startle reflex task. During PPI in healthy subjects, a non-startling sound (prepulse) will inhibit the startling effect of a subsequent startling sound (pulse). Previously, in vivo and in vitro animal studies have shown that the brainstem caudal pontine reticular nucleus (PnC) is at the core of the PPI pathway, relaying sensory inputs from several brain regions directly to spinal and cranial motor neurons. In fact, the PnC has been shown to receive cholinergic inputs from the pedunculopontine tegmental nucleus (PPTg). Initially thought to be an essential connection for PPI, recent in vivo rat studies suggest that the PPTg-PnC cholinergic connection is no longer considered critical for PPI. Therefore, we hypothesized that other brain pathways contribute to sensorimotor gating, and need to be investigated. The central nucleus of the amygdala (CeA) is another region directly connected to the PnC. Interestingly, the CeA modulates PnC neuronal activity, and lesions to the amygdaloid complex can disrupt PPI. However, the potential role of the CeA-PnC connection in sensorimotor gating remains to be determined. Therefore, here, we investigated the contribution of the CeA-PnC connection to PPI in mice using immunohistochemistry and electrophysiological recordings paired with optogenetics in acute brain slices (N=7 mice). Our results suggest that the CeA sends monosynaptic glutamatergic projections to the PnC. In addition, when the PPI task was mimicked in vitro in PnC slices, PPI was modified by the photo-stimulation of the CeA inputs. Our results will contribute to better understand the neural pathways underlying PPI, and allow us to identify potential therapeutic targets for diseases associated with sensorimotor gating deficits.

Disclosures: J.C. Cano: None. K. Fenelon: None.

Poster

311. Auditory Processing: Circuits, Synapses, and Neurotransmitters I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: D.06. Audition

Support: NIH Grant DC013073

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Title: A novel mutual information estimator to measure spike train correlations in a model of the thalamocortical network

Authors: *E. D. GRIBKOVA^{1,2}, B. A. IBRAHIM^{1,3}, D. A. LLANO^{1,2,3} ²Neurosci. Program, ³Mol. and Integrative Physiol., ¹Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: The impact of the various states of the thalamus on information transmission to the cortex remains poorly understood. This limitation exists due to the rich dynamics displayed by thalamocortical networks and because of the inadequate tools available to investigators to characterize those dynamics. Herein, we introduce a novel estimator of mutual information between spike trains and use this estimator to determine the impact of a computational model of thalamic state on information transmission. A limitation of previously employed mutual information estimators is their reliance on fixed partitions of spike trains. Therefore, we developed a mutual information estimator with an adaptive partition for signals with different time scales and used this estimator to compare input spike trains to output spike trains. It was observed that this estimator was superior to other mutual information estimators with fixed partitions when used to analyze simulated spike train data with different mean spike rates, as well as electrophysiological data from simultaneously recorded neurons, since it provided estimates that were closer to expected values and trends. When the estimator was applied to a thalamocortical model, it was found that thalamocortical (TC) cell calcium T-current conductance (T-current) influences mutual information between the input and output from this network. In particular, a T-current of about 50 nS appears to produce maximal mutual information between the input to this network (conceptualized as an afferent input to the TC cell) and the output of the network at the level of a cortical layer 4 neuron. Furthermore, at particular combinations of inputs to TC and thalamic reticular nucleus (TRN) cells, thalamic cell bursting correlated strongly with recovery of mutual information between thalamic afferents and L4. These studies suggest that a novel mutual information estimator using adaptive partitions has advantages over previous estimators, and that TRN activity can enhance mutual information between thalamic afferents and thalamorecipient cells in the cortex, while the cortex can recover mutual information between these thalamic afferents and thalamorecipient cells that is otherwise lost due to thalamic bursting.

Disclosures: E.D. Gribkova: None. B.A. Ibrahim: None. D.A. Llano: None.

Poster

311. Auditory Processing: Circuits, Synapses, and Neurotransmitters I

Location: Halls A-C

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Program#/Poster#: 311.06/DD5

Topic: D.06. Audition

Support: AIIMS, NEW DELHI, Intramural Grant No. A-499

Title: Age-related changes in the expression of glutamic acid decarboxylase (GAD67) and NMDA receptor (NMDAR1) in the human inferior colliculus

Authors: *I. PAL¹, T. G. JACOB¹, R. DADA¹, D. N. BHARDWAJ², T. S. ROY¹ ¹Dept. of Anat., ²Forensic Med. & Toxicology, All India Inst. of Med. Sci., New Delhi, India

Abstract:

The inferior colliculus (IC) is an important nucleus of auditory pathway, where all projections from ascending auditory nuclei converge, it is also connected with several descending pathways that is essential for normal hearing. GABA and glutamate are the major inhibitory and excitatory neurotransmitters in the IC respectively and critically involved in the temporal processing of acoustical stimuli. The balance between excitatory and inhibitory neurotransmission here is crucial for normal hearing. Declining levels of GABA have been found in the auditory region of patients and in animal models presbycusis. Earlier, we have reported decline in the number of neurons in the cochlear nucleus occurring from 6th decade of life. Therefore in the present study, we planned to investigate the changes in the transcription and translation of GAD67 and NMDAR1 in human IC at various ages by using quantitative real-time PCR and immunohistochemistry that was quantified by unbiased stereology (optical fractionator probe), respectively. For this study, 27 human IC were obtained from the mortuary with proper ethical clearance and divided into three age groups- 11-30 years, 31-50 years and >51 years (n=9 in each group). The 11-30 years' age group served as control group for comparison with the other two groups. We observed a significant decline in the number and expression of GAD67 and NMDAR1-immunoreactive neurons and their relative genes in the age group 51 years and above (p=0.001). The relative expression of GAD67 and NMDAR1 mRNA in the 31-50 age groups were 0.82 and 0.42 (p=0.02) and in the old age group was 0.56 and 0.55 (p=0.02) respectively. Hence, the balance between excitatory and inhibitory neurotransmission in IC is altered in the human IC with age and this may be affecting the normal hearing leading to pathogenesis of presbycusis.

Disclosures: I. Pal: None. T.G. Jacob: None. R. Dada: None. D.N. Bhardwaj: None. T.S. Roy: None.

Poster

311. Auditory Processing: Circuits, Synapses, and Neurotransmitters I

Location: Halls A-C

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Program#/Poster#: 311.07/DD6

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NIH,NIDCD R01DC01508

Title: Differential contribution of presynaptic calcium channels to synaptic transmission at the mouse medial olivocochlear-outer hair cell synapse at two developmental stages

Authors: L. VATTINO¹, A. B. ELGOYHEN^{1,2}, *E. KATZ^{1,3}

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Abstract: The mammalian auditory epithelium contains two types of mechanosensory cells, inner and outer hair cells (IHCs and OHCs, respectively). IHCs transduce sound stimuli into electrical signals that travel to the central nervous system while the OHCs, by virtue of their electromotile properties, are mainly involved in sound amplification. OHC electromotility is regulated by descending medial olivocochlear (MOC) fibers. The MOC-OHC synapse is cholinergic and inhibitory and is mediated by the $\alpha 9\alpha 10$ cholinergic nicotinic receptor coupled to the activation of Ca²⁺-activated K⁺ channels that hyperpolarize the OHCs. During development, before reaching the OHCs, MOC fibers transiently innervate the IHCs (from birth to hearing onset, postnatal day (P)12 in mice). The ion channels coupled to transmitter release at the MOC-IHC synapse have been previously described (Zorrilla de San Martin et al., 2010), however, nothing is known about the ion channels coupled to this process at the MOC-OHC synapse. Using acutely isolated mouse cochleas we show, by whole-cell voltage-clamp recordings in OHCs while electrically stimulating the efferent axons, that ACh release is mediated by both P/Q and R-type voltage-gated Ca²⁺ channels (VGCC) at P11-13 and by P/Q- and N-type VGCCs at P20-22. In addition, we show that at P11-13, large conductance Ca²⁺-activated K⁺ channels (BK) are functionally expressed at the MOC-OHC synapse as block by iberiotoxin (200 nM) significantly increases release (control 0.16±0.02; Ibtx 0.30±0.05, n=7; p<0.01). Interestingly, both the L-type VGCC antagonist nifedipine $(3 \mu M)$ and the agonist BayK $(10 \mu M)$ significantly increased release (control 0.19±0.06; Nife 0.30±0.07, n=6; p<0.001; Control 0.32±0.10; BayK 0.66 ± 0.25 , n=6, p<0.05). Occlusion experiments with iberiotoxin and BayK indicate that Ca²⁺ flowing in through L-type VGCC is both activating BK channels and partially supporting ACh release at P11-13. In addition, after loading the efferent terminals with EGTA-AM the contribution of L-type VGCC to release was completely abolished. At P20-22, however, BayK decreased release (control 0.47 \pm 0.16; BayK 0.22 \pm 0.07, n=6, p<0.05), indicating that Ca²⁺ influx through L-type VGCC is only activating BK channels at this stage. These results show that the VGCCs coupled to ACh release at the MOC-OHC synapse at P11-13 are the same as those at the MOC-IHC synapse at early developmental stages (P4-7; Kearney et al., ARO Abstracts 2014), while at P20-22, they resemble those of the MOC-IHC synapse at more mature stages (P9-11; Zorrilla de San Martín et al., 2010). Our results suggest that the MOC-OHC synapse is still functionally immature at the onset of hearing.
Disclosures: L. Vattino: None. A.B. Elgoyhen: None. E. Katz: None.

Poster

311. Auditory Processing: Circuits, Synapses, and Neurotransmitters I

Location: Halls A-C

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Topic: D.06. Audition

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Kirchgessner Foundation

Title: A reticular-limbic ascending pathway for transmitting aversive auditory signals

Authors: *G. ZHANG^{1,2}, W. SUN⁴, B. ZINGG³, H. TAO⁵, L. I. ZHANG² ¹Dept. of Neurobio., Third Military Med. Univ., Chongqing City, China; ³Neurosci. Grad. Program, ²USC, Los Angeles, CA; ⁴City Univ. of HongKong, Hong Kong, China; ⁵USC Keck Sch. Med., Los Angeles, CA

Abstract: It is well recognized that in the mammalian brain auditory information is processed along a central ascending pathway into the auditory cortex (AC). However, whether there exist any major auditory pathways beyond this canonical neuraxis remains unclear. In awake mice, we found that neurons in the entorhinal cortex (EC) exhibited auditory responses with so short latencies that cannot be explained by a previously proposed relay from AC. Screening input sources of EC with combined anatomical/functional tracing, optogenetic and pharmacological manipulations, we discovered that EC received auditory input primarily from the medial septum (MS). A previously uncharacterized auditory pathway was then revealed: it branched from the cochlear nucleus, via caudal pontine reticular nucleus, pontine central gray, MS, and reached EC. Interestingly, neurons along this non-canonical pathway all exhibited selective responses to high-intensity noise, rather than tone stimuli. Such reticular-limbic pathway may be specialized in transmitting aversive auditory signals for memory functions.

Disclosures: G. Zhang: None. W. Sun: None. B. Zingg: None. H. Tao: None. L.I. Zhang: None.

Poster

311. Auditory Processing: Circuits, Synapses, and Neurotransmitters I

Location: Halls A-C

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Topic: D.06. Audition

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Title: Tuning of cortical gain, sound frequency selectivity and auditory-driven behaviors by synaptically released zinc

Authors: *M. KUMAR¹, C. T. ANDERSON¹, S. XIONG¹, T. TZOUNOPOULOS² ¹Dept. of Otolaryngology, ²Otolaryngology and Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Activity-dependent tuning of sensory processing modifies behavior advantageously, but the underlying signaling mechanisms remain unknown. Here, we imaged sound-evoked neuronal activity in the auditory cortex of awake mice, and discovered that synaptically released zinc reduced the gain of sound-evoked responses of layer (L) 2/3 principal neurons and interneurons and sharpened sound frequency selectivity of L2/3 principal neurons. In the absence of cortical synaptic zinc, mice showed reduced ability in detecting behaviorally meaningful signals from their auditory environment, such as changes in sound frequencies. By establishing a previously unknown link between synaptically released zinc and auditory cortical processing, our findings advance understanding about cortical synaptic mechanisms and create a new framework for approaching and interpreting the role of the auditory cortex in normal and pathological neuronal processing.

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Poster

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UConn Spring 2017 Health Research Program (N.M.)

Title: Hearing with an oversized inferior colliculus - is bigger better?

Authors: ***A. BURGHARD**¹, N. MOREL², D. L. OLIVER¹ ¹Neurosci., UConn Hlth., Farmington, CT; ²Univ. of Connecticut, Storrs, CT

Abstract: The inferior colliculus (IC) is a major hub of auditory processing. After different aspects of an acoustic signal are processed in the auditory brainstem nuclei, this information converges in the central nucleus of the IC (ICC) onto one tonotopic map. According to the synaptic domain theory, the inputs from different auditory brainstem nuclei to the ICC cluster in specific sub-regions and, thus, form functional zones superimposed on the single tonotopic map. These sub-regions may send this information via the thalamus to distinct areas of the core auditory cortex with different functions. One approach to the detailed study of function and anatomy in the ICC sub-regions is to genetically manipulate the IC during development. In the present study, we used a mouse model with an oversized IC. Developed by Dee et al. (2016), MEK1 (also referred to as mitogen-activated protein 2 kinase 1, MAP2K1) is overexpressed in this mouse in the tectal stem cell zone from which the IC originates. Consequently, the stem cells remain in the proliferation stage longer, thus increasing the number of stem cells, but also delaying neurogenesis. This results in more, but later developed IC neurons. The gross anatomy shows that in all MEK1 mice the IC is massively enlarged compared to littermate controls. We tested the hearing thresholds of MEK1 mice using the click-evoked auditory brainstem response (ABR) and the amplitude-modulated frequency following response (AMFR). To obtain the AMFR audiogram, we used narrow-band noise (0.3 octave) centered at frequencies 2-40 kHz and a modulation frequency of 42.9 Hz. We tested 5 and 13 week old MEK1 mice as well as age-matched littermate controls. Our preliminary electrophysiological evaluation of the MEK1 mice showed a diverse phenotype. In comparison to the audiograms of littermate controls, some MEK1 mice had an almost normal audiogram, while others showed elevated thresholds. We also measured the growth in the AMFR signal amplitude in response to increasing sound level intensity. In some MEK1 mice there was a reduced growth function especially close to threshold. Interestingly, the hearing threshold did not predict the amplitude growth function or vice-versa. We also measured the peak synchrony of the AMFR signal and found that synchrony was slightly degraded in most MEK1 mice in comparison to littermates. In summary, our preliminary observations of a mouse with a massively enlarged IC suggest that this structural change may result in more than one type of alteration in the circuitry of the auditory midbrain and more than one hearing phenotype. Bigger may not be better.

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Poster

311. Auditory Processing: Circuits, Synapses, and Neurotransmitters I

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Support: NIH grant R0DC3013396

Title: Principal neurons in the anteroventral cochlear nucleus express cell-type specific glycine receptor subunits

Authors: S. LIN, *R. XIE

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Abstract: Cochlear nucleus is the first station in the central auditory system that gates all sound information from the periphery. Signal processing in the principal neurons of the anteroventral cochlear nucleus (AVCN), including bushy, T-stellate and D-stellate neurons, are modulated by extensive inhibition mediated by glycine receptors. It has been shown that glycinergic inhibition in AVCN exhibits cell-type specific IPSC kinetics that differentially shapes the neural responses of the target cells. However, it remains unclear what glycine receptor subunits are involved in the inhibitory transmission to these AVCN principal neurons. In this study, we performed whole-cell patch clamp recording in AVCN brain slices from CBA/CaJ mice (either sex), electrophysiologically identified these three types of principal neurons, and characterized the properties of IPSCs in these neurons evoked by stimulating the inhibitory inputs from the dorsal cochlear nucleus. Neurons were filled with Alexa Fluor 488 during the recording, followed by fixation of the brain slices and standard immunostaining with primary antibodies against different types of glycine receptor subunits, and secondary antibodies conjugated with Alexa Fluor 594. Images of the filled neurons and the expression patterns of glycine receptor subunits were obtained using confocal microscope. We found that bushy neurons with slow IPSC kinetics express glycine receptor alpha4 subunit, whereas both T-stellate and D-stellate neurons that show fast IPSC kinetics express glycine receptor alpha1 subunit. The results suggest that the differential effects of glycinergic inhibition in AVCN rely on the distinct expression of cell-type specific glycine receptor subunits in the principal neurons.

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Poster

311. Auditory Processing: Circuits, Synapses, and Neurotransmitters I

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Support: the program for Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) from Japan Agency for Medical Research and development, AMED

Title: Axonal projection map of auditory areas in the common marmoset

Authors: *H. ABE¹, T. TANI¹, H. MASHIKO¹, N. KITAMURA¹, K. SAKAI³, T. HAYAMI³, N. MIYAKAWA³, W. SUZUKI³, H. MIZUKAMI⁴, A. WATAKABE², T. YAMAMORI², N. ICHINOHE³

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Abstract: Auditory processing is important for verbal communication in our social environment. In order to study its neural mechanisms in more detail, its anatomical connection must be revealed. The auditory cortex in the primate is thought to be divided into three regions: core, belt, and parabelt. Each region further contains subregions. As a first step, we aimed to reveal axonal projections from the auditory belt regions of a New World monkey, the common marmoset (Callithrix jacchus), which has rich vocal communication with other conspecifics. We injected an AAV, which works as an anterograde tracer by expressing either green or red fluorescent proteins in infected neurons, to three sites in the auditory belt regions of two adult marmosets. Each injection site was chosen based on a tonotopy map made by optical imaging while presenting auditory stimuli to the animal under anesthesia. After a three week waiting period, the animal was perfused and the post-mortem brain was sectioned in a thickness of 50 um. The sections were served to obtain fluorescent images to examine fluorescently labeled axonal projections and processed with myelin and Nissl substance staining to identify brain areas. The brain area annotations were based on a marmoset brain atlas (Paxinos et al 2012). Each injection site was histologically confirmed in either AL, ML or CL in the auditory belt regions. The three belt regions had projections to the entire auditory cortex (core, belt, and parabelt regions), except AL case which had a weak projection to the layer 1 of the core region. Projections to other areas in the temporal cortex were also prominent, including STR, TPO, FST, FSTv and MST (except AL case). The insular cortex, DI, Ipro and Tpro (except ML case) received the projections. For the frontal cortex, projections to 120 and A10 (except ML case) were commonly found. Thus the AL, ML, and CL regions had projections to similar regions with

some variation. This tendency in the projection might reflect the auditory processing done in each area.

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Poster

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Title: Cell type-specific long-range connections of the higher-order auditory thalamus

Authors: *D. CAI¹, Y. YUE¹, X. SU², Y. WANG¹, M. LIU¹, F. DENG², L. YOU¹, F. XIE¹, F. CHEN², M. LUO⁴, K. YUAN³

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Abstract: Although many traditional anatomical studies have shown the connectivity between the higher-order auditory thalamus and other brain areas, data showing cell type-specific connectivity for different subdivisions has been lacking. Previous immuno-histological studies demonstrated localized expression of two types of calcium binding proteins, calbindin (CB) and calretinin (CR), in the higher-order auditory thalamus. Using anterograde and retrograde viral tracing in CB- and CR-Cre mice, we found largely distinct cortical and subcortical connection patterns for the three subdivisions of the higher-order auditory thalamus: MGBd, MGBm and PIN. In addition, although CB and CR neurons exhibited generally similar connection patterns, they do show some differences depending on the specific subdivision injected. The most significant difference is in their connectivity with subcortical structures. Our results show that, due to the localized expression of CB and CR in the higher-order auditory thalamus, diffusion of traditional tracers into the first-order part is not an issue anymore. Most importantly, our results suggest that CB- and CR-Cre mice can be used as a powerful tool to explore the functions of specific pathways between the higher-order auditory thalamus and other brain areas. These anatomical findings and potential functional studies will deepen our understanding of the role of the high-order auditory thalamus in information processing and animal behavior.

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Poster

311. Auditory Processing: Circuits, Synapses, and Neurotransmitters I

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Topic: D.06. Audition

Support: NSF Grant 1456298

Title: Functional topography of raphe-collicular projections: Contributions of dorsal raphe subregions to the inferior colliculus of the mouse

Authors: *C. L. PETERSEN, L. M. HURLEY

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Abstract: The ability to process auditory signals in a context-dependent manner is highly adaptive and pervasive in vertebrate animals. One mechanism through which mice (Mus *musculus*) are able to add context to acoustic processing is via the release of the neuromodulator serotonin into the inferior colliculus (IC) within the auditory midbrain. Serotonin release into the IC is context dependent. Levels of serotonin increase within the IC of mice during sexual and non-sexual social encounters. Interestingly, within individual sexual encounters, levels of IC serotonin relate negatively to the amount of rejection-like behavior male mice receive. The vast majority of serotonergic projections to IC arise from the dorsal raphe nucleus (DRN), while other raphe nuclei such as the median raphe account for the rest. However, the rodent DRN can be broken down into at least 7 anatomically and functionally distinct sub-regions, and whether each DRN sub-region projects to the IC is unknown. Elucidating how individual sub-regions contribute to serotonergic innervation of IC is the first step in understanding the neural pathways that facilitate context-dependent release of serotonin in the auditory system. To this end, we injected the retrograde tract tracer Fluoro-Gold (FG) into the IC of CBA/J mice, which were perfused 5-7 days post surgery. To test the hypothesis that sub-regions of the DRN differentially innervate IC, we visualized neurons immunoreactive (-ir) for FG as well as tryptophan hydroxylase (TPH; the rate-limiting enzyme in serotonin production) throughout the extent of DRN. We report that FG-TPH co-labeled neurons were localized to several DRN sub-regions, specifically the dorsal part (DRd), and the "lateral wings" which includes the lateral DRN (DRl) as well as the posterodorsal raphe. Additionally, we found FG-ir neurons that are nonserotonergic, providing support for the observation that not all modulatory input to IC from DRN is serotonergic. Given that DRd and DRl respectively receive projections from distinct forebrain and brainstem nuclei, we can begin to establish a network model of how different DRN afferents may gate serotonin involvement in auditory processing.

Disclosures: C.L. Petersen: None. L.M. Hurley: None.

Poster

311. Auditory Processing: Circuits, Synapses, and Neurotransmitters I

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Support: College of Natural Sciences, University of Texas at Austin

Title: Role of Kv1 channels in regulating the excitability and firing patterns of neurons in the medial geniculate body

Authors: A. M. PEDERSON, A. B. KARAGOZ, D. DEAN, K. E. DEMBNY, M. DODLA, L. DUNCAN, R. FAHMY, A. KUO, V. RAMIREZ, D. B. HAIMES, *N. L. GOLDING Dept. of Neurosci., Univ. of Texas at Austin, Austin, TX

Abstract: Potassium channels play a vital role in controlling the threshold and pattern of action potential firing in neurons of the medial geniculate body of the thalamus. In other thalamic regions, previous experiments have implicated fast-inactivating Kv4 family potassium channels (so-called "A-current") in raising spike threshold and delaying the onset of action potential firing during depolarizations. However, other studies suggest that low threshold, Kv1 family potassium channels play the dominant role in shaping the timing of firing. Thus the respective roles of these channels in medial geniculate neurons remains unclear. To address this question, we performed whole cell current-clamp recordings in neurons of the medial geniculate body in the rat auditory thalamus (near-horizontal slices, 17 postnatal day animals, 24-26°C). Recordings were made in all subdivisions of the nucleus. In a fraction of recordings 1% biocytin was included in the pipette solution for subsequent morphological analysis. In response to one second long current pulses, medial geniculate neurons typically exhibited a ramping depolarization and a delayed onset of action potential firing, the magnitude of which was dependent on the amplitude of the stimulus. These delays could be sharply reduced by the injection of 500 ms pre-pulses to the cell that produced depolarizations spanning the subthreshold voltage range (max. decrease in delay from 332.16 ± 74.3 ms to 57.25 ± 7.8 ms, n=5). This delay to firing was also strongly reduced by the application of 80nM -dendrotoxin (DTX), a selective blocker of the Kv1 family of potassium channels (Ctl, 332.16 ± 74.3 ms delay; DTX, 159.76 ± 38.6 ms, n=5). While DTX did not alter the input resistance of cells significantly, DTX typically lowered action potential threshold to more hyperpolarized values and reduced spike afterhyperpolarizations. The slopes of frequencyintensity curves were steeper in DTX, with firing saturating at lower values of current injection. These results indicate that Kv1 channels play a critical role in controlling the excitability of neurons throughout the medial geniculate nucleus. Activation of Kv1 channels in responses to sound stimuli in vivo would be expected to increase both their frequency selectivity and dynamic

range, as well as enable medial geniculate neurons to encode a broader range of sound intensities.

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Poster

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Title: Modularity of intrinsic inputs within the lateral cortex of the mouse inferior colliculus

Authors: *A. M. LESICKO¹, D. LLANO²

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Abstract: The lateral cortex of the inferior colliculus contains a network of modules characterized by dense labeling for glutamic acid decarboxylase-67 (GAD-67) and other neurochemical markers. Previous studies from our laboratory have shown that the extrinsic sensory inputs to the lateral cortex are patterned: somatosensory inputs terminate within these neurochemical modules, while auditory inputs target the extramodular regions of the lateral cortex. While the topography of extrinsic inputs to the lateral cortex is well defined, the degree and directionality of intrinsic connectivity between neurons in this region remains unknown. In the present study, we sought to characterize the intrinsic inputs to neurons in the lateral cortex of the mouse inferior colliculus and determine if these connections also exhibit modularity. Experiments were performed in brain slices from the GAD-67-GFP knock-in mouse, in which modular and extramodular areas of the lateral cortex can be clearly distinguished. GAD-67positive and GAD-67-negative cells in both regions were recorded from in either a single or dual-channel whole-cell voltage clamp configuration while potential pre-synaptic sites throughout the ipsilateral colliculus were stimulated using laser photostimulation of caged glutamate. Preliminary results include a heterogeneous set of input maps. Presynaptic stimulation generated both excitatory and inhibitory responses in cells within the lateral cortex; excitation resulted predominately from direct stimulation of the recorded cell, while strong synaptic inhibition was seen for the majority of cells. Neurons within modular regions of the lateral cortex received inputs primarily from presynaptic sites within a module, while extramodular cells were predominately activated from sites outside of the modules. These preliminary data indicate that

the intrinsic connections within the lateral cortex may also exhibit modularity that is predicted by the underlying neurochemical modularity within this structure. Potential interconnectivity between modular and extramodular regions of the lateral cortex could have important implications for processing of multisensory information in the lateral cortex.

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Poster

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Title: A Cre mouse line labeling stellate cells of the ventral cochlear nucleus

Authors: *G. E. ROMERO¹, L. O. TRUSSELL²

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Abstract: T-Stellate (planar multipolar) cells of the ventral cochlear nucleus (VCN) are excitatory neurons that receive frequency specific input from auditory nerve and whose axons have wide ranging targets, including local collaterals, superior olive, dorsal cochlear nucleus and inferior colliculus. We have been interested in identifying mouse lines that mark t-stellate cells in order to perform cell specific anatomical, electrophysiological, and optogenetic studies. Here we report an analysis of labeling of t-stellate cells in a somatostatin-cre mouse line. This line was crossed to an Ai9 tdTomato reporter to generate mice with tdTomato in cre positive cells. In histological sections, labeled cells were observed throughout the VCN, with only sparse somata found in the dorsal cochlear nucleus (DCN). This line was then crossed to a GlyT2-GFP BAC transgenic line to double label for cre and glycinergic neurons. In the VCN, most cells were not double labeled, suggesting that the majority of cre+ cells were excitatory neurons. A few large, double-labeled cells were observed, consistent with labeling of D-stellate neurons, a large inhibitory projection cell. In the medial nucleus of the trapezoid body (MNTB), an occasional calyx of Held was apparent in each section, suggesting that relatively few globular bushy cells label. Thus, the majority of tdTomato labeled VCN cells are neither bushy cells nor inhibitory. To probe their identity further, we conducted patch clamp recordings on labeled cells in brain slices to assess their intrinsic firing properties, and related those results to known properties of different VCN cell classes. We found that 81% (71 of 88) of tdTomato+ cells exhibited repetitive firing in response to steady inward current steps. Peristimulus time histograms of spike firing in these repetitively firing cells closely resembled the chopping profile of t-stellate cells recorded in vivo in a variety of species. Dye in injections into cells generally revealed a dendritic layout

consistent with the t-stellate cell's narrow band acoustic input and morphology. Finally, dense concentrations of labeled boutons were present both in the superior olivary nuclei and in the DCN, two known targets of t-stellate projections. Additional observations in this study are that an apparently random subset of MNTB neurons are also cre positive, as are a large set of neurons throughout the inferior olive. We propose that this line may be of use in virally driven tract tracing and functional optogenetic studies of auditory circuitry.

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Poster

311. Auditory Processing: Circuits, Synapses, and Neurotransmitters I

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Title: Neuronal organization in the inferior colliculus revisited with cell-type dependent monosynaptic tracing

Authors: *C. CHEN¹, M. CHENG¹, T. ITO², S. SONG¹ ¹Tsinghua Univ., Beijing City, China; ²Kanazawa Med. Univ., Kanazawa, Japan

Abstract: The inferior colliculus (IC) is viewed as the first integration center in auditory pathway. It is generally accepted that core IC receives ascending inputs, while shell IC receives descending and core inputs, and inputs are combined to innervate groups of neurons. Moreover, IC is composed of excitatory and inhibitory projection neurons.

Here, we utilized the monosynaptic rabies tracing technique, combined with excitatory and inhibitory Cre driver mouse lines to characterize the brain-wide inputs to specified IC neuron types within specific subdivisions. Furthermore, with the *in situ* hybridization method, we clarified the cell type of commissural inputs on specific cell types.

First, we uncovered two long-range disinhibitory connections in core and shell IC. The core inhibitory neurons received more inhibitory inputs from ventral nucleus of lateral lemniscus than excitatory neurons, suggesting this inhibitory feedforward tectothalamic circuit could be effectively inhibited. In addition, shell inhibitory neurons received more ascending ventral cochlear nucleus inputs than excitatory neurons. Furthermore, inhibitory neurons preferentially received inhibitory inputs from contralateral shell IC. We suppose that the candidate neurons for multisensory integration of shell IC are subsets of GABAergic neurons.

Second, with covariance analysis of input nuclei, we found that core excitatory neurons received

modular ascending inputs, while shell excitatory neurons received the other modular inputs, which was composed of descending, neuromodulatory nuclei and contralateral inputs. On the other hand, inhibitory neurons received mixed inputs. The differential pattern of inputs suggests excitatory and inhibitory neurons play different roles in auditory pathway. Lastly, we revealed that shell excitatory and inhibitory neurons both received dominant ascending inputs, rather than descending or core inputs. This is consistent with anterograde tracing studies which have clearly shown that the deep layer of shell is innervated by numerous ascending fibers. Similar with the excitatory neurons between core and shell, within shell, we found excitatory neurons which received modular ascending inputs distinct from those received other inputs. Together, we suggest that tripartite pathways may exist within IC. Primary lemniscal pathway locates within core, secondary nonlemniscal pathway locates within shell, and third polysensory pathway locates within shell deep layer.

This is the first systematic and cell-type defined study on the IC, and lays the foundation for further physiological and behavioral experiments.

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Poster

311. Auditory Processing: Circuits, Synapses, and Neurotransmitters I

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Title: OHC glutamate signaling: OHC VGLUTS and cochlear nucleus neurons activated by Type-II afferents in response to sound

Authors: *C. J. WEISZ¹, C. S. ECKARD², C. B. DIVITO³, K. N. FANTETTI⁴, S. A. DETTWYLER², M. E. RUBIO², K. KANDLER⁵, R. P. SEAL³ ¹Natl. Inst. on Deafness and Other Communication Disorders, NIH, Bethesda, MD; ³Neurobio., ²Univ. of Pittsburgh, Pittsburgh, PA; ⁴Cook MyoSite, Inc., Pittsburgh, PA; ⁵Dept. Otolaryngology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA

Abstract: Outer hair cells (OHCs) are critical for normal hearing and work through an electromotile mechanism, which amplifies sound and sharpens the frequency tuning curve. However OHCs also form synapses with centrally projecting type II afferents and release vesicular glutamate. Unlike inner hair cells (IHCs), which clearly show the presence of VGLUT3 by immunostaining, the VGLUT(s) used by outer hair cells have been more difficult to discern. Here we used a number of approaches including RT-PCR, distribution of floxed reporter proteins in VGLUT-Cre expressing mice and patch clamp recordings of OHC-type II afferent synapses in VGLUT knockout mice to demonstrate that VGLUT3 is the predominate isoform in the OHCs, but that VGLUT2 is also expressed at least at early postnatal ages. In addition, we examined the distribution of cochlear nucleus neurons activated as a consequence of specifically inducing OHCs to signal in response to sound. For this study, we took advantage of a Cre mouse line that expresses the recombinase in only IHCs and not OHCs and crossed it to our conditional VGLUT3 knockout mice to eliminate VGLUT3 from inner and not outer hair cells. After exposure of the mice to a 115 dB SPL broadband noise, we assessed the distribution of c-Fos, a marker of neuronal activity, within the cochlear nucleus. Post-hoc analyses of the cochleae demonstrate no loss of hair cells or cilia. The study shows the functional recruitment of neurons specifically within the granule cell layer of the cochlear nucleus. Data presented here provide important new information about OHC-type II afferent glutamate signaling that will aid in determining the role of this signaling pathway in auditory function.

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Title: A layer-specific corticofugal input to the mouse superior colliculus

Authors: *H. ZURITA¹, C. ROCK², J. PERKINS³, A. APICELLA¹ ¹Univ. of Texas At San Antonio, San Antonio, TX; ²Biol., ³Univ. of Texas at San Antonio, San Antonio, TX

Abstract: In the auditory cortex (AC), corticofugal projections arise from each level of the auditory system and are considered to provide feedback "loops" important to modulate the flow of ascending information. It is well established that the cortex can influence the response of neurons in the superior colliculus (SC) via descending corticofugal projections. However, little is known about the relative contribution of different pyramidal neurons to these projections in the SC. We addressed this question by taking advantage of anterograde, retrograde neuronal tracing, and optogenetics to directly examine the laminar distribution, long-range projections, electrophysiological properties, and thalamic synaptic input of pyramidal neurons projecting from the AC to the SC of the mouse brain. Here we show that layer 5 cortico-superior-collicular pyramidal neurons act as bandpass filters, resonating with a broad peak at \sim 3 Hz, whereas layer 6 neurons act as low-pass filters. The dissimilar subthreshold properties of layer 5 and layer 6 cortico-superior-collicular pyramidal neurons can be described by differences in the hyperpolarization-activated cyclic nucleotide gated cation h-current (Ih). Ih also reduced the summation of short trains of artificial excitatory postsynaptic potentials injected at the soma of layer 5, but not layer 6, cortico-superior-collicular pyramidal neurons, indicating a differential dampening effect of Ih on these neurons.

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Poster

312. Visual Cortical Streams: Mouse and Primate

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Support: NIH Grant EY022428

Title: Laminar differences in responses to naturalistic texture in macaque V1 and V2

Authors: *R. K. PEREZ, J. PAI, C. M. ZIEMBA, L. E. HALLUM, C. SHOONER, J. G. KELLY, J. A. MOVSHON Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: We recently reported that neurons in V1 and V2 of visual cortex are differentially selective to the naturally occurring higher-order statistics of visual textures. Most single units recorded from macaque V2 respond with higher firing rates to synthetic texture images containing "naturalistic" higher-order statistics than to spectrally-matched "noise" images lacking these statistics. In contrast, few single units recorded from macaque V1 show this property. To better understand the possible origins of sensitivity to the statistics of naturalistic textures, we explored how the strength and dynamics of response vary across the different layers of visual cortex.

We recorded neuronal activity in V1 and V2 of four anesthetized macaque monkeys using a laminar probe with 32 contacts spaced 0.1 mm apart. We briefly presented samples from a large set of synthetic images matched to the statistics of 32 different ancestral photographic images. The stimulus set included noise images, matched only in their power spectra, as well as naturalistic textures, matched for a rich set of higher-order statistics. The areal and laminar location of recording contacts was estimated from analysis of current source density and histology.

We measured multi-unit and gamma-band activity in response to naturalistic textures and spectrally-matched control images. We defined the sensitivity to the difference in response at each contact and time point in units of d'. As expected, recordings in V2 showed consistently stronger responses to naturalistic texture, and correspondingly robust values of d'. In contrast to earlier single unit work, V1 multi-unit activity showed some sensitivity to texture statistics, and gamma-band activity showed even higher sensitivity, comparable in magnitude to V2. Sensitivity to naturalistic texture in V1 was evident in the upper and lower layers, but weak in middle layers, suggesting that it might result from feedback from V2. To test this, we measured multi-unit visual response latency, and the time course with which reliable multi-unit sensitivity for the naturalistic images emerged. Visual responses were first evident in the middle layers of V1, then propagated to upper and lower layers of V1, and then emerged in V2. Texture sensitivity emerged 10-30 ms later, first in the upper layers of V2, then in the remainder of V2, and finally in V1.

Our results demonstrate laminar differences in the encoding of higher-order statistics of natural texture in both V1 and V2. Response dynamics suggest that sensitivity first arises in V2 and is fed back to weakly modulate the firing of individual V1 neurons.

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Poster

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Topic: D.07. Vision

Title: Organization of binocular signals in macaque V1 and V2

Authors: *L. E. HALLUM, C. SHOONER, J. G. KELLY, R. RAGHAVAN, M. J. HAWKEN, J. A. MOVSHON Ctr. for Neural Science, New York Univ., New York, NY

Abstract: The cortical organization of binocular signals is only partly understood. We recorded multi-unit signals using a 32-contact linear probe with 0.1 mm contact spacing implanted normal

to the opercular surface in V1 and V2 of two anesthetized, paralyzed, visually normal macaques. We presented large sinusoidal grating patches of optimal orientation and spatial frequency to each eye. At 100 ms intervals, we independently randomized patch contrast (0, 50, 100%) and spatial phase (0, 45, ... 315 deg). Using responses to 100%-contrast stimuli, we quantified ocular dominance on a seven-point scale. We measured relative phase-selectivity by plotting response against interocular phase difference and calculating the modulation ratio (F1/F0). To capture linear responses to modulations around mean contrast, we pooled over stimulus phase and computed the spike-triggered average (STA) of the stimulus appearing in each eye (0, 50 and 100% contrasts were represented by regressor values -1, 0 and 1, respectively). We also computed a spike-triggered average of the product of the regressors representing the stimuli in the two eyes, capturing nonlinear interactions of eye signals. We reconstructed the recording locations histologically. We recorded from 260 V1 sites and 89 V2 sites. Approximately 75% of V1 sites were binocular and 95% of V2 sites were binocular. Comparing binocular sites, V2 was more selective for relative phase than V1. At binocularly activated sites in V1, STAs revealed that responses to contralateral eye stimulation peaked, on average, 8 ms earlier than those of the ipsilateral eye, whereas in binocular V2 we found no such latency difference. At approximately 15% of sites in V2, we observed two response types not seen in V1: stimulation of one eye suppressed cortical responses to stimulation of the other eye; and the nonlinear response to interactions between signals from the two eyes was comparable in magnitude to the linear response to contrast. The diverse patterns of response we have observed presumably reflect processes that support specific binocular functions, including stereopsis and interocular suppression.

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Poster

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Title: Curvature sensitivity of V2 neurons in amblyopic monkeys

Authors: *Y. WANG¹, B. ZHANG², X. TAO³, G. SHEN³, J. M. WENSVEEN¹, I. OHZAWA⁴, E. L. SMITH, III¹, Y. M. CHINO¹

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Abstract: Experiencing binocular imbalance early in life leads to binocular vision disorders and often amblyopia. Amblyopic humans show the reduced capacity for judging the relative position of a visual target in reference to nearby stimulus elements (position uncertainty) and often experience visual image distortion. Abnormal pooling of local stimulus information by neurons beyond striate cortex (V1) is often suggested as a neural basis of these deficits. The receptive field (RF) of neurons in visual area V2 in normal monkeys is made up of multiple subunits that are thought to reflect V1 inputs and are capable of encoding the spatial relationship between local stimulus features. We previously found that unlike in normal monkeys, the subunit maps of V2 neurons in amblyopic monkeys were severely disorganized: the overall difference in preferred orientations and spatial frequencies between subunits were far greater than that in normal monkeys (Tao et al, 2013). This discovery suggests that the ability of V2 neurons to encode the degree and direction of curved contours in image sub-regions may be compromised in amblyopic monkeys. In this study we investigated the sensitivity of V2 neurons to curvature (degree and direction) in anesthetized amblyopic monkeys that were reared with chronic monocular defocus. We used dynamic dense noise stimuli and a novel analysis method of quantifying the neuron's selectivity to stimulus orientation and curvature (Transform Domain Reverse Correlation or TDRC)(Arai et al, 2017). The TDRC computes spike-triggered average of stimuli after transforming them into curvature domain. We found that 1) the proportion of curvature sensitive neurons driven by the amblyopic eye was slightly lower compared to that driven by the fellow eye, 2) the average signal strength and reliability (z-score) of curvature sensitive neurons driven by the amblyopic eye was significantly lower than that for neurons driven by the fellow eye, 3) although the spatial organization of curvature selectivity within RF (subunit map) was generally similar between amblyopic and fellow neurons, the curvature selectivity index for each neuron (defined as an absolute value of summed z-scores of all nonzero curvature subunits divided by the sum of all z-scores) was significantly lower for amblyopic neurons compared to that for fellow neurons, 4) there was no difference between "amblyopic neurons" and "fellow neurons" with respect to the distribution of preferred direction. These results suggest that the curvature sensitivity of V2 neurons is lower for those V2 neurons driven by the amblyopic eye compared to that for the fellow eye.

Disclosures: Y. Wang: A. Employment/Salary (full or part-time):; University of Houston. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH Research Grants EY-008128 (YC), EY-003611, CORE grant EY-007551. B. Zhang: A. Employment/Salary (full or part-time):; Nova Southeastern University College of Optometry. X. Tao: None. G. Shen: None. J.M. Wensveen: A. Employment/Salary (full or part-time):; University of Houston. I. Ohzawa: A. Employment/Salary (full or part-time):; Osaka University. E.L. Smith: A. Employment/Salary

(full or part-time):; University of Houston. **Y.M. Chino:** A. Employment/Salary (full or part-time):; University of Houston.

Poster

312. Visual Cortical Streams: Mouse and Primate

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Title: Spiking noise in V2 neurons of infant monkeys

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Abstract: We previously reported that spiking of neurons in visual area V2 of amblyopic monkeys is noisy; the square of the coefficient of variation in inter-spike intervals (CV^2) and the trial-to-trial fluctuation in spiking (m-FF) are abnormally elevated in monkeys reared with monocular defocus (Wang et al, 2017). Moreover the noisiness of spiking was stimulus contrast dependent and correlated with the level of binocular suppression measured for the same V2 neurons. These results suggest that interocular decorrelations of cortical inputs by experiencing early monocular chronic defocus disrupted the normal maturation of cortical circuitry in V2. However, to better understand how abnormal spiking noises emerge in V2 of amblyopic monkeys, it is necessary to know how noisy the spiking of V2 neurons is in normal infant monkeys around the time when binocular imbalance was introduced in our previous study (3-4 weeks of age). In this study, therefore, we analyzed the spike trains of V2 neurons in 4- and 8week-old 'normal' infant monkeys. More specifically, we quantified the spiking noise in response to brief (640 ms) sine wave gratings that were optimized for orientation and spatial frequency for each neuron and varied for contrast between 0% and 80%. We calculated CV² and mean matched Fano Factor (m-FF) for stimulus contrasts 0%, 10%, 25%, 50% and 80%. We found that both the magnitude of spiking irregularity (CV²) and the trial-to-trial fluctuation (m-FF) was much *lower* in spike trains of V2 neurons in infant monkeys than those in adult monkeys for all stimulus contrasts. Our findings suggest that the enhanced noise in spiking of

amblyopic V2 neurons in our previous study resulted from 'active' disruptions of developing cortical connections due to abnormal visual experience instead of passively maintaining ('freezing') the immature state of cortical connections around 3-4 weeks of age.

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Poster

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Title: Three organizing principles for feature selectivity in V2

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Abstract: Object recognition relies on a series of transformations among which only the first cortical stage is relatively well understood. Already at the second stage, the visual area V2, the complexity of the transformation precludes a clear understanding of what specifically this area computes. Previous work has found multiple types of V2 neurons, with neurons of each type selective for multi-edge features. Here we analyze responses V2 neurons to natural stimuli using a quadratic convolutional model along with linear and non-convolutional alternatives. We found that the quadratic convolutional model outperformed the predictive power of linear model on a reserved dataset by a factor of 3.9. The quadratic convolutional models had an average of 7.6 excitatory and 5.8 suppressive features, with the number of each strongly correlated.Further analysis of the models shows three organizing principles. First, the relevant edges for V2 neurons can be grouped into quadrature pairs, indicating invariance to local translation. Second, the

excitatory edges have nearby suppressive edges with orthogonal orientations. Third, the resulting multi-edge patterns are repeated in space to form textures or texture boundaries. The cross-orientation suppression increases the sparseness of responses to natural images based on these complex forms of feature selectivity while allowing for multiple scales of position invariance. Finally, we could divide the neurons into two subpopulations based on the spread of the orientations that excited the neurons.

Disclosures: R. Rowekamp: None. T.O. Sharpee: None.

Poster

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EY019684

Title: A deep convolutional energy model of ventral stream areas V1, V2 and V4

Authors: *M. D. OLIVER¹, J. L. GALLANT²

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Abstract: The ventral stream areas V1, V2 and V4 are crucial for visual object recognition. While V1 responses have been extensively and successfully modeled using a variety of approaches, there has been much less success building general models of areas V2 and V4. In order to build models that can generalize to any stimulus set, we recorded from neurons while awake animals viewed clips of large, full color natural movies. The chronic recordings were stable enough that neurons could often be held for several days. This allowed us to collect responses to hundreds of thousands (up to over 1 million) distinct movie frames, for hundreds of different V1, V2 and V4 neurons. This large volume of data allowed us to fit a highly flexible, yet biologically inspired and constrained model to the responses of each neuron. The model incorporates insights from the Adelson-Bergen energy model, the scattering transform and deep convolutional neural networks. We call this the deep convolutional energy model. A two-stage version of the model is used to model V1 and V2. To model V4, the first stage of the model is simply repeated to create a three-stage model. In addition, for the V4 model stimuli are preprocessed with a log-polar transform to account for the fact that the large receptive fields in V4 are warped by V1's cortical magnification of the visual field. These models are simple and interpretable, and predict V1, V2 and V4 responses significantly better than previous models. In particular, deep convolutional energy models fit to V1 and V2 neurons stimulated with natural

movies approach the noise-ceiling of prediction performance. For the first time, a model can predict most of the variance in most of the recorded neurons in V1 and V2. In V4, the model can not only predict responses to natural movies better than previous models but can also accurately predict V4 responses to various types of synthetic curvature stimuli similar to those used in previous studies of V4. Furthermore, the models can also be used to interpret the response properties of each neuron by generating optimal excitatory and inhibitory stimuli. This allows the direct visualization of usually invisible features such as the inhibitory surround. The deep convolutional energy model thus presents a unified framework for modeling and understanding the ventral stream.

Disclosures: M.D. Oliver: A. Employment/Salary (full or part-time):; University of California Berkeley. J.L. Gallant: A. Employment/Salary (full or part-time):; University of California Berkeley.

Poster

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Title: Different cell types project from L4B of V1 to V2 in macaque monkey

Authors: *J. T. YARCH¹, H. LARSEN², M. CHEN², M. FIEDEL², A. ANGELUCCI³ ¹Moran Eye Inst., ²Ophthalmol, Moran Eye Inst., Univ. of Utah, Salt Lake City, UT; ³Ophthalmol, Moran Eye Inst., Univ. of Utah, Salt Lake Cty, UT

Abstract: Layer (L) 4B of the macaque primary visual cortex includes two morphological types of excitatory projection neurons: pyramidal cells, which receive functional inputs from both magno- (M) and parvocellular (P) divisions of L4C, and spiny stellate cells, which receive only

M inputs from L4Ca. Previous studies found that area MT receives 80% of its L4B inputs from spiny stellates, while area V2 receives 80% of its L4B inputs from pyramids (Nassi & Callaway, 2007). As V2 consists of distinct cytochrome oxidase stripes, which receive segregated projections from V1 L4B, we asked whether projections to distinct stripes arise from distinct L4B cell types. L4B neurons were labeled by injecting a GFP-expressing G-deleted rabies virus into either the thick or thin stripes of V1 (n= 6 injections in 5 animals), identified in vivo by optical imaging, and the somata and dendrites of 63 cells (42 thick-projecting and 21 thinprojecting) were fully reconstructed. We found a larger amount of spiny stellate input from L4B to both thick and thin stripes than in previous studies, with greater stellate input to thick (55% of total) than to thin (40% of total) stripes. This finding suggests a greater M contribution from V1 to V2 than previously thought and, therefore, to both the dorsal and ventral visual streams, to which thick and thin stripes project, respectively. We also measured several morphological properties for these cells: soma area, soma perimeter, total dendritic length, dendritic perimeter, dendritic area, and dendritic complexity. Unbiased cluster and PCA analyses, using these metrics, revealed that both spiny stellate and pyramidal cells separate into 3 different groups (small, medium, large). Only the largest stellates showed stripe-type specificity, projecting only to thick stripes, and resembling those previously reported to project only to area MT. These large stellates constitute only 7% of all the stellate cells we characterized. The other cell groups projected equally to thin or thick stripes. Our results indicate that L4B stellate and pyramidal cells projecting to V2 each consist of 3 different types differing in size and dendritic complexity, but not in their targeted V2 stripe, except for the giant stellates that project only to thick stripes. Neurons with different dendritic field sizes and complexity suggest different abilities for visuospatial integration, and may correlate with the different known functions of L4B neurons including orientation, direction, and disparity tuning.

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Poster

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Title: Kv3.1b expression in putative excitatory subpopulations in macaque V2: Quantitative laminar investigation of Kv3.1b, PV, and GABA co-immunoreactivity

Authors: *J. G. KELLY, M. J. HAWKEN

Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: The Kv3.1b potassium channel subunit, which facilitates the fast-spiking phenotype characteristic of parvalbumin (PV)-expressing inhibitory interneurons, is also expressed by a subpopulation of excitatory neurons in macaque V1 that are concentrated in layers $4C\alpha$ and 4B. It was unknown whether this pattern was specific to V1 and/or to the magnocellular (M) pathway. We examined whether there were also Kv3.1b⁺ excitatory neurons in area V2 and quantified their laminar distribution. Parasagittal sections from dorsal V2 of three macaque monkeys were immunofluorescence labeled for Kv3.1b, PV, and GABA and examined using confocal microscopy and an automated image analysis-based counting method. Densities of neurons of each type were measured across cortical depth and compared with overall neuronal densities measured previously. Results were compared to our previous observations from V1. The GABAergic population comprised 13% of all neurons, lower than previous estimates. Across layers 2-6 the proportion of GABAergic neurons ranged from 10-13%. In V2, Kv3.1b and PV were frequently co-expressed: 80% of Kv3.1b⁺ neurons and 88% of PV⁺ neurons expressed both Kv3.1b and PV. In a previous study in V1 we found that Kv3.1b⁺ neurons expressing either GABA or PV almost always co-expressed the other substance. In V2, this was often, but not always, the case: 87% of Kv3.1b⁺, PV⁺ neurons were also GABA⁺, and 91% of Kv3.1b⁺, GABA⁺ neurons were PV⁺. Kv3.1b⁺, GABA⁺, PV⁻ neurons were most frequently observed in layers 2 and 3.

Kv3.1b⁺, GABA⁻, PV⁻ neurons were present in dorsal V2 and were largely restricted to layer 3B, comprising 4% of the total neuronal population and 34% of all Kv3.1b⁺ neurons in this layer. Many of these neurons could be identified as pyramidal neurons. Therefore, there is a population of putative excitatory neurons in layer 3B that are likely to show relatively narrow spike widths and that may be capable of high firing rates, the signature of Kv3.1b⁺ excitatory subpopulations are not specific to area V1 and may be common across primate cortical areas.

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Poster

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Title: Properties of layer 1 inputs to layer 1 neurons of the medial secondary visual cortex in mice

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Abstract: Layer 1 of the neocortex consists of loosely packed GABAergic neurons scattered among apical tufts of pyramidal neurons and horizontally oriented axons. These axons derive from the "matrix" thalamus and other cortical areas. Last year we reported on local inputs to these layer 1 cells from lower-layers in slice preparations from the medial secondary visual cortex of the mouse brain. Here we study the responses of these cells to stimulation of their afferents at a horizontal distance within layer 1 in coronal slice preparations of the medial secondary visual cortex.

Stimulation was achieved via a concentric bipolar electrode placed in layer 1 100 to 500 µm lateral to the recording site. Such stimulation evoked biphasic responses in voltage-clamp mode. When the excitatory currents were isolated by including the GABAergic antagonist, 4,4'-Dinitro-stilbene-2,2'-disulfonic acid (DNDS), in the intracellular solution, these currents exhibited paired-pulse depression, and they were blocked by application of iGluR blockers (DNQX & AP5); further high frequency stimulation failed to evoke mGluR responses in two experiments, suggesting that these glutamatergic inputs, whether from thalamic or cortical origin, have "driver" properties. In other experiments, inhibitory currents remained in the presence of iGluR antagonists and showed pair-pulse depression; these currents were blocked by a GABAA antagonist, gabazine.

A long-lasting excitatory current was revealed after both glutamatergic and GABAergic currents were blocked. This current was partially inhibited by a serotonergic antagonist, methiothepine maleate (10 μ M), whereas muscarinic (10 μ M scopolamine) and dopaminergic (10 μ M clozapine) antagonists had no effect. More experiments will be conducted to study the composition of this current.

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Poster

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HHMI

Title: Functional relationship between primary and higher-order visual cortices

Authors: *R. BELTRAMO, M. SCANZIANI

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Abstract: The mouse visual cortex consists of a primary visual area (V1) surrounded by at least 9 retinotopically organized higher-order visual areas which are functionally tuned to specific features of the visual scene. Unraveling how information flows between primary and higher cortical areas is a crucial step in understanding the mechanisms of visual perception. V1 receives retinal inputs through the dorsal lateral geniculate nucleus of the thalamus, and heavily projects to higher order visual areas. However, despite the dense projections, the extent to which V1 actually *drives* the activity of higher-order cortical areas is a matter of debate. To address this issue, we optogenetically silenced the primary visual cortex of awake mice and simultaneously recorded, with multichannel electrodes, the visual areas was heavily affected by V1 silencing, the activity of others was minimally influenced. These results show that the neural activity of higher-order visual cortices differentially relies on V1 inputs. Characterizing the source of V1-independent visual information that reaches higher cortices will expand our knowledge on potential V1-bypassing pathways in the mouse visual system.

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Poster

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Topic: D.07. Vision

Title: The transformation of visual information through the mouse visual cortex

Authors: *S. E. DEVRIES¹, M. A. BUICE¹, J. LECOQ², G. K. OCKER³, D. MILLMAN³, N. H. CAIN¹, D. FENG¹, R. REID⁴ ¹Allen Inst. For Brain Sci., Seattle, WA; ²Structured Sci., Allen Inst., Seattle, WA; ⁴Neural Coding, ³Allen Inst. for Brain Sci., Seattle, WA

Abstract: A prominent question of sensory processing is how information is transformed by the neural circuit in order to create perceptions and ultimately guide behavior. Physiological recordings from different stages in the circuit can shed light on these transformations, but often suffer from using different stimulus sets or experimental paradigms. The Allen Brain Observatory is a public dataset of neural activity in the mouse visual cortex created by the Allen

Institute for Brain Science. Using high throughput 2-photon calcium imaging of transgenically expressed GCaMP6f under the control of specific Cre drivers, we have captured the neural activity of specific neuron populations in response to a standard set of visual stimuli, including gratings, noise stimuli, natural images and movies. To date, we have released over 200 datasets collected from neurons in six cortical visual areas using six excitatory Cre lines spanning from layer 2/3 to layer 5. Neurons are individually characterized by their spatial receptive field structure, their temporal dynamics, and their orientation, spatial and temporal frequency tuning. Further, responses to natural images and movies serve to compare the spatial and temporal response properties to activity in a more naturalistic context. Using this dataset, we examine single cell responses and population coding to explore how the representation of visual information changes through the mouse cortical circuit. We observe that many response features remain largely unchanged through the circuit, while others show marked changes. For example, while the orientation selectivity of neurons measured in response to grating stimuli is largely the consistent across distinct visual areas and layers, direction selective responses are more prominent in V1 than in the higher visual areas. Similarly, population decoding of natural scenes performs better in V1 than in higher visual areas. We will explore these differences in visual representation throughout the mouse visual cortex, and consider the implications this has on prevailing models of cortical computation in the visual cortex.

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Poster

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Title: Large-scale imaging and functional parcellation of mouse visual cortex

Authors: ***M.** HU¹, R. V. RIKHYE², A. RAMANUJAN³, M. G. M. KUMAR³, H. SUTHAR³, M. J. GOARD⁴, M. HEMA³, M. SUR¹

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Abstract: Although it is known that mice have roughly ten distinct visual areas, the specific function of each area and interactions between areas remain poorly understood. In this study, we used wide-field imaging from awake, head-fixed mice, which transgenically expressed GCaMP6f, to segment the entire visual cortex into functionally different areas and investigate each areas' responses to a battery of visual stimuli, including simple drifting gratings (varying orientation, spatial frequency and temporal frequency), random moving dots and natural movies. Despite the well-known "salt-and-pepper" distribution of orientation preference at a microscopic scale in mouse V1, we found a retinotopically dependent mesoscale bias of averaged orientation preference and spatial and temporal frequency preferences, in response to drifting gratings. In addition, we found higher orientation selectivity in the binocular region. Representation of the amplitude and phase of natural movies were biased towards the binocular zone and monocular zone respectively. In addition to these conventional input-output characterizations, we also applied various machine learning techniques on this dataset to explore its intrinsic structure. Specifically, we used supervised Gaussian Mixture Models (GMM) and unsupervised agglomerative clustering of Maximum a Posteriori (MAP) model to estimate area borders. The generated models in both cases predicted area borders consistent with that of the physiologically identified retinotopic map, suggesting that each area had consistent and correlated responses to visual stimuli. In another experiment, we observed that spatial temporal change points in the visual stimuli correlated well with the change points in the cortical responses. Altogether, by integrating tools developed from mouse genetics, large-scale imaging and modern statistical analysis, we obtained novel insights into the functional organization of mouse visual cortex. These hypotheses are being verified with further experiments.

Disclosures: M. Hu: None. R.V. Rikhye: None. A. Ramanujan: None. M.G.M. Kumar: None. H. Suthar: None. M.J. Goard: None. M. Hema: None. M. Sur: None.

Poster

312. Visual Cortical Streams: Mouse and Primate

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 312.13/DD32

Topic: D.07. Vision

Support: NIH Grant R01 EY016184

NIH Grant R01 EY022090

Title: A hierarchical organization of mouse visual cortical areas

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Abstract: The mammalian visual cortex is a mosaic of multiple, distinct areas that communicate with each other through the axons of pyramidal neurons. Previous studies in monkeys (Maunsell and Van Essen, 1983; Felleman and Van Essen, 1991) and rats (Coogan and Burkhalter, 1993) have shown that the laminar termination patterns in the target area formed by these axons depend on the hierarchical organization of the connecting areas. The interareal pathways have been classified as being feedforward if they transmit signals from lower to higher areas of the cortical hierarchy, feedback if they send descending inputs from higher to lower areas, or lateral if they belong to the same hierarchical level. Due to the tractability of the mouse model in examining cortical circuit physiology and function, constructing the hierarchy of mouse visual cortex is a crucial step in understanding how sensory processing is achieved across a distributed neocortical system. To do this, we first injected the anterograde tracer, biotinylated dextran amide, into each of ten previously identified visual areas, and examined the axonal termination patterns in each of the other nine. Areas were identified by the locations of the axonal projections relative to retrogradely-labeled callosal landmarks, and by their locations relative to each other. Feedforward projections from primary visual cortex (V1) terminated strongest in layers 2 to 4 (L2-4) while selectively avoiding layer 1 (L1) in each of the target areas. On the other hand, feedback projections from each of the higher areas to V1 showed a strong preference for terminating in L1 and only weakly targeting L2-4. We therefore reasoned that the ratio of the density of axonal afferents in L2-4 to that in L1 ('density ratio', DR) would provide for a quantitative measure of hierarchical distance between two connecting areas. We generated a 10x10 connectivity matrix of 80 interareal pathways, which included 37 bidirectional areal pairs. A beta regression analysis of all 80 DRs showed that the ten areas occupy seven hierarchical levels, with areas POR (postrhinal area) and AM (anteromedial area) at the highest level of the hierarchy. We further separated the areas depending on whether they belong to the dorsal or ventral stream (Wang et al., 2012), and propose a hierarchy for each stream. Additionally, electrophysiological mapping of receptive fields of neurons in each area showed that receptive field sizes increase with increasing hierarchical position, indicating that the structural organization of cortical hierarchy is consistent with increasing convergence of feedforward inputs.

Disclosures: R. D'Souza: None. Q. Wang: None. W. Ji: None. A. Meier: None. H. Kennedy: None. K. Knoblauch: None. A. Burkhalter: None.

Poster

312. Visual Cortical Streams: Mouse and Primate

Location: Halls A-C

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Topic: D.07. Vision

Support: CSC Scholarship

Title: Area specialization of feature selectivity in the mouse visual cortex

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Abstract: The mouse visual cortex is composed of primary sensory area (V1) and multiple secondary visual areas that receive distinct inputs from V1. So far mouse visual cortical responses were probed mainly with drifting grating stimuli. While all visual areas show highly selective responses for orientation, selectivity for spatiotemporal frequency differs widely across visual areas, indicative of functional specialization. There is little data on how these properties impact responses to more complex visual stimuli.We used cellular imaging and visual noise stimuli to probe the responses of neurons in layer 2/3 of V1 and four higher visual areas (LM, AL, AM and PM) of awake mice. To measure cellular activity, we used Thy1-GCaMP (GP4.12) mice, which express the calcium indicator GCaMP6s in a subset of cortical excitatory neurons. We stimulated neurons with spectrally-controlled noise stimuli with different peak spatiotemporal frequencies and peak orientations. To activate a broad population, we used noise stimuli of variable orientation power, ranging from isotropic (non-oriented) to anisotropic (oriented).Consistent with previous studies, around 70 percent of spatiotemporally-tuned cells showed responses to oriented stimuli. Two-thirds of these cells also responded to isotropic stimuli. Surprisingly, over a quarter of tuned cells were driven by isotropic but not anisotropic stimuli, showing a strong preference for non-oriented stimuli. Neurons responding to anisotropic and isotropic stimuli showed similar spatiotemporal tuning. We used spectral clustering to compare response patterns across visual areas. Neural responses fell into broad groups that were differentially represented across areas. V1 had the largest fraction of cells tuned to low frequencies and of cells responding to non-oriented stimuli. In contrast to V1, higher visual areas were comprised of distinctly tuned groups with mixed preferences for oriented or non-oriented stimuli. LM had fewer low frequency tuned cells and were biased toward mid frequencies (0.1 cpd, 2 Hz). A good fraction of neurons in AL and AM were tuned for low spatial frequencies, high temporal frequencies (0.025 cpd, 8 Hz). PM was composed of two main groups responding to low or high spatial frequencies (0.025 and 0.32 cpd) and low temporal frequencies (1 Hz). Taken together, our results demonstrate rich feature selectivity and functional specialization in the mouse visual cortex. We are currently investigating the circuitry underlying these responses.

Disclosures: X. Han: None. B. Vermaercke: None. V. Bonin: None.

Poster

312. Visual Cortical Streams: Mouse and Primate

Location: Halls A-C

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Topic: D.07. Vision

Support: NIH Grant RO1 EY022090

NIH Grant R21 EY027946

Title: Modular organization of inputs to mouse postrhinal cortex involved in contextual visual processing

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Abstract: Postrhinal cortex (POR) is a higher visual cortical area upstream of the entorhinal cortex and hippocampus which is essential for contextual learning and affective responses gated by input from the amygdala. We found that POR shows modular organization, in which muscarinic acetylcholine receptor 2 (M2) is strongly expressed in repeating 'patches' in cortical layer 1 (L1) interdigitating with weakly M2-expressing 'interpatches.' In primary visual cortex (V1), which is also modularly organized, layer 2/3 neurons lying below patches have distinct spatiotemporal tuning properties from those lying below interpatches. Here we tested the hypothesis that inputs from areas involved in motion and affective processing, rostrolateral cortex (RL) and lateral amygdala (LA), target different modules in POR than the dLGN and lateral posterior nucleus (LP) which are involved in bottom-up and attention-related processing. We first used retinotopic mapping of projections from V1 to POR to determine the location and borders of POR relative to M2 expression. Using transgenic mice expressing M2 tagged with tdTomato (ChM2-tdT), we found that POR lies within a region of higher M2 expression surrounded by areas P, ECT, PORa, LM, and LI. Next, we examined the projection pattern of inputs from LA, dLGN, LP, and RL to L1 of POR. LA receives cortical projections driving aversive and reward-related behaviors. dLGN provides retinotopically precise bottom-up input to L1 of V1. LP, a homologue of the pulvinar, is a higher thalamic nucleus carrying contextual information extracted from self-motion and optic flow. RL is an extrastriate area specialized for processing motion and is a possible homologue of primate MT. We injected adeno-associated virus expressing EGFP, an anterograde tracer, into these four areas to determine the projections to L1 of POR. After injections, mice were perfused with fixative and the cortex was removed and flattened. Cortices were cleared using the ScaleS protocol, then EGFP-labeled projections and ChM2-tdT expression were imaged with confocal microscopy. We found that dLGN and LP projections are biased toward targeting patches, with dLGN projections being more patchspecific. LA and RL inputs to POR more strongly targeted POR interpatches, with LA projections showing greater interpatch specificity. These projection patterns demonstrate that the patchy organization of POR serves to cluster L1 inputs from specific sets of areas: patches receive more inputs from visual thalamic structures (dLGN and LP), while interpatches receive inputs from areas related to fear and reward (amygdala) and moving objects (RL).

Disclosures: A.M. Meier: None. Q. Wang: None. A.H. Burkhalter: None.

Poster

312. Visual Cortical Streams: Mouse and Primate

Location: Halls A-C

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Program#/Poster#: 312.16/DD35

Topic: D.07. Vision

Title: Visual cortex activation modulated by relative head direction changes is stronger in secondary areas

Authors: *M. KANG, H. YANG, Y. JEONG

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Abstract: Integrating visual information with relative head direction is essential for forming coherent representation of environment. For instance, static visual stimuli move in the opposite direction when head is rotated whereas moving visual stimuli does not. This can also be seen in virtual navigation setups where motor input and somatosensory inputs from head-to-body angle changes are coupled with changes in visual stimuli. Indeed, recent studies have reported motor activity driven modulation of visual cortex. However, little is known about which cortical regions/projections mediate the integration of somatosensory information such as changes in head-to-body angle and visual stimuli at mesoscopic level. To identify the regions activated and to characterize the dynamics of brain activity, we modulated head-to-body angle in awake, behaving Thy1-GCaMP6s transgenic mice while the cortical activity was recorded at mesoscopic level. We observed sequential activation of the cortex starting from somatosensory trunk area to secondary motor cortex, retrosplenial cortex and visual cortices. Modulation of head-to-body angle alone could drive the sequence of activity. However, the activation level was clearly higher in secondary visual cortices compared to primary visual cortex. This pattern was dependent on the depth of focus. This suggests that secondary visual cortices receive higher level of somatosensory input compared to primary visual cortex. We speculate that the selective activation of secondary visual cortices in response to head-to-body angle modulation may be mediated by the midline brain regions that send disproportionate projections to secondary visual cortices

Disclosures: M. Kang: None. H. Yang: None. Y. Jeong: None.

Poster

312. Visual Cortical Streams: Mouse and Primate

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 312.17/DP06/DD36 (Dynamic Poster)

Topic: D.07. Vision

Title: The Allen Brain Observatory: A standardized and ongoing survey of evoked neuronal activity in the mouse visual cortex

Authors: *J. LECOQ¹, J. D. LARKIN², K. ROLL², C. WHITE², F. GRIFFIN², T. NGUYEN², S. CROSS², R. VALENZA², S. CALDEJON², E. K. LEE², A. STEGER², S. E. DEVRIES², M. A. BUICE³, M. STOECKLIN², P. A. GROBLEWSKI², D. SULLIVAN², W. WAKEMAN², N. BOWLES², J. PERKINS², A. WILLIFORD², A. HO², L. KUAN², D. FENG², C. FARRELL², C. SLAUGHTERBECK², H. ZENG², C. KOCH²

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Abstract: Understanding the brain requires more than a census of its genes or a catalog of its parts--we need to watch it in action as it performs the tasks that give rise to activity and perception

In 2016, the Allen Institute for Brain Science launched the Allen Brain Observatory: the first tool of its kind to provide a highly standardized survey of cellular-level activity in the mouse visual system. With the data and tools in this resource, researchers around the world are empowered to investigate how circuits in the mouse brain process a wide range of visual stimuli.

The first data released in June 2016 in the Allen Brain Observatory surveyed four areas in the mouse visual cortex at multiple depths, sampling more than 18,000 neurons in total. The mice were presented with a large variety of visual stimuli to determine the "tuning," or preference, of each individual cell to visual features like motion and shape orientation, as well as complex images like natural scenes and movies. Two additional data releases were carried out in 2016 and 2017, extending the scope of the observatory dataset with the inclusion of new visual areas as well as new cre-lines. In addition, we also added new metrics to quantify both previously and newly released datasets.

The Allen Brain Observatory is also a foundational platform for future system neuroscience efforts both inside and outside of the Allen Institute. As such, it is essential that we carry out careful, iterative and data driven improvements to our operational, software and hardware infrastructure. Here we describe some of these improvements along with their supportive analysis. In many such instances, we leveraged our access to a large amount of data to capture biological variability. For instance, we systematically assessed the eye gaze location to position our visual stimulation screen in an optimal location or we leveraged our platform to adjust the locally sparse noise stimulation parameters for Higher Visual Areas (HVAs).

Our final goal is to provide a comprehensive functional characterization of layers, cell types and visual areas in the mouse visual cortex. This dataset will provide key landmarks to guide future research on cortical computation during behavior task.

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Poster

312. Visual Cortical Streams: Mouse and Primate

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 312.18/EE1

Topic: D.07. Vision

Title: Relationship among sensory tuning profiles across stimulus sets in mouse primary visual cortex

Authors: *D. MILLMAN, N. CAIN, G. OCKER, P. LEDOCHOWITSCH, C. REID, J. LECOQ, M. BUICE, S. DE VRIES Neural Coding Group, Allen Inst. For Brain Sci., Seattle, WA

Abstract: When characterizing the selectivity of visual sensory neurons, an exhaustive sampling of all possible stimulus features is not tractable. Instead, one or a few classes of stimuli are typically used to characterize specific aspects of a cell's selectivity, often in an attempt to efficiently sample along the presumed principal dimensions of the feature space. Significant selectivity within a stimulus class can be illustrated, for instance, through the ability to map spatial receptive fields or derive an orientation tuning curve. We investigated the relationships in the selectivity of individual neurons across multiple classes of visual stimuli with 2-photon calcium imaging in mouse primary visual cortex, including data in the Allen Brain Observatory that surveys multiple cre-lines and cortical layers. Although cells are not expected to maintain similar feature selectivity across stimulus classes with very different visual statistics (e.g. locallysparse noise versus natural scenes), cells would be expected to have similar selectivity across stimulus classes that have similar visual statistics (e.g. locally-sparse noise versus static gratings). However, we found that the presence or absence of selectivity within one stimulus class did not predict whether the same cell would exhibit selectivity within another class of stimuli, even in cases where visual statistics were similar across the stimulus classes. We explored this phenomenon in detail by comparing the responses of single neurons to locallysparse noise and static gratings in order to leverage existing knowledge about the relationship

between tuning for these two types of stimuli. Surprisingly, we found a substantial fraction of neurons that had significant receptive fields as mapped with locally-sparse noise, but lacked selectivity among the set of static gratings or failed to respond to them at all. Furthermore, we found a substantial fraction of cells that reliably responded to a particular class of stimuli overall, such as locally-sparse noise, without exhibiting selectivity among the stimuli within that class. Our results highlight important considerations for the design of sensory stimulus sets as well as the interpretation of neuronal selectivity within and across stimulus sets.

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Poster

312. Visual Cortical Streams: Mouse and Primate

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 312.19/EE2

Topic: D.07. Vision

Title: Hierarchical models of processing in the mouse visual cortex

Authors: *N. H. CAIN¹, R. IYER¹, Y. N. BILLEH², A. ARKHIPOV¹, M. A. BUICE¹, S. MIHALAS¹

¹Allen Inst. For Brain Sci., Seattle, WA; ²Allen Inst., Seattle, WA

Abstract: How do cell types and connectivity give rise to experimentally recorded responses in the mouse visual cortex? A data-driven modeling approach can help dissect the laminar and cell type circuit that gives rise to cortical activity. By combining two large-scale data resources, the Allen Mouse Brain Connectivity Atlas (http://connectivity.brain-map.org/) and the Allen Brain Observatory (http://observatory.brain-map.org/visualcoding/), we develop a framework for testing models of feedforward columnar computation.

We first construct models of neuronal visual responses in dorsal lateral geniculate nucleus (LGd) parameterized using data from electrophysiological recordings [1]. The model contains multiple cell classes modeled as separable spatio-temporal filters. Cell classes were determined on the basis of the spatial profile and temporal kinetics of responses to sparse noise and drifting grating stimuli. For each cell-class, model parameters were optimized by minimizing the sum of squares of differences between experimental and model response metrics based on these stimuli. This model can transform a wide range of visual stimuli into a putative input for primary visual cortex (VISp).

We then construct models of VISp responses; preliminary results indicate that these models can be fit to responses from the Allen Brain Observatory when the processing of the early visual system is included. A staged approach in which we characterize the set of neuronal responses in a population (area and layer) followed by populations which directly receive its inputs can then be iteratively applied to the Allen Brain Observatory. Such an approach requires a large sample of responses throughout the cortical hierarchy, such as the systematic recordings provided in the Allen Brain Observatory.

[1] Durand, S., Iyer, R., Mizuseki, K., de Vries, S., Mihalas, S., & Reid, R. C. (2016). A Comparison of Visual Response Properties in the Lateral Geniculate Nucleus and Primary Visual Cortex of Awake and Anesthetized Mice. Journal of Neuroscience, 36(48), 12144-12156. http://doi.org/10.1523/JNEUROSCI.1741-16.2016

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Poster

313. Sensorimotor Transformation: Behavior and Whole-Animal Processing

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 313.01/EE3

Topic: D.09. Visual Sensory-motor Processing

Support: CCLI-Brooklyn College

Title: Developing a virtual environment for studying spatial orientation in rotating visual environments

Authors: H. DRILLICK¹, E. DOROKHIN¹, L. GOETZ¹, *T. RAPHAN² ¹Brooklyn Col. of CUNY, Brooklyn, NY; ²Brooklyn Col. of the City Univ. of New York, Brooklyn, NY

Abstract: Vertigo and motion sickness can be generated by moving visual stimuli in the same way that head and body movement can induce vertiginous and motion sickness sensations. The same stimuli that induce motion sickness have also been shown to be effective in the habituation of motion sickness following repeated exposure to these stimuli. The purpose here is to describe the development of a virtual reality head mounted system that can be used to determine spatial orientation in the presence of a rotating visual environment. The system was developed using the Oculus Rift virtual reality display and the OpenGL graphics API to generate rotating environments in three dimensions. The system generates stripes whose width can be set by a simple user interface. The speed of rotation of the stripes and the axis about which they rotate can also be set through the user interface. The program was developed in Windows using C++ together with Oculus Rift 64-bit libraries. The program was compiled and linked using the 64-bit open source MinGW compiler. The spatial orientation estimate was determined by having subjects give a haptic representation of the spatial vertical by pointing the thumb in the direction of the spatial vertical by pointing the thumb in the direction of the spatial vertical. The Leap Motion hand sensor was used for this purpose since it is capable of determining the directions of the finger tips relative to its coordinate frame. Our initial

investigation in a single subject shows that thumb pointing is stable for at least ten seconds when the subject is asked to point towards the spatial vertical. The initial data also suggest that motions of the environment induce disorientations, especially in roll. Thus, a virtual reality display and a Leap Motion hand sensor can potentially be coordinated to study perception of the vertical in static and visual motion environments with the head in different orientations relative to gravity.

Disclosures: H. Drillick: None. E. Dorokhin: None. L. Goetz: None. T. Raphan: None.

Poster

313. Sensorimotor Transformation: Behavior and Whole-Animal Processing

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 313.02/EE4

Topic: D.09. Visual Sensory-motor Processing

Support: HHMI Janelia Research Campus

Title: Descending interneurons controlling visually-evoked escape in Drosophila

Authors: *M. Y. PEEK, S. NAMIKI, P. BREADS, W. R. WILLIAMSON, J. M. ACHE, G. M. CARD

HHMI Janelia Res. Campus, Ashburn, VA

Abstract: To understand rapid sensorimotor processing underlying a behavior essential to survival, we investigated the role of descending interneurons in the control of loom-evoked escape behavior in Drosophila. Using the an automated behavioral assay, we performed a neural activation and silencing screen across a collection of fly lines targeting specific descending interneuron cell types. A subset showed robust escape phenotypes, including seven with putatively shared visual inputs. To test whether these neurons are active in the context of loom-evoked escape, we characterized their visual tuning using in vivo somatic whole-cell patch clamp electrophysiology. Recordings show strong excitatory loom responses which suggests that these neurons serve as parallel descending pathways to downstream takeoff motor centers that coordinate escape.

Disclosures: M.Y. Peek: None. S. Namiki: None. P. Breads: None. W.R. Williamson: None. J.M. Ache: None. G.M. Card: None.
Poster

313. Sensorimotor Transformation: Behavior and Whole-Animal Processing

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 313.03/EE5

Topic: D.09. Visual Sensory-motor Processing

Support: HHMI

Title: Looming sensitivity in Drosophila visual projection neurons

Authors: *N. C. KLAPOETKE, A. NERN, M. PEEK, E. ROGERS, G. RUBIN, M. REISER, G. CARD

Janelia Res. Campus, Ashburn, VA

Abstract: Animal early visual systems process primitive information from the photoreceptor level to encode higher-level representations of ethologically relevant visual features. It is thought that looming motion should be encoded by the early visual system in order to detect imminent collision or dangers such as an approaching predator. Recently Wu et al. (2016 eLife) described more than 20 different classes of Drosophila visual projection neurons that transmit information from the early visual system to higher order brain regions. We here examined looming sensitivity in a subset of these visual projection neurons using in vivo two-photon calcium imaging. We found that different classes of visual projection neurons encode distinct spatiotemporal features of looming motion. Furthermore, we characterized visual response properties on a single cell level and found a strong correspondence between an individual neuron's anatomy and its functional properties. Our results suggest that looming motion is encoded by parallel channels in the Drosophila early visual system.

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Poster

313. Sensorimotor Transformation: Behavior and Whole-Animal Processing

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Program#/Poster#: 313.04/EE6

Topic: D.09. Visual Sensory-motor Processing

Support: HHMI

Janelia Research Campus

Title: Control of landing in Drosophila

Authors: *J. M. ACHE, S. NAMIKI, A. LEE, K. BRANSON, G. M. CARD HHMI/Janelia Res. Campus, Ashburn, VA

Abstract: Animals respond to sensory cues in a context-specific manner, an ability that is critical for survival. Even innate sensorimotor responses are flexible, such that an identical cue elicits different actions in different situations. How the brain achieves this context-dependent flexibility is unclear. Here, we describe the sensorimotor pathways controlling visually-evoked landing in *Drosophila*. Landing exemplifies a context-specific behavior because flies only initiate landing actions in response to expanding visual cues if they are flying, and the landing motor sequence is suppressed during other behaviors.

Using patch-clamp recordings, optogenetics and quantitative behavioral analysis, we identified the most important components of the landing pathways and their role in extracting visual features and controlling the landing motor sequence. We show that descending neurons mediating landing integrate visual and mechanosensory cues and control leg extensions in a graded fashion while the fly is flying. Our recordings reveal that visual responses in these descending pathways are shut down when the fly is not flying. Moreover, we found that this gating of visual input occurs via two different mechanisms in two different landing descending neuron cell types. Our findings suggest that state-dependent gating of descending pathways is one mechanism that controls the brain's access to different motor networks, thus enabling flexible, context-dependent action selection.

Disclosures: J.M. Ache: None. S. Namiki: None. A. Lee: None. K. Branson: None. G.M. Card: None.

Poster

313. Sensorimotor Transformation: Behavior and Whole-Animal Processing

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 313.05/EE7

Topic: D.09. Visual Sensory-motor Processing

Title: A cell type specific driver line library targeting wing motor circuits in the ventral nerve cord of *Drosophila*

Authors: *E. E. EHRHARDT, S. NAMIKI, H. OTSUNA, I. COHEN, D. STERN, M. DICKINSON, W. KORFF, G. M. CARD Card Lab., HHMI Janelia Res. Campus, Ashburn, VA

Abstract: A primary function of a fly's wings is to produce rapid, agile aerial maneuvers. Yet male flies also use their wings to create a precise, species-specific courtship song required for reproduction. The patterns of activity in the 12 wing steering muscles during flight and courtship must be distinct. However, how potentially overlapping neural circuits in the ventral nerve cord drive distinct patterns is unclear. In order to enable the systematic analysis of neural circuits underlying flight and courtship song, we created cell-type specific driver lines targeting cells in the wing and haltere neuropils using the split-GAL4 system. In split-GAL4 lines, two different enhancers each drive the expression of one domain of the split-GAL4 transcription factor. Only cells that express both domains generate a functional GAL4 protein and thus drive expression of the gene of interest. Well-chosen combinations of enhancers can produce very sparse lines with expression limited to one or a few specific cells. To rapidly screen existing enhancer expression patterns for potential overlap, we created a library of over 14,000 auto-brightness adjusted maximum intensity projections (MIPs) of expression patterns in Janelia Generation 1 GAL4 lines. These were registered to a common ventral nerve cord template and color-coded to indicate the position of each point on the dorsal-ventral axis. We developed a Fiji plugin which enables users to draw an area of interest around a cell or neurite, and then uses this mask to search the MIP library for GAL4 lines which including matching cells. Depending on the mask and settings used, the program can narrow down the search to a few hundred suggested matching images (1-3% of the original images), which can then be used as candidates in choosing split intersections. Using this system, we have made over 100 split-GAL4 lines targeting sensory neurons of the wing and haltere, haltere motoneurons, direct wing muscle motoneurons, indirect or power motoneurons, descending interneurons, ascending interneurons, local interneurons, bilateral interneurons, and intersegmental interneurons. We are currently using these cell-specific drivers to screen for activation and silencing effects on flight or courtship song.

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Poster

313. Sensorimotor Transformation: Behavior and Whole-Animal Processing

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Topic: D.09. Visual Sensory-motor Processing

Title: An apparatus for automated, high-throughput, and detailed assessment of individual *Drosophila* free behavior

Authors: *W. R. WILLIAMSON, M. PEEK, P. BREADS, C. BRIAN, G. M. CARD HHMI Janelia Res. Campus, Ashburn, VA

Abstract: New genetic methods in *Drosophila* make it possible to modulate the activity of single neuronal cell types. However, the behavioral consequences of such modifications are often context-specific, may occur over small temporal and spatial scales, and are sometimes incompletely penetrant. One can enhance detection of such behavioral changes by assaying more animals, by reducing noise in a given assay (for example, eliminating background behavior generated by social interactions), or by monitoring the motor patterns at higher temporal and spatial resolutions. Here we present a new, automated apparatus that simultaneously implements all three of these approaches to quickly characterize large populations of unrestrained individual flies during visual or optogenetically-induced behaviors. As a proof of principle for detailed description of behavior, we use flyPEZ to observe the sequence of optomotor behaviors in response to a rotating grating, and we characterize the direction of takeoff in response to looming disk stimuli. FlyPEZ is also equipped with red LEDs for optogenetic activation of neurons, and we show how different patterns of light stimulation affect behavior. Finally, to demonstrate flyPEZ's temporal sensitivity and ability to detect loss-of-function phenotypes, we genetically silence visual output neurons and identify a novel phenotype which occurs on a millisecond timescale.

Disclosures: W.R. Williamson: None. M. Peek: None. P. Breads: None. C. Brian: None. G.M. Card: None.

Poster

313. Sensorimotor Transformation: Behavior and Whole-Animal Processing

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 313.07/EE9

Topic: D.09. Visual Sensory-motor Processing

Support: NIH Grant P400D018537

Title: Quantitatively tuned internal predictions orchestrate visual signaling in Drosophila

Authors: ***A. J. KIM**¹, L. M. FENK¹, C. LYU¹, G. MAIMON² ²Lab. of Integrative Brain Function, ¹The Rockefeller Univ., New York, NY

Abstract: Vision influences behavior, but ongoing behavior also modulates vision in animals ranging from insects to primates. The function and biophysical mechanisms of most such modulations, however, remain unresolved. We studied how motor-related inputs dynamically influence visual signaling in flying *Drosophila*. We first showed via genetic silencing experiments that a network of eighteen identified motion-sensitive visual neurons participates in gaze-stabilizing head movements. We then performed patch-clamp electrophysiology to comprehensively measure motor-related inputs arriving to these neurons during rapid flight turns in tethered, flying *Drosophila*. We found that the motor-related inputs arrive to all visual

neurons, but the strength of the input varied systematically across cell types. Why are some visual neurons modulated more strongly than others? Insight into this question arose from examining each cell's visual responses to rotational optic flow. We found that the stronger a neuron responded to optic flow about the yaw axis, the larger was the measured motor-related modulation in that cell. This result supported the hypothesis that the motor-related inputs selectively abrogate the yaw stability function in this neural network, while leaving the stability function for other rotational axes active. This idea, however, appeared incompatible with the body kinematics during turns, which consist of stereotyped banked turns that would induce optic flow feedback about all axes of rotations. In order to resolve the paradox, we measured the head movements via high-speed videography, while magnetically tethered, flying flies make rapid turns. We found that Drosophila attempt to maintain their heads stable along roll axes during turns, in agreement with previous studies in freely flying larger flies, which can act to confine the effective visual feedback in the yaw rotational axis. Together, our data support the hypothesis that these optic-flow sensitive neurons regulate gaze-stabilizing head movements and that motorrelated inputs aim to mute the predicted visual responses to yaw visual motion in this population during turns, thus facilitating an anti-stability yaw head movement toward the new gaze direction. This work proposes a function for a behavioral modulation of visual processing and illustrates how the brain can remove one sensory signal from a neural network carrying multiple related signals.

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Poster

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Program#/Poster#: 313.08/EE10

Topic: D.09. Visual Sensory-motor Processing

Support: NIH Grant 1R35NS097287-01

Title: Cortical sensorimotor transformations in a mouse visual detection task

Authors: *D. B. SALKOFF¹, E. ZAGHA², D. A. MCCORMICK³ ¹Neurosci., Yale Med. Sch., New Haven, CT; ²Dept. of Psychology, Univ. of California Riverside, Riverside, CA; ³Dept. Neurobiol, Yale Univ. Sch. Med., New Haven, CT

Abstract: Sensory to motor transformations in cerebral cortex evolve over both time and space. The spatio-temporal properties of cortical processing and contributions of diverse cortical regions to sensory-guided behaviors are still under investigation. We recorded wide-field calcium (GCaMP6s) activity in mice trained to perform a visual Go/No-Go detection task. Mice were trained to lick a reward port in response to a visual stimulus (target) and ignore an auditory

stimulus (distractor). Mice performed the task well (d' 1.4 to 3.5) while head-fixed and free to run on a wheel. During task performance, we imaged contralateral dorsal cortex transcranially at 20 Hz. Calcium activity increased first in V1 ~100 ms after target stimulus presentation. Frontal cortex was highly correlated with all licking (hits, false-alarms, spontaneous licking). Surprisingly, a parietal region spanning trunk/limb somatosensory cortex was active prior to the response and was not associated with running or spontaneous licking, suggesting a role in decision making. To understand how behavioral and brain state affects task performance, we compared running speed, pupil diameter, and pre-stimulus calcium activity on hit and miss trials. On average, pre-stimulus calcium activity in parietal and occipital regions was higher on miss trials than hit trials, suggesting that high spontaneous activity interferes with the generation and/or propagation of sensorimotor representations. The magnitude and timing of activity in disparate cortical regions is indicative of the function of these cortical regions during learned behavior and may further reveal how sensorimotor transformations occur.

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Poster

313. Sensorimotor Transformation: Behavior and Whole-Animal Processing

Location: Halls A-C

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Program#/Poster#: 313.09/EE11

Topic: D.09. Visual Sensory-motor Processing

Title: Hiding in plain sight: Neuronal integration of specific changes in luminosity encode perceived threat and leads to behavioral freeze

Authors: *J. A. JELLIES¹, M. R. KING², V. PARIKH³, T. K. H. GROVES² ²Biol. Sci., ¹Western Michigan Univ., Kalamazoo, MI; ³Kalamazoo Area Math and Sci. Ctr., Kalamazoo, MI

Abstract: Animals distinguish between threats and innocuous events using sensory computations. Leeches are counter-shaded annelids that are active during the day and are therefore themselves vulnerable to visually guided predators. They will often undulate in a rhythmic motion with the rear sucker attached, and such "ventilation" behavior may attract unwanted attention and render the leech vulnerable. Accordingly leeches suddenly "freeze" when a shadow passes over. To characterize this we used small groups of juvenile leeches in defined light environments and exposed them to either a decrease in luminosity (shadow, lights-off) or a comparable increase (lights-on) using a calibrated green LED. Each of 20 groups received 20 trials of lights-off and lights-on while individuals (tracked by video) were ventilating. Shadow proved a much more effective stimulus (74.6%) than lights-on (16.9%) at inducing a freeze. Refinements in stimulus presentation allowed us to determine optimal shadow durations and rates of change, suggesting that the system also encodes temporal cues. Since photoreceptors

produce action potentials in response to lights-on, we hypothesized that there must be discrete interneurons selectively activated to encode the "off" response. To examine this we assessed the shadow response of a known visually responsive interneuron, the S-cell. There is a well-characterized S-cell response to lights-on and so we used this feed-forward interneuron as an assay for visual activity evoked within the CNS. Examining midbody S-cells using intracellular electrophysiology in semi-intact preparations we found that shadows elicited a depolarizing response in almost half (48%) of the preparations, sometimes causing spiking. Typically there was a barrage of complex depolarizing PSPs with a long, variable latency (200-800 ms), consistent with a polysynaptic pathway. This was in contrast to the lights-on response (as the shadow was removed) that occurred 100% of the time with shorter, more consistent latency (~200 ms). We suggest that there must be interneurons that invert the sign of visual information by spiking in response to lights-off stimuli having particular temporal characteristics, and that these interneurons then inhibit the ventilation circuitry. Thus, synaptic interactions between photoreceptors and interneurons in the CNS may provide substrates for synaptic computation for visual feature extraction by a system lacking image-forming eyes.

Disclosures: J.A. Jellies: None. M.R. King: None. V. Parikh: None. T.K.H. Groves: None.

Poster

313. Sensorimotor Transformation: Behavior and Whole-Animal Processing

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Topic: D.09. Visual Sensory-motor Processing

Support: Sackler Fellowship in Biophysics

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The Allen Institute founders, Paul G. Allen and Jody Allen

Title: A link between brains, learning and recurrent neural networks

Authors: *M. STERN^{1,2}, K. CHAMPION¹, A. S. ROKEM³, D. R. OLLERENSHAW², S. MANAVI², S. R. OLSEN², E. T. SHEA-BROWN¹ ¹Applied Mathematics, Univ. of Washington, Seattle, WA; ²Allen Inst. For Brain Sci., Seattle, WA; ³eScience Inst., The Univ. of Washington, Seattle, WA

Abstract: New brain-wide data acquisition techniques provide the opportunity to explore how activity patterns change over the course of learning complex tasks. We use models of neural networks to understand essential changes in the dynamical trajectories of networks during learning. We look for evidence of these learning-related changes in brain activity. We train models of recurrent neural networks to perform various tasks using reservoir computing [Jaeger

and Haas 2004, Sussillo and Abbott 2009]. The tasks span from learning a sinusoidal wave output to more complicated change-detection tasks that parallel behavioral tasks we use in behavioral experiments conducted in mice. We search for common footprints in the altered posttraining dynamics across tasks. To this end we analyze the dominant low-dimensional latent variables. First, we find changes in the number of latent dimensions: specifically, fewer dimensions are required to explain the bulk of the activity after learning. The correlations responsible for this collapse in dimensions, in reservoir computing, are created via feedback. They then allow for controlled dynamics, from which we can read the desired output. Indeed, correlated activity of single neurons in a specific brain area, while the animal is engaged in a task, has been shown to rise during learning [Huber et al 2012, Peters et al 2013]. Here we explore this phenomenon in brain recordings that span multiple areas. We train mice to perform a visual change detection task and use wide-field calcium imaging to measure mesoscale activity across mouse cortex over the course of task learning - from the naïve mouse to expert. Our recordings show that higher-order brain areas correlate their activity with the visual cortex in a mouse that has successfully learned the task. As our reservoir computing results suggest, the input to higher areas is altered, giving rise to correlated activity driven by the stimulus. We expect the latent variables that describe the activity to change accordingly as well.

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Poster

313. Sensorimotor Transformation: Behavior and Whole-Animal Processing

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Topic: D.09. Visual Sensory-motor Processing

Support: JSPS KAKENHI JP16H06566

Title: Automatic adjustment of walking speed by optic flow benefits from binocular vision

Authors: *S. TAKAMUKU¹, T. NAGASAWA², H. GOMI¹ ¹NTT Communication Sci. Labs., Kanagawa, Japan; ²Nagaoka Univ. of Technol., Nagaoka, Japan

Abstract: How we estimate self-motion from optic flow has been an important topic for decades. While numerous studies have revealed the computational process underlying our perception of self-motion (i.e., vection), less is known on the process underlying our implicit visuo-motor control. This is especially the case for walking speed adjustments based on optic flow. Several earlier studies on postural response induced by optic flow assumed error correction mechanism which minimizes retinal motion (and in some cases retinal motion parallax).

However, such mechanisms could fail in explaining the walking speed adjustment because retinal motion velocities depend on the distances of our surroundings and do not directly represent the physical walking velocities per se. Here, to understand how our brain deals with the issue, we measured head velocity of participants walking in a virtual corridor. The corridor was built upon the HTC Vive system which enables free walking in virtual reality environments. When the side walls of the corridor, painted with checkerboard patterns of white and black squares, moved occasionally either towards or against the walking direction with a velocity proportional to the head velocity, their walking velocity increased and decreased respectively as previously been reported. In our first experiment, we measured the walking velocity under different velocities (gain of 0.5 or -0.5 or 0.0) and distances (0.5 and 1.0 m) of the same side walls (identical checkerboard patterns). Two-factor-repeated-measures ANOVA on average walking velocity revealed statistically significant effect of only the physical velocity of the walls (but neither of the distance of the walls nor the interaction between the distance and the velocity). In the second experiment, we changed the pattern of the near wall so that the spatial frequency as well as the area of the checkerboard patterns on the retina closely mimic that of the far wall. Furthermore, we had the participants walk with either monocular or binocular vision, the order of the two conditions counterbalanced among the participants. In this case, the effect of distance was statistically significant in both monocular and binocular conditions, but the effect was smaller in the case of binocular vision (statistically significant effect of interaction between vision condition and distance). While relevance of depth cues remains rather controversial in case of posture control, our findings suggest that the walking speed adjustment based on optic flow is robust to changes in distances of our surroundings and that the robustness depends on the accuracy of our depth estimation.

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Poster

313. Sensorimotor Transformation: Behavior and Whole-Animal Processing

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Program#/Poster#: 313.12/EE14

Topic: D.09. Visual Sensory-motor Processing

Title: Neuronal integration of spectral contrast information: Receptive field properties of visually sensitive neurons in the medicinal leech

Authors: *T. K. GROVES, J. A. JELLIES Dept. of Biol. Sci., Western Michigan Univ., Kalamazoo, MI

Abstract: For animals lacking image-forming eyes, the role of visual circuitry in extracting visual features remains to be characterized. Our central hypothesis is that two-dimensional pixel arrays of simple eyes can be used to extract low-resolution image features. We examined visual

circuitry of the medicinal leech, *Hirudo verbana*, to learn how extraction of distributed visual input drives adaptive behavior. Hirudo possesses 5 pairs of pigmented cephalic eyecups at the margin of the anterior sucker and a distributed, segmentally iterated array of 7 pairs of dermal sensilla positioned dorsal to ventral along the central annulus of each mid-body segment. With this photosensory input, Hirudo discriminates between green and near ultraviolet (UV) light and rotates to minimize ventral, but not dorsal UV exposure. We argue that the distributed array of dermal sensilla can act as a "spectral statocyst" to maintain 3-D body position. Combining light stimulation with extracellular recordings, we characterized sensillar responses across wavelength and luminosity to determine the contribution of primary sensillar photoreceptors in discriminating spectral information. Ventral sensilla appeared to be preferentially narrowly responsive to UV light, while dorsal sensilla were more broadly responsive to green light. Therefore, we suggest that spatially discrete spectral contrast information is established at the primary sensory level. Interactions between sensillar axons and interneurons in the CNS may form the substrates for synaptic computation and extraction of visual features that inform behavior. Considering the location-specific spectral sensitivity documented in the S-cell, we predicted that intracellular inspection of select paired and unpaired visually sensitive CNS neurons would reveal receptive field patterns that reflect the asymmetrical rotation to reduce ventral UV exposure. To determine if the response properties of these neurons can be modulated by exposure to specific wavelengths from spatially constrained inputs, we are comparing their electrophysiological profiles in leeches having all input pathways with those in animals receiving constrained stimuli (i.e. unilateral ventral or dorsal input). Coupled with a similar characterization of motor and premotor neurons that may coordinate body rotation, receptive field properties of the neurons characterized herein should reflect the observed asymmetric rotational behavior and facilitate information circuit identification in the leech visual system.

Disclosures: T.K. Groves: None. J.A. Jellies: None.

Poster

313. Sensorimotor Transformation: Behavior and Whole-Animal Processing

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Topic: D.09. Visual Sensory-motor Processing

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Title: Phototactic responses of histamine-deficient Drosophila mutants

Authors: *J. BORYCZ¹, J. A. BORYCZ¹, I. A. MEINERTZHAGEN^{1,2} ¹Dept. Psychology & Neurosci., ²Dept. Biol., Dalhousie Univ., Halifax, NS, Canada

Abstract: The advanced genetics, short life cycle and anatomy of its compound eye make the fruit fly a perfect model to study visual processes. Histamine (HA) is released at Drosophila photoreceptor terminals. Most is recycled, depending on two enzymes: Ebony, expressed in surrounding epithelial glia, where it conjugates HA with β -alanine to form β -alanylhistamine (carcinine); and Tan in photoreceptors, which mediates hydrolysis of carcinine to liberate HA and β -alanine. Mutant *tan* and *ebony* have abnormal electroretinograms (Hotta & Benzer, 1969); both also have significantly reduced head HA (Borycz et al., 2002). Phototaxis paradigms have been widely applied to Drosophila, conveniently simple methods that complement molecular and biochemical techniques. Our goals were to assess: 1) whether tan and ebony, known to recycle histamine abnormally, can still detect light; and 2) the effect of a HA supplement on phototactic behaviour. We used wild-type Oregon R; hdc, which is unable to synthesize HA; tan and ebony. Phototaxis was measured using a T-Maze and two illumination paradigms: 1) a light/dark choice; and 2) a green/blue choice using 525 nm and 467 nm LEDs. Wild-type flies mostly explored the lit arm (72%) in a light/dark choice; only 22% entered the dark arm and 6% remained in the loading chamber. In a green/blue choice 62% of wild-type chose the blue-lit arm, 20% the greenlit arm, and 18% remained in the loading chamber. In hdc, which cannot synthesize HA and is reportedly blind, 67% of flies remained in the loading chamber in a light/dark choice; only 17% and 16% explored either lit or dark arm. In a green/blue choice, the number of immobile hdc flies increased to 85%; only 5% and 10% entered either green or blue arm, respectively. tan showed some phototactic behaviour entering either the lit- (33%) or dark- (24%) arm, the remaining 43% being immobile, whereas ebony had 43, 5 and 52%, respectively. In a green/blue choice 31% of tan entered the green arm, 45% the blue and 24% remained inside the loading chamber. Corresponding numbers for *ebony* in the same task were 15, 48 and 37%. A 5% HA supplement for 24 h completely rescued-wild type phototactic behaviour in hdc either in a dark-light paradigm or a blue/green choice. The same treatment increased by 12% the number of wild-type entering the lit-arm with a parallel reduction of flies entering the dark-arm. Drinking 5% HA improved T-maze performance in tan whereas it was a median lethal dose for ebony, with approximately 50% flies dead after 24h access to HA. Thus, despite lacking HA recycling, tan and *ebony* still show some phototactic responses, possibly from *de novo* HA synthesis.

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Poster

313. Sensorimotor Transformation: Behavior and Whole-Animal Processing

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Title: Processing of passive and motion-induced visual percepts in the rat dorsomedial striatum

Authors: *A. J. NAGY¹, Y. TAKEUCHI², A. BERENYI^{2,3} ¹Univ. of Szeged, Fac. of Med., Szeged, Hungary, Hungary; ²Univ. of Szeged, Szeged, Hungary;

³Neurosci. Inst., New York Univ., New York, NY

Abstract: One of the inevitable gains of the evolutionary development of vision is the capacity to detect moving objects. The utility is two-fold: the concordant shift of the elementary visual features reflects the voluntary or involuntary movement of the observer and discordant changes can be identified as independently moving 'third party' objects. Making a distinction between these percepts and recognizing the moving prays or predators are essential for survival. Such sensory information allows focusing the attention, adapting behavior and adjusting the motor patterns; however, we have very scarce knowledge on how the appropriate motor output is generated by integrating the sensory information. Motor habits and action selection are often associated with the basal ganglia, and especially the striatum. This process requires continuous monitoring of ongoing behavior to provide its meaningful interpretation, but the nature of the striatal multisensory integrative functions during dynamic behavior is still largely unknown. In the current set of experiments, we set out to decipher that which aspects of the visual information are reaching the dorsomedial caudate nucleus (CPu) of freely moving rats. We also explored how these inputs shape the activity of the striatal neurons by simultaneously performing large-scale high-density extracellular recordings in the CPu and in the visual cortex, while the animals perform various behavioral tasks involving visual stimulation. We found that fastspiking interneurons are particularly sensitive to visual motion, and this sensory information may have a cortical origin. Our data show that besides the presence of space and reward related activity, discrete gamma bursts generated by the visual cortex also appear in the CPu with a few tens of ms delay, and modulate the firing patterns of the same striatal neurons. Consequently, our results suggest that the CPu acts also as an integrator, and adjusts the procedural motor functions by the internal representation of the past experiences and the present circumstances.

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Poster

313. Sensorimotor Transformation: Behavior and Whole-Animal Processing

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SyNergy, EXC 1010, Munich

Title: Generalization of rat spontaneous behavior between real world and virtual reality environments in a CAVE experimental setup

Authors: *N. A. DEL GROSSO^{1,2,3}, J. J. GRABOSKI⁴, E. BLANCO-HERNÁNDEZ⁴, W. CHEN⁴, A. SIROTA^{4,5}

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Abstract: Virtual reality (VR) experimental behavior setups enable cognitive neuroscientists to study the integration of visual depth cues and self-motion cues into a single percept of threedimensional space. Rodents can navigate a virtual environment when locomotion is simulated by running on a spherical treadmill; however, two-dimensional navigation is markedly reduced when their ability to turn freely is restricted. Besides making movement more difficult, this reduced exploration may also stem from sensory conflict between the visual and vestibular systems, as head translation produces no change in the visual perspective of the virtual environment. In humans, vestibulo-visual conflict reduces the subject's immersion in the virtual environment and produces sensations of nausea. Updating the virtual environment via the subject's head movements solves both the vestibulo-visual and sensorimotor conflict issues, however, and in 2014, we demonstrated the utility of real-time VR via rodent head tracking in a proof-of-principle CAVE system. Here, we show that these freely-moving rats demonstrate immersion in virtual environments by displaying height aversion to virtual cliffs, exploration preference of virtual objects, and spontaneously modify their locomotion trajectories near virtual walls. These experiments help bridge the classic behavior and virtual reality literature by showing that rats display similar behaviors to virtual environment features without training.



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Poster

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Topic: D.09. Visual Sensory-motor Processing

Title: Cerebellar granule cells mediate transient sensory-evoked locomotor inhibition in larval zebrafish

Authors: *A. A. BHANDIWAD, H. A. BURGESS Div. of Developmental Biol., NIH/NICHD, Bethesda, MD

Abstract: Sensorimotor behaviors are strongly influenced by the behavioral state of an animal. In this study, we demonstrate that exposure to a strong low frequency stimulus results in a transient inhibition of locomotor activity, which persists for 1-2 min after cessation of the stimulus. The magnitude of inhibition and its recovery time are both stimulus-dependent. During the inhibitory period, selective auditory and visual escape responses are also suppressed, suggesting that this is a novel behavioral state in zebrafish. Inhibition is abolished by prior bath application of 300 mM ethanol, and the inhibitory effect is exaggerated after ethanol washout. We also demonstrate that this effect can be disrupted by ablating the lateral line neuromasts, but disruption of the visual or auditory/vestibular systems has no effect. In order to identify the neural substrates mediating this inhibitory state, we utilized a circuit breaking screen using a library of gal4 enhancer trap lines. We identified a gal4 line, Et(REx2-SCP1:kGal4ff)y318, which labels cerebellar neurons that co-express *vglut2a* and *gata1*, suggesting that these are glutamatergic granule cells. Genetic ablation of these cells results in a ~33% reduction of inhibitory state, and recovery time is also unchanged. Further investigation will explore the role of these neurons in the zebrafish cerebellum in processing lateral line information and regulating this novel behavioral state.

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Poster

313. Sensorimotor Transformation: Behavior and Whole-Animal Processing

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Jane Coffin Childs Memorial Fund for Medical Research

Title: Sensori-motor processing during heat perception in larval zebrafish

Authors: *M. HAESEMEYER¹, D. N. ROBSON³, J. M. LI³, A. SCHIER², F. ENGERT² ¹Harvard Univ. Biolabs, ²Mol. and Cell. Biol., Harvard Univ., Cambridge, MA; ³Rowland Inst. at Harvard, Cambridge, MA

Abstract: Thermosensation provides us with crucial information about our environment. It allows us to judge whether the drink we are holding has a pleasant temperature, while sensing noxious heat or cold enables us to avoid dangerous environmental conditions. While temperature sensation is well understood at the cellular-molecular level, especially in vertebrates fairly little is known about central processing of heat information.

The larval zebrafish is an ideal model to identify temperature processing centers and strategies in

the brain. Like most other animals, larval zebrafish need to avoid extreme hot and cold to maintain a suitable internal temperature. Indeed, from five days post fertilization zebrafish readily navigate thermal gradients seeking out a suitable temperature range. To understand the sensori-motor transformations underlying temperature sensation we previously used random white-noise stimuli to characterize heat perception in larval zebrafish (Haesemeyer et al., Cell Systems, 2015). This approach revealed that zebrafish modulate swimming by integrating temperature information over short timescales and that both changes in temperature and absolute heat levels influence swim initiation.

Using brain-wide functional calcium imaging in head-restrained larval zebrafish we have now identified stereotyped responses related to temperature processing in the central nervous system. Our data indicate that temperature is represented in the brain on different timescales from very slow dynamics on the order of minutes in the pre-optic area to quickly adapting neurons in the hindbrain.

At the periphery, trigeminal sensory neurons detect fluctuations in external temperature and encode these in ON and OFF channels. While temperature representation in the trigeminal ganglion is simple and highly correlated, responses in their hindbrain target area become highly diversified. This decorrelation of responses seems to underlie the generation of behavioral output by hindbrain motor centers and is hence a critical step in the sensori-motor transformation.

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Poster

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Title: Visual guidance of flight mode transitions in birds

Authors: *D. L. ALTSHULER, R. DAKIN

Zoology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Vision is the primary sense that many animals use to develop a dynamic map of the surrounding environment. Visual information is interpreted through guidance algorithms that are specific to different modes of locomotion. For example, recent studies with flying birds have revealed that, like in humans, visual motion (specifically, pattern velocity) is interpreted

differently when stationary (i.e., hovering) than when moving forward. During hovering flight, hummingbirds drift to compensate in relation to the magnitude of pattern velocity in all three major axes. During forward flight, these animals ignore fore- and aft- pattern velocity with respect to flight trajectory, but will adjust altitude to compensate for vertical pattern velocity. The lateral aspect of trajectories for forward flight control are more consistent with a guidance strategy based on balancing visual expansion. The ability of birds to smoothly transition between forward and hovering flight when docked at a flower requires a shift in visual guidance strategy that reflects changes in behavior and in neural signals. How animals control locomotor transitions to accomplish complex movements is not well understood. As a first step to determining the neural mechanisms underlying smooth transitions between locomotor modes, we investigated the flight trajectories of hummingbirds with the sequence: takeoff, cruise, deceleration, and hovering. Experiments were performed in a virtual reality tunnel that allowed for separate stimuli on the sidewall and frontal field. We systematically tested the combined effects of visual pattern velocity and expansion cues on flight mode transitions. Flights were tracked using machine vision that permitted automated tracking of body position and velocity. Collectively, the results reveal how and when hummingbirds shift their use of frontal and lateral cues as they transition between flight modes. These results are interpreted relative to the welldescribed circuits between the avian retina and pre-motor areas.

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Poster

314. Cerebellum Purkinje Cells and Plasticity

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 314.01/EE21

Topic: E.02. Cerebellum

Support: NSF IOS-1208029

Title: The Purkinje cell climbing fiber calcium response increases after induction of parallel fiber long term depression

Authors: *F. SANTAMARIA¹, Z. YANG²

¹Biol., Univ. Texas San Antonio, San Antonio, TX; ²Biol., UTSA, San Antonio, TX

Abstract: We performed simultaneous Purkinje cell whole-cell patch-clamp recordings and calcium imaging obtained from 16-23 day-old mice cerebellar slices. We induced parallel fiber to Purkinje cell long term depression (LTD) in voltage clamp mode by pairing parallel fiber stimulation and somatic depolarization. We measured the changes in intracellular calcium in response to climbing fiber stimulation before and after LTD induction. We found that the calcium response to climbing fiber activation increases in regions near and around the synaptic

LTD induction site, while dendritic regions far from the conditioning site are not affected. Furthermore, this enhanced calcium response after LTD induction are localized in spiny dendrites, but not in smooth main dendrites, even when they are in close vicinity to conditioning sites. Thus, our results show that the climbing fiber evoked calcium response increases in spiny dendrites around the synaptic LTD induction site. Given the dependence of synaptic plasticity on calcium concentration, this could facilitate synapses that underwent LTD to reach the threshold for further synaptic plasticity.

Disclosures: F. Santamaria: None. Z. Yang: None.

Poster

314. Cerebellum Purkinje Cells and Plasticity

Location: Halls A-C

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Program#/Poster#: 314.02/EE22

Topic: E.02. Cerebellum

Support: Welcome Trust DBT India Alliance

Department of Biotechnology (DBT), Govt. of India.

NCBS Core Funding

Title: Dual mode representation of efference copy in purkinje neurons

Authors: *M. SENGUPTA¹, S. NARAYANAN¹, C. WYART², V. THIRUMALAI¹ ¹Natl. Ctr. For Biol. Sci., Bangalore, India; ²Inst. Cerveau Et Moelle Epiniere, Paris, France

Abstract: The cerebellum is important for maintaining balance, for generating co-ordinated locomotion and for motor adaptation and learning. Purkinje neurons in the cerebellum are critical for cerebellar function yet how they shape cerebellar output is not yet clearly understood. We recently established that Purkinje neurons in vivo exhibit membrane potential bistability. In the 'up state they generate sodium spikes tonically, interspersed with calcium spikes while in the 'down' state, they are quiescent and fire bursts of sodium action potentials. Yet, how such single neuron dynamics like membrane potential bistability is used by the whole population of Purkinje neurons to influence behavior remains unknown. We wanted to address this question by using calcium imaging to monitor populations of Purkinje neurons during behavior. Specifically we wanted to ask whether Purkinje neurons switched mode during motor episodes. For this, one first needs to establish the relationship between calcium signals recorded optically and membrane potential. Therefore, we first performed combined imaging and whole-cell patch clamp from single Purkinje neurons. We observed that the optical signals corresponded to calcium spikes in the up state and bursts in the down state. These results demonstrated that calcium imaging in Purkinje neurons yields information regarding different types of cellular signals, depending on

the membrane potential of the neuron. We also discovered that calcium spikes in the up state and bursts in the down state were driven by CF input and also correlated with motor bout initiation. As such the GCaMP signals in Purkinje neurons faithfully follow the motor code. We next monitored fictive swim episodes during open loop optomotor responses (OMR) while imaging populations of Purkinje neurons in their somata and dendrites. We report interesting spatiotemporal dynamics within ensembles of these neurons during sensory stimulus and motor activity. Taken together, our results show that Purkinje neurons represent motor bouts using two modes (tonic spiking vs. bursting) depending on their membrane potential. Thus bistability in Purkinje neurons seems to endow them with an additional degree of freedom for the representation of motor outputs.

Disclosures: M. Sengupta: None. S. Narayanan: None. C. Wyart: None. V. Thirumalai: None.

Poster

314. Cerebellum Purkinje Cells and Plasticity

Location: Halls A-C

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Program#/Poster#: 314.03/EE23

Topic: E.02. Cerebellum

Support: NIH Grant NS083894

Title: Mechanisms of Purkinje cell-dependent instructive signaling in the cerebellum

Authors: *J. M. CHRISTIE¹, C. A. BAKER³, M. G. PINO¹, M. BOLTON², A. BONNAN⁴ ¹Synapse Physiol. Group, ²Disorders of Neural Circuit Function, Max Planck Florida Inst., Jupiter, FL; ³Allen Inst. For Brain Sci., Seattle, WA; ⁴MPFI, Jupiter, FL

Abstract: The cerebellum is known to play a crucial role in motor learning. Purkinje cells (PC) give rise to the sole output of the cerebellar cortex and are therefore pivotal in this process. PCs can fire two different types of spikes: simple spikes (SS) in response to parallel fiber (PF) stimulation and complex spikes (CS) as a result of climbing fiber input. The CS is a multi-component response that includes a burst of sodium spikes at the soma and a dendrite-wide Ca²⁺ transient. A classic theory of cerebellar function postulates that CFs carry instructive signals to PCs that guide learning. However, recent findings indicate that learning may occur independent of CF activity suggesting the existence of other candidate instructive signals. To study the mechanistic basis of instructive signaling, we used the vestibulo-ocular reflex (VOR), a compensatory eye movement in response to head motion that is subject to re-calibration through cerebellar-dependent plasticity. We used optogenetics to selectively manipulate PC activity during vestibular stimulation and probe for the consequences of this activity by examining the gain of the VOR. Using several techniques to induce PC activity either with or without dendritic

 Ca_{2+} influx during vestibular stimulation, we found that PC activity associated with dendritic Ca^{2+} was sufficient to induce a change in VOR gain after repeated pairing with vestibular stimulation. However learning did not occur when Purkinje cells were activated without a dendritic Ca^{2+} transient. Our results suggest that Ca^{2+} influx in PC dendrites is a key component for learning and point to PC dendrites as the initial site of plasticity as predicted by early theories.

Disclosures: J.M. Christie: None. C.A. Baker: None. M.G. Pino: None. M. Bolton: None. A. Bonnan: None.

Poster

314. Cerebellum Purkinje Cells and Plasticity

Location: Halls A-C

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Topic: E.02. Cerebellum

Support: NIH Grant NS083127 (MJMR)

NIH Grant NS083894 (JMC)

Title: Inhibitory control of climbing fiber-mediated plasticity and motor learning in the cerebellum

Authors: *M. J. ROWAN¹, A. BONNAN¹, S. B. AMAT¹, E. M. PATINO², J. M. CHRISTIE¹ ¹Max Planck Florida Inst., Jupiter, FL; ²Florida Atlantic Univ., Jupiter, FL

Abstract: The synaptic properties of Purkinje cell dendrites will contribute to the effectiveness of converting climbing fiber (CF)-mediated complex spikes into meaningful instructive signals that guide motor learning. Molecular layer interneurons (MLIs) inhibit Purkinje cell dendrites yet their role in modulating dendritic excitability related to climbing fiber instruction is poorly understood. Using optogenetics to precisely control MLI output, we found that molecular layer inhibition potently diminished dendritic Ca²⁺ spiking in Purkinje cells evoked by CFs. Because Ca²⁺ spikes provide the biochemical trigger for inducing long-term depression (LTD) at coincidently active parallel fiber synapses, suppressing CF-mediated Ca²⁺ influx prevented LTD and, instead, revealed opposite direction plasticity indicative of multiplexed instructive signaling through distinct mechanisms. To evaluate the effect of ML inhibition on CF-mediated learning we used a dual-color optogenetic approach allowing for the independent actuation of both MLIs and CF. Examining for adaptation of the vestibulo-ocular reflex (VOR), optogenetic activation of CFs in the flocculus during vestibular stimulation in the dark could be used to implant an artificial motor memory resulting in a long-lasting learned increase in the VOR. Interestingly, when MLIs were co-activated with CFs, learning occurred in the opposite direction; there was a

decrease in eye movement relative to head rotation. These findings point to a potential profound role for MLIs in directing (or ensuring) the accuracy (or appropriateness) of learning through their potent influence on Purkinje cell dendritic excitability and plasticity.

Disclosures: M.J. Rowan: None. A. Bonnan: None. S.B. Amat: None. E.M. Patino: None. J.M. Christie: None.

Poster

314. Cerebellum Purkinje Cells and Plasticity

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Program#/Poster#: 314.05/EE25

Topic: E.02. Cerebellum

Support: NIH Grant NS073919

Title: Capturing the functional importance of graded climbing fiber bursts using calcium imaging in awake mice

Authors: *A. FANNING^{1,2}, J. SIEGEL^{1,2}, R. CHITWOOD^{1,2}, D. JOHNSTON^{1,2}, H. NISHIYAMA^{1,2}

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Abstract: Climbing fibers (CFs) are the axons originating from excitatory neurons in the inferior olive that provide instructive signals driving cerebellar-dependent motor learning. CFs are activated by erroneous movement or unexpected sensory activation providing error feedback to Purkinje cells (PCs) in cerebellar cortex and triggering plasticity that makes way for a learned motor response to be expressed. CFs exhibit variable, high-frequency bursts of 1-6 action potentials, yet the consequence of this variable activity has been largely ignored. This is due to the fact that even a single action potential can produce a massive all-or-none postsynaptic PC response. Recent evidence suggests CF burst size may encode parametric features of a sensory stimulus and possibly determine the speed and direction of learning. However, in previous studies, CF burst size has been inferred based on PC recordings, which may be affected by factors other than CF activity, such as non-CF inputs into and the intrinsic excitability of the PC. Using calcium (Ca²⁺) imaging to directly measure CF activity in awake mice we show that CF burst size serves multiple functions. CFs differentiate unexpected sensory events from spontaneous activity by increasing burst size. During eyeblink conditioning, CFs are initially more reliably responsive to the unconditioned stimulus (US; air puff) before learning occurs, but switch to become more reliably responsive to the conditioned stimulus (CS; a light) as learning progresses. As the switch to become primarily CS-responsive occurs, the magnitude of Ca²⁺ transients increases, whereas repeatedly presenting only the CS (unpaired) results in a decrease in the magnitude of CF Ca^{2+} transients. These data suggest that CF burst size serves multiple

functions: to encode unexpected sensory stimuli, provide positive error feedback, and to attach saliency to cues that reliably predict aversive stimuli presentation.

Disclosures: A. Fanning: None. J. Siegel: None. R. Chitwood: None. D. Johnston: None. H. Nishiyama: None.

Poster

314. Cerebellum Purkinje Cells and Plasticity

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Topic: E.02. Cerebellum

Support: NRF-MSIP 2012R1A5A2A44671346

NRF-2013H1A2A1034318

Title: Long-term depression of intrinsic excitability accompanied by the synaptic depression in the cerebellar Purkinje cells

Authors: *H. SHIM¹, D. JANG², S. KIM¹

¹Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of; ²Col. of Science, Dept. of Brain and Cognitive Sci., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: One remarkable question in neuroscience is how the brain stores novel information from the environment and makes modification in behavior. Of interest, many implications reveal that the activity-dependent plasticity of synaptic efficacy and intrinsic excitability underlie the learning rule. The long-term depression (LTD) at the parallel fiber (PF) and Purkinje cell (PC) synapses has been found to be cellular basis of the cerebellum-dependent motor learning. In order to transfer information formed at cerebellar cortex to the postsynaptic regions such as the vestibular nuclei (VN) and the cerebellar nuclei (CN), the activity-dependent changes in the intrinsic excitability might be manifested for appropriate signal transduction within motorlearning circuitry. Here, we show that pairing of the PF and climbing fiber (CF) for PF-PC LTD induction evokes long-term depression of intrinsic excitability (LTD-IE) in the cerebellar PCs from male C57BL/6 mice. Interestingly, this intrinsic plasticity showed different kinetics from synaptic plasticity, but both forms of plasticity share Ca^{2+} signaling and protein kinase C (PKC) pathway as their underlying mechanism. While small-conductance Ca²⁺-activated K⁺ channels (SK channels) play important roles in long-term potentiation of intrinsic excitability (LTP-IE), no direct implication was found in LTD-IE. After PF-PC LTD induction, neither the temporal summation of dendritic EPSP nor the power of spike frequency adaptation is changed, indicating that cerebellar LTD executes the information processing in a quantitative way without quality changes of synaptic integration and generation of output signals. Our results suggest that LTD-IE may have a synergistic effect with synaptic depression on the total net output of neurons by amplifying the modification of PF synaptic transmission.

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Poster

314. Cerebellum Purkinje Cells and Plasticity

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Topic: E.02. Cerebellum

Support: National Institute of Neurological Disorders and Stroke (NS-062771 to CH and JD)

National Institute of Health (R37 – AG008796 to JFD)

Title: Enhanced intrinsic excitability in cerebellar Purkinje cells following delay eyeblink conditioning in mice

Authors: *G. V. WATKINS¹, H. K. TITLEY¹, C. LIN², C. WEISS², J. F. DISTERHOFT², C. HANSEL¹

¹Neurobio., Univ. of Chicago, Chicago, IL; ²Physiol., Northwestern Univ., Chicago, IL

Abstract: Cerebellar learning is canonically thought to rely on synaptic plasticity, particularly at the inputs to Purkinje cells. Recently, however, other complementary mechanisms have been identified. Intrinsic plasticity is one such mechanism, and depends on a down-regulation of calcium-dependent SK-type K channels, which is associated with an increase in neuronal activity. In the hippocampus, SK-mediated intrinsic plasticity has been shown to play a role in trace eyeblink conditioning learning; however, it is not yet known how intrinsic plasticity contributes to a cerebellar learning task such as delay eyeblink conditioning. Here we show that after delay eyeblink conditioning, Purkinje cells in lobule simplex / lobule HVI were more excitable than controls consistent with an increase of intrinsic excitability. Whole cell recordings were obtained from acute cerebellar slices from mice 48 hours after the final training session. Groups of mice received over a period of repeated training sessions either distinctly paired trials of a tone co-terminating with a periorbital shock (conditioned mice), unpaired trials of only a tone or a shock (pseudoconditioned mice) or neither a tone or a shock (naïve mice). We found that Purkinje cells from conditioned mice exhibited more evoked activity than cells from control animals, resulting in a shift to the left in the I-F curve following a gradual current injection protocol. The current and potential threshold needed to evoke spikes was decreased in the conditioned mice. Furthermore, following the stimulation of parallel fibers or climbing fibers, the Purkinje cell responses from conditioned mice were more excitable showing a increased number of spikelets in complex spikes, a greater area and reduced duration of depolarization, a

reduced afterhyperpolarization, and shorter latency to the minimum amplitude in both complex spikes and bursts of parallel fiber activity. This increase in excitability may indicate that SK-dependent intrinsic potentiation occurs during eyeblink conditioning, as the conditioned mice also showed reduced changes in excitability following the application of an intrinsic plasticity protocol, suggesting an occlusion effect in conditioned mice.

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Poster

314. Cerebellum Purkinje Cells and Plasticity

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Topic: E.02. Cerebellum

Support: Marie Skłodowska-Curie fellowship

Title: Functional coordination of parallel fiber and climbing fiber inputs during multi-sensory association and timed motor behavior in cerebellar cortex

Authors: *S. TSUTSUMI, O. CHADNEY, M. HÄUSSER

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Abstract: Cerebellar Purkinje cells (PCs) form the sole output of the cerebellar cortex and receive two major excitatory synaptic inputs: parallel fibers (PFs), the axons of granule cells (GCs), and a climbing fiber (CF). GCs integrate multi-modal information from multiple sources to deliver patterns of inputs via PFs, whereas CFs send instructive signals such as sensorimotor error signals and/or timing signals to PCs. Coincident PF and CF inputs cause plastic changes in PC firing, a process which is thought to be crucial for multi-modal integration and precise adaptation of motor action. However, how PF and CF inputs interact to regulate PC output during behavior is not well understood. To address this question, we used two functional fluorescent probes with different colors to monitor PF and CF inputs simultaneously during a multi-sensory association task, to visualize how these inputs are spatiotemporally coordinated to achieve precisely timed behavior. RCaMP2 was expressed in PCs to monitor CF inputs, while GCaMP6f was expressed in GCs to directly monitor PF inputs to the PCs. Imaging was performed in Crus I, the region responsible for orofacial sensorimotor processing. Head-fixed mice were trained on a multi-sensory go/no-go task in which they had to lick during brief (0.5 s) combined sensory stimuli with different modalities such as air-puff, LED flash, or a tone, but not to single-modality sensory stimulus. Mice refined their lick behavior during learning so that licking in response to combined stimuli became quicker, shorter, faster and more regular. Simultaneous imaging of PF and CF inputs to the same PCs revealed that these inputs became

more coupled during performing the task. Along with learning, PF responses to cues became temporally precise, whereas CF responses for non-rewarded trials were diminished. Both PF and CF inputs became bimodal in licking trials: the first peak appeared just after the cue and the second peak was observed after lick offset. Muscimol injection into the Crus I in expert mice deteriorated task performance through a decrease in hit rate and increase in latency of licks after the combined cue (lick onset latency: 0.236 ± 0.006 s to 0.363 ± 0.007 s, p < 0.001). These results suggest that spatiotemporal coupling of PF and CF inputs to PCs is important for immediate multi-sensory processing necessary for rapid motor initiation and reliable suppression of unnecessary movement. Optogenetic manipulation of PCs, PF and CF inputs will allow further dissection of the roles of individual inputs and PC outputs in this form of cerebellum-dependent motor adaptation.

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Poster

314. Cerebellum Purkinje Cells and Plasticity

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Title: Ablation of TFR1 in Purkinje cells inhibits mGlu1 trafficking and impairs motor coordination, but not autistic-like behaviors

Authors: *J. ZHOU¹, L. ZHOU¹, C. DE ZEEUW^{2,3}, Y. SHEN¹

¹Inst. of Neurosci. Zhejiang Univ., Hangzhou Zhejiang, China; ²Dept. of Neuroscience, Erasmus MC, Rotterdam, Netherlands; ³Netherlands Inst. for Neuroscience, Royal Dutch Acad. for Arts & Sci., Amsterdam, Netherlands

Abstract: Group 1 metabotropic glutamate receptors (mGlu1/5) are critical to synapse formation and participate in synaptic long-term potentiation (LTP) and long-term depression (LTD) in the brain. mGlu1/5 signaling alterations have been documented in cognitive impairment, neurodegenerative disorders, and psychiatric diseases, but the underlying mechanisms for its modulation are not clear. Here, we report that transferrin receptor 1 (TFR1), a trans-membrane protein of the clathrin complex, modulates the trafficking of mGlu1 in cerebellar Purkinje cells

(PCs). We show that conditional knockout of TFR1 in PCs does not affect the cyto-architecture of PCs, but reduces mGlu1 expression at synapses. This regulation by TFR1 acts in concert with that by Rab8 and Rab11, which modulate the internalization and recycling of mGlu1, respectively. TFR1 can bind to Rab proteins and facilitate their expression at synapses. PC ablation of TFR1 inhibits parallel fiber-PC LTD, whereas parallel fiber-PC LTP and PC intrinsic excitability are not affected. Finally, we demonstrate that PC ablation of TFR1 impairs motor coordination, but does not affect social behaviors in mice. Together, these findings underscore the importance of TFR1 in regulating mGlu1 trafficking, and suggest that mGlu1 and mGlu1-dependent parallel fiber-LTD are associated with regulation of motor coordination, but not autistic behaviors.

Disclosures: J. Zhou: None. L. Zhou: None. C. De Zeeuw: None. Y. Shen: None.

Poster

314. Cerebellum Purkinje Cells and Plasticity

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Topic: E.02. Cerebellum

Support: EMBO

Wellcome Trust

Title: Population coding in the Purkinje cell network during execution of goal directed action

Authors: *D. KOSTADINOV, M. BLANCO POZO, M. BEAU, M. HAUSSER Wolfson Inst. for Biomed. Res., Univ. Col. London, London, United Kingdom

Abstract: The activity of Purkinje cells (PCs), the sole output neurons of the cerebellar cortex, is important for both execution of accurate motor programs and learning of new actions. Activation and suppression of many PCs is capable of directly inhibiting and driving movements, respectively, and coincident activation of climbing fibre and parallel fibre input pathways to PCs drives plastic changes in firing that is a major locus of cerebellar learning. Our goal is to uncover how PC populations acquire the response properties necessary for driving goal-directed motor execution, and how these representations change under altered sensorimotor conditions. To address these questions, we have recorded the activity of groups of PCs in mice trained to perform a virtual-reality based sensorimotor integration task. Mice were head-fixed in front of an array of monitors and trained to use a steering wheel placed in front of their forepaws to translate a virtual object from eccentric positions to the midline to get a reward. Thus, trial outcomes consisted of undershoots, correct trials, and overshoots. Over several weeks of training, mice learned to make accurate movements at rates well above chance. To monitor activity in PCs

during behaviour, we virally expressed GCaMP6f in these neurons and used resonant-scanning 2-photon microscopy to record dendritic calcium transients, which predominately reflect complex spiking activity, simultaneously across ~200 PCs while mice performed the task. In well-trained mice, movement initiation produced calcium transients in similar groups of PCs, irrespective of trial outcome. However, PC populations exhibited a stereotyped activation sequence in correct trials that was not present in undershoot and overshoot trials: PC calcium signals synchronized just prior to movement onset, paused during movement execution, and resynchronized at movement termination. We also observed that many PCs are active upon delivery of reward on correct trials and absence of reward delivery on incorrect trials. PC populations encoding movement dynamics were not independent populations from the ones encoding reward and error related activity. Thus, calcium signals in PCs contain information about the execution of actions as well as the evaluation of their outcome. We are currently using chronic imaging of PC populations over days to explore how PCs acquire these response properties as animals learn the task, determine the reliability of PC responses on single trials, and track the dynamics of responses as animal adapt their actions under changing sensorimotor coupling.

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Poster

314. Cerebellum Purkinje Cells and Plasticity

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Topic: E.02. Cerebellum

Support: ZonMW (project # 10-10400-98-008)

Stichting Coolsingel

Title: BDNF Val66Met and transcranial direct current stimulation interact in cerebellardependent motor learning

Authors: *M. A. FRENS¹, R. VAN DER VLIET¹, S. LOUWEN¹, M. HOOG¹, L. DE VREEDE¹, G. M. RIBBERS², C. I. DE ZEEUW¹, O. DONCHIN³, R. W. SELLES¹, J. N. VAN DER GEEST¹

¹Erasmus MC Rotterdam, Rotterdam, Netherlands; ²Rijndam Rehabil. Ctr., Rotterdam, Netherlands; ³Ben Gurion Univ. of the Negev, Be'er Sheva, Israel

Abstract: Cerebellar tDCS has been reported to enhance motor associative learning and motor adaptation, holding promise for future clinical application in patients with movement disorders.

However, behavioral benefits from cerebellar tDCS are highly inconsistent across the literature and further characterization of predictive factors of treatment success is necessary. Therefore, we studied the role of BDNF Val66Met in classical eyeblink conditioning and VOR adaptation and the interaction with cerebellar tDCS. We undertook two large tDCS studies in genotyped subjects performing either an classical eyeblink conditioning task (N=117, between design) or a VOR adaptation task (N=46 subjects, within-design). Our results show faster eyeblink conditioning for carriers compared to non-carriers (βCarriers=20.7 [1.3 38.0]) with a supportive role for anodal tDCS only in the slower non-carrier group βAnodal,Non-carriers=19.7 [2.8 36.1] but not in the faster carrier group βAnodal, Carriers=-1.0 [-21.6 17.3]. In addition, cathodal tDCS was ineffective in both genetic groups (βCathodal,Non-Carriers=-1.6 [-19.6 21.1], βCathodal, Carriers=2.7 [-13.8 18.7]). The VOR adaptation experiment did not show an effect of BDNF Val66Met, tDCS or the interaction (βCarriers=-4.8 [-11.8 2.0], βAnodal, Carriers=5.0 [-1.4 11.0], βAnodal, Non-Carriers=0.7 [-3.6 4.5]). These results suggest that BDNF Val66Met (1) can determine effectiveness of cerebellar tDCS by modifying learning rate in some cerebellardependent tasks and (2) has a currently unknown role in cerebellar-dependent associative learning.

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Poster

315. Striatal Physiology

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Topic: E.03. Basal Ganglia

Support: NIAAA AA021505

R01AA024659

Title: Alcohol induces input-specific aberrant synaptic plasticity in the rat dorsomedial striatum

Authors: *T. MA, B. BARBEE, X. WANG, J. WANG Texas A&M Hlth. Sci. Ctr., Bryan, TX

Abstract: Accumulated evidence suggests that the dorsomedial striatum (DMS) of the basal ganglia plays an essential role in pathological excessive alcohol consumption. The DMS receives multiple glutamatergic inputs. However, whether and how alcohol consumption distinctly affects these excitatory afferents to the DMS remains unknown. Here, we used optogenetics to selectively activate the rat medial prefrontal cortex (mPFC) and basolateral amygdala (BLA)

inputs in DMS slices, and measured the effects of alcohol consumption on glutamatergic transmission in these corticostriatal and amygdalostriatal circuits. We found that excessive alcohol consumption increased AMPA receptor- and NMDA receptor (NMDAR)-mediated neurotransmission, as well as the GluN2B/NMDAR ratio, at the corticostriatal input to the DMS. The probability of glutamate release was increased selectively at the amygdalostriatal input. Interestingly, we discovered that paired activation of the mPFC and BLA inputs using dual-channel optogenetics induced robust long-term potentiation (LTP) of the corticostriatal input to the DMS. Taken together, these results indicate that excessive alcohol consumption potentiates glutamatergic transmission via a postsynaptic mechanism for the corticostriatal input and via a presynaptic mechanism for the amygdalostriatal input.

Disclosures: T. Ma: None. B. Barbee: None. X. Wang: None. J. Wang: None.

Poster

315. Striatal Physiology

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Support: NIAAA R01AA021505 (JW)

NINDS NS074895 (FS)

Development Funds from TAMHSC-VPR (FS and JW)

Title: Stroke triggers nigrostriatal plasticity and increases alcohol consumption in rats

Authors: *J. WANG, C. HUANG, T. MA, E. HELLARD, X. WANG, A. SELVAMANI, J. LU, F. SOHRABJI

Dept. of Neurosci. & Exptl. Therapeut., Texas A&M Hlth. Sci. Ctr., Bryan, TX

Abstract: Excessive alcohol consumption is a known risk factor for stroke, but the effect of stroke on alcohol intake is unknown. The dorsomedial striatum (DMS) and midbrain areas of the nigrostriatal circuit are critically associated to stroke and alcohol addiction. Here we sought to explore the influence of stroke on alcohol consumption and to uncover the underlying nigrostriatal mechanism. Rats were trained to consume alcohol using a two-bottle choice or operant self-administration procedure. Retrograde beads were infused into the DMS or midbrain to label specific neuronal types, and ischemic stroke was induced in the dorsolateral striatum (DLS). Slice electrophysiology was employed to measure excitability and synaptic transmission in DMS and midbrain neurons. We found that ischemic stroke-induced DLS infarction produced significant increases in alcohol preference, operant self-administration, and relapse. These

increases were accompanied by enhanced excitability of DMS and midbrain neurons. In addition, glutamatergic inputs onto DMS D1-neurons was potentiated, whereas GABAergic inputs onto DMS-projecting midbrain dopaminergic neurons was suppressed. Importantly, systemic inhibition of dopamine D1 receptors attenuated the stroke-induced increase in operant alcohol self-administration. Our results suggest that the stroke-induced DLS infarction evoked abnormal plasticity in nigrostriatal dopaminergic neurons and DMS D1-neurons, contributing to increased post-stroke alcohol-seeking and relapse.

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Poster

315. Striatal Physiology

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David Lehr Research Award from American Society for Pharmacology and Experimental Therapeutics

Title: Chronic intermittent ethanol potentiates goal-directed behavior via dorsomedial striatal adenosine A2A receptor

Authors: *S.-I. HONG¹, S.-Y. CHANG², D.-S. CHOI¹ ¹Mol. Pharmacol. and Exptl. Therapeut., Mayo Clin. Col. of Med., Rochester, MN; ²Mayo Clin., Rochester, MN

Abstract: Chronic intermittent ethanol (CIE) exposure promotes voluntary ethanol consumption. Previously we have reported that inhibition of the adenosine A2A receptor ($A_{2A}R$) in the dorsomedial striatum (DMS) increases reward seeking when mice were tested in random ratio (RR) paradigm of nose-poke operant chamber, which is implicated in goal-directed behavior. However, the mechanisms underlying how CIE exposure regulates the goal-directed behavior under devalued and valued conditions remain unknown. Here, we investigated whether the $A_{2A}R$ in the DMS contributes to goal-directed behavior in mice exposed to CIE. Our results show that CIE exposure elicited anxiety-like behavior in elevated plus maze test without affecting working memory in y-maze test. During acquisition of instrumental behavior training in C57BL/6J mice, CGS21680 (0.3 mg/kg, i.p.), A_{2A}R agonist, and optogenetic stimulation of A_{2A}R-expressing neurons in the DMS abolished goal-directed behavior for sweetened 10% ethanol, whereas ZM241385 (20 mg/kg, i.p.), A_{2A}R antagonist, did not impede goal-directed behavior under valued condition. Interestingly, A_{2A}R activation prevented voluntary ethanol consumption in operant chamber even after 24 hours following CGS21680 (0.3 mg/kg, i.p.) injection. Taken together, activation of the A_{2A}R in the DMS dampens goal-directed behavior and alcohol drinking, suggesting that activation of DMS A_{2A}R could be a potential target for the treatment of alcohol use disorder.

Disclosures: S. Hong: None. S. Chang: None. D. Choi: None.

Poster

315. Striatal Physiology

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 315.04/FF6

Topic: E.03. Basal Ganglia

Support: NIAAA RO1AA021505

Title: Retrograde mapping of afferent inputs to direct- and indirect-pathway neurons in the dorsomedial striatum

Authors: *Y. CHENG, J. LU, B. BARBEE, X. WANG, J. WANG Dept. of Neurosci. and Exptl. Therapeut., Texas A&M Univ. Hlth. Sci. Ctr., Bryan, TX

Abstract: The dorsomedial striatum (DMS) is crucial for goal-directed behavior and heavily implicated in drug and alcohol addiction. Our recent study indicated an opposite role of DMS dopamine D1 receptor (D1R)- and D2R-expressing medium spiny neurons (MSNs) on alcohol consumption (Cheng et al. 2016). However, the cell type of presynaptic neurons that project to DMS D1- versus D2-MSNs remains unknown. To assess whether these presynaptic neurons also contain D1Rs or D2Rs, we used a state-of-the-art rabies viral-based monosynaptic retrograde tracing technology together with D1-/D2-Cre;Ai14 transgenic mice to map these neurons brainwide. We found abundant DMS-projecting neurons in the different cortical regions, amygdala, thalamus, and midbrain. Interestingly, we found that most D1-MSN-projecting neurons did not express D1Rs, and similarly, most D2-MSN-projecting neurons did not express D2Rs. We only observed a few D1-MSN-projecting neurons in the cortex and thalamus that contained D1Rs; a few D2-MSN-projecting neurons in the same brain regions that expressed D2Rs. In addition, using optogenetic slice electrophysiology and D1-/D2-Cre;Ai32 mice, we found that DMS D1-MSNs received glutamatergic inputs mainly from D2-neurons outside of the striatum, whereas

glutamatergic inputs onto DMS D2-MSNs were predominately from D1-neurons. These results suggest that the D1-D2 and D2-D1 projections are the dominant connections in brain-wide, which is an important question in the addiction field that has not been addressed before. Since drugs of abuse and alcohol affect dopaminergic system brain-wide, understanding these connections will improve our knowledge of the DMS circuits in drug addiction.

Disclosures: Y. Cheng: None. J. Lu: None. B. Barbee: None. X. Wang: None. J. Wang: None.

Poster

315. Striatal Physiology

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Program#/Poster#: 315.05/FF7

Topic: E.03. Basal Ganglia

Support: NARSAD

Ike Muslow Predoctoral Fellowship

Title: Pharmacologic and genetic targeting of the neural extracellular matrix to ameliorate aging-related cognitive decline

Authors: *A. D. RICHARD¹, X. TIAN³, X. W. YANG⁴, X. LU²

¹Pharmacology, Toxicology, and Neurosci., ²Dept. of Pharmacology, Toxicology & Neurosci., Louisiana State Univ. Hlth. Sci. Ctr., Shreveport, LA; ³Pharmacol., LSU, Shreveport, LA; ⁴Psychiatry, UCLA Hlth. Syst., Los Angeles, CA

Abstract: The percentage of the global population aged 65 years and older is projected to double from 8% to 16% by the year 2050. With no effective prevention and treatment, cognitive decline is a major concern of the aging population, presenting a large financial burden on public health care systems worldwide. The brain's extracellular matrix (ECM) has been implicated in mediating neural structural stability by enwrapping synaptic connections fundamental for long term memory storage and other cognitive functions. We hypothesize that aging-dependent accumulation of neural ECM impairs the structure and function of cognitive neural circuitry (e.g, striatum), therefore pharmacologic and genetic modulations of the neural ECM can restore/reverse aging-dependent cognitive decline. Young (6-7 month old) and aged (22-24 month old) mice were analyzed for multiple cognitive functions. Aged mice manifested significant decline in motor skill learning, working memory, object recognition memory, and adaptive learning compared to younger counterparts. The cognitive decline was accompanied by a significant aging-dependent accumulation of the striatal neural ECM. To establish a functional link between aging-dependent striatal ECM accumulation and cognitive decline, we performed

stereotaxic infusions of chondroitinase ABC (ChABC) into striatum to eliminate CSPGs in aged mice, in order to reverse aging-related decline of object recognition memory and motor skill learning. Furthermore, to identify an endogenous genetic target of the striatal neural ECM, we have genetically targeted an orphan sugar-nucleotide transporter as a potential modulator of neural ECM proteoglycans synthesis. AAV-mediated overexpression was shown to drive striatal ECM accumulation. Our study unravels a causal link between cognitive decline and neural ECM accumulation, affording new therapeutic targets for guided neuroplasticity to reverse age dependent cognitive decline.

Disclosures: A.D. Richard: None. X. Tian: None. X.W. Yang: None. X. Lu: None.

Poster

315. Striatal Physiology

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Topic: E.03. Basal Ganglia

Support: Human Frontier Science Program

JSPS Grant-in-Aid for Challenging Exploratory Research

JSPS Grant-in-Aid for Young Scientists (A)

Title: Anatomical evidence that multiple striatal regions influence motor cortex

Authors: *S. AOKI^{1,2}, M. IGARASHI¹, P. COULON³, J. R. WICKENS⁴, T. J. RUIGROK² ¹Okinawa Inst. of Sci. and Technol., Okinawa, Japan; ²Erasmus MC Rotterdam, Rotterdam, Netherlands; ³Inst. de Neurosciences de La Timone, Marseille Cedex 05, France; ⁴Neurobio. Res. Unit, OKINAWA INSTITUTE OF SCIENCE AND TECHNOLOGY, Onna-son, Japan

Abstract: Striatum receives monosynaptic cortical input and sends multisynaptic output back to the cortex. Dorsolateral striatum (DLS) receives motor cortical input but dorsomedial (DMS) and ventral striatum (VS) do not. However, accumulated evidence suggests the involvement of DMS and VS in motor action such as goal-directed and Pavlovian approach behaviors. Those findings raise the possibility of output from DMS and VS to motor cortex, despite an absence of motor cortical input. We address this possibility by retrograde transneuronal tracing using rabies virus (RABV). RABV was co-injected with an anterograde monosynaptic tracer (cholera toxin b-subunit, CTb) into the rat motor cortex, which resulted in third-order RABV+ striatal neurons and CTb+ cortico-striatal fibers. DLS showed both RABV and CTb labeling, suggesting a closed-loop between DLS and motor cortex. Surprisingly, a few clusters of RABV+ neurons were observed in DMS, VS, and the tail of striatum. These areas were devoid of CTb labeling but made output connections to motor cortex, suggesting an open-loop structure. In another set of

rats, injection of RABV/CTb into prefrontal cortex resulted in RABV labeling in DMS and VS but not in DLS. Since these RABV-labeled regions receive prefrontal input, this suggests a closed-loop structure. These results demonstrate co-existence of closed- and open-loop structures between the striatum and motor cortex, but a closed-loop structure for the striatum and prefrontal cortex. The present study provides an anatomical basis for understanding how the striatum can access motor cortex.

Disclosures: S. Aoki: None. M. Igarashi: None. P. Coulon: None. J.R. Wickens: None. T.J. Ruigrok: None.

Poster

315. Striatal Physiology

Location: Halls A-C

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Program#/Poster#: 315.07/FF9

Topic: E.03. Basal Ganglia

Support: NIH Grant NS047085

NIH Grant NS097185

Title: Predicting the PSTH for inhibitory conductance input to substantia nigra pars reticulata

Authors: *D. SIMMONS, M. H. HIGGS, C. J. WILSON

Univ. of Texas At San Antonio, San Antonio, TX

Abstract: Rhythmically firing neurons in substantia nigra pars reticulata (SNr) receive inhibitory synaptic input from direct pathway striatal spiny neurons. To investigate how direct pathway input could affect spike timing in SNr neurons, we characterized the direct pathway synaptic input, measured the post-stimulus time histogram (PSTH) for simulated inhibitory input, and interpreted the results using a model based on the phase resetting curve (PRC). To characterize direct pathway inhibition, a virus containing a Cre-inducible channelrhodopsin was injected into the striatum of Tac1-IRES2-Cre-D mice. The 20 - 80 % rise time and decay time constant were measured from photo-evoked currents in whole-cell voltage-clamp mode. The reversal potential (Erev) was measured from photo-evoked IPSPs recorded in perforated-patch current-clamp mode. To measure PRCs and characterize the spike responses to simulated direct pathway inhibition, we recorded from SNr neurons in perforated-patch configuration. To obtain the PRC, we injected zero-mean current noise and analyzed the spike responses by a previously described multiple linear regression method. To investigate the spike responses to direct pathway input, we used dynamic clamp to apply trains of simulated inhibitory post-synaptic conductances (IPSGs) with kinetics and Erev based on our experimental data. The PSTH was computed with respect to the onset of each IPSG. The main feature of the PSTH was a rapid dip in firing rate typically

lasting on the order of 10 ms. In some cells the dip was followed by a period of firing above the baseline rate. To understand the PSTH shapes, we developed a PRC-based phase model augmented with an approximation of the phase-dependent membrane potential, Vm(phi). Given the experimental Erev, Vm(phi) provides a phase-dependent driving force to convert input conductance into current, which changes the cell's phase according to the PRC. The same IPSG trains injected into each neuron were applied to each phase-voltage model, and the model PSTH was calculated. The models generally produced a PSTH closely resembling the cell data. Our data indicate that, rather than only reducing firing rate, direct pathway inhibition can produce dynamic, bidirectional changes in spike probability. These changes were well explained by our phase-voltage models, providing a tool to explore the potential impact of more complex patterns of input arriving from multiple pathways.

Disclosures: D. Simmons: None. M.H. Higgs: None. C.J. Wilson: None.

Poster

315. Striatal Physiology

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Program#/Poster#: 315.08/FF10

Topic: E.03. Basal Ganglia

Support: NIH Grant NS047085

NIH Grant NS097185

Title: Predicting the PSTH for excitatory current input to substantia nigra pars reticulata neurons

Authors: *M. H. HIGGS, D. V. SIMMONS, C. J. WILSON Biol., Univ. of Texas at San Antonio, San Antonio, TX

Abstract: Autonomous oscillator neurons in substantia nigra pars reticulata (SNr) integrate synaptic input from the direct, indirect, and hyperdirect pathways, including synaptic excitation from the subthalamic nucleus (STN). Synaptic input perturbs the oscillation phase of SNr cells, producing changes in spike timing that can be quantified by the phase resetting curve (PRC). When a rhythmically firing neuron receives inputs that do not cause immediate spiking, small changes in spike timing underlie the average response measured by the post-stimulus time histogram (PSTH). Thus, we tested the ability of phase models based on the PRC to predict the PSTH of SNr neurons in response to excitatory currents. Perforated-patch, current-clamp recordings were obtained from SNr neurons in mouse brain slices. Episodes of noise stimulation used to estimate the PRC were alternated with episodes of EPSC waveforms designed to mimic synaptic input from the STN. PRCs were computed by a previously described multiple linear regression method. The PRCs were type-1, showing only shortening of the inter-spike interval

(ISI) by depolarizing stimulus pulses. Most of the PRCs had a strong rightward skew, indicating greater sensitivity to stimuli arriving late in the ISI, and many had a prominent peak at late phase. Phase models were constructed based on the PRC for each neuron and run with the same EPSC trains delivered to the real cell. The PSTH was then measured for each cell and the corresponding model. Overall, the models captured the major features of the experimental PSTHs, including a large, brief peak shortly after EPSC onset, often followed by a dip below the baseline rate. However, some quantitative differences between the cells and models were observed. In particular, cells with very high sensitivity at late phases often showed a larger PSTH peak and a more prominent dip than predicted by the models. Thus, nonlinear mechanisms deviating from ideal phase-oscillator behavior increase the sensitivity and temporal precision of SNr neurons' responses to fast EPSCs. These properties of SNr neurons may facilitate the transmission of fast signals along the hyperdirect pathway from cortex to STN to SNr.

Disclosures: M.H. Higgs: None. D.V. Simmons: None. C.J. Wilson: None.

Poster

315. Striatal Physiology

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Topic: E.03. Basal Ganglia

Support: NIH Grant NS047085

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Title: Oscillatory entrainment of striatal low threshold spike interneurons

Authors: *J. C. MORALES, C. J. WILSON Biol., Univ. of Texas At San Antonio, San Antonio, TX

Abstract: Striatal low-threshold spike (LTS) interneurons are autonomous oscillators that receive input from the cortex, the thalamus, and local neurons within the striatum. Oscillatory activity in the cortex and thalamus is transmitted to the striatum, where it produces oscillatory synaptic currents in striatal neurons. A previous study found that striatal LTS interneurons show a spiking resonance with frequencies between 5 and 30 Hz when stimulated with broadband noise, suggesting that they might amplify oscillations in that frequency range and become synchronized by these inputs. We characterized the oscillatory entrainment of striatal LTS interneurons by applying small (10-20 pA) sinusoidal intracellular currents to mimic oscillatory synaptic currents over a frequency range from 1 to 30 Hz. LTS neurons were identified in B6.FVB-Tg(Npy-hrGFP)1Lowl/J transgenic mice, in which they express green fluorescent protein. The applied sinusoidal currents did not alter the average firing rate of the cells, but had
strong effects on their firing patterns at all stimulus frequencies. LTS cells were most strongly phase-locked to the stimulus when its frequency matched the cells' unperturbed firing rate. To determine whether a shared oscillation might synchronize the population of LTS cells, we targeted channelrhodopsin to LTS cells by viral delivery in a somatostatin-cre (Sst^{tm2.1(cre)Zjh}/J) mouse line. Sinusoidal blue light was used to generate an oscillatory current in all the LTS cells in the region of the injection, and single cells were recorded in cell-attached configuration. Application of the sinusoidal current produced no change in firing rate, and changes in firing pattern were indistinguishable from those seen with intracellular current, indicating that the network did not synchronize. To determine whether oscillations in the LTS cell population could be transferred to postsynaptic spiny projection neurons (SPNs), we measured spontaneous inhibitory postsynaptic currents in SPNs during sinusoidal optogenetic stimulation of the LTS cell population. The data showed a strong transfer of oscillations from entrained presynaptic LTS cells to the synaptic currents produced in SPNs. These results may suggest that presynaptic entrainment of LTS cells will generate an oscillating input to SPNs, which could interact either constructively or destructively with oscillating inputs reaching SPNs directly from the cortex or thalamus.

Disclosures: J.C. Morales: None. C.J. Wilson: None.

Poster

315. Striatal Physiology

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 315.10/FF12

Topic: E.03. Basal Ganglia

Support: JSPS Research Fellow

KAKENHI 16K08486

KAKENHI 17H05543

Title: Involvement of the store operated calcium entry in the long-lasting calcium transient in the striatal GABAergic neuron

Authors: S. KIKUTA^{1,2}, Y. YANAGAWA³, N. HOMMA^{2,4}, M. TAKADA¹, *M. OSANAI^{2,4} ¹Primate Res. Institute, Kyoto Univ., Inuyama, Japan; ²Tohoku Univ. Grad Sch. Med., Sendai, Japan; ³Gunma Univ. Grad. Sch. of Med., Maebashi, Japan; ⁴Grad Sch. Biomed Eng, Tohoku Univ., Sendai, Japan

Abstract: We previously reported that the long-lasting spontaneous Ca^{2+} transients (spontaneous $[Ca^{2+}]_i$ transients), which lasted up to about 300 s, were observed in striatal cells. These Ca^{2+} transients were mainly caused by the Ca^{2+} release from the intracellular Ca^{2+} store (ER) via IP3

receptors. But, the mechanisms that underlie the spontaneous $[Ca^{2+}]_i$ transients remain unclear. Store operated Ca^{2+} entry (SOCE) is thought to be activated by the depletion of Ca^{2+} in ER. Thus, we investigated whether SOCE involved in the spontaneous $[Ca^{2+}]_i$ transients or not. At first, we confirmed the presence of SOCE in the striatal neurons using Ca^{2+} imaging in the striatal slice preparations. In the striatum more than 95% neurons are GABAergic, thus, the GAD67-GFP knock-in mice were used for visualization of the GABAergic neurons. After depletion of Ca^{2+} in the ER by the application of thapsigargin, $[Ca^{2+}]_i$ was elevated when the extracellular Ca²⁺ concentration returned to normal level from the Ca²⁺-free condition. This $[Ca^{2+}]_i$ elevation was blocked by the application of the blocker of the SOCE, SKF96365. Thus, striatal GABAergic neuron has SOCE. Next, we investigated the contribution of SOCE in the spontaneous $[Ca^{2+}]_i$ transients. To quantify the amount of the Ca^{2+} influx, we used the method of the Mn^{2+} quenching of the fluorescent Ca^{2+} indicator. The time constant of the quenching was faster in the neurons exhibiting the spontaneous $[Ca^{2+}]_i$ transients compared with the neurons not exhibiting the [Ca²⁺]_i transients under of TTX administration. SKF96365 tended to decrease the frequency of the spontaneous $[Ca^{2+}]_i$ transients. These results suggested that the spontaneous $[Ca^{2+}]_i$ transients led to activate SOCE in the striatal cells, and that SOCE might contribute to the maintenance of the high $[Ca^{2+}]_i$ levels during the spontaneous $[Ca^{2+}]_i$ transients.

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Poster

315. Striatal Physiology

Location: Halls A-C

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Topic: E.03. Basal Ganglia

Support: NIAAA ZIA AA000416

Title: Dopaminergic modulation of striatal cholinergic interneurons governs sequence learning

Authors: *J. H. CHANCEY¹, D. M. LOVINGER²

¹LIN, NIH-NIAAA, Rockville, MD; ²Chief, Lab. Integrative Neurosci, Natl. Inst. on Alcohol Abuse and Alcoholism Rockville Office, Rockville, MD

Abstract: Learning action sequences is necessary for normal daily activities, such as tying your shoes. The dorsal striatum (dStr) has been shown to encode learned action sequences. Specifically dorsal striatal medium spiny neurons (MSNs), change their firing rate at the start and/or stop of action sequences and are able to encode entire learned action sequences as a single event through sustained changes in firing throughout the sequence. Acetylcholine (ACh), released from tonically active cholinergic interneurons (CINs), regulates striatal function by

acting on a diverse group of ACh receptors found on MSNs, presynaptic glutamate and dopamine terminals, and GABAergic interneurons, which regulate MSN excitability, neurotransmitter release, and striatal synaptic plasticity. CINs in the dStr pause their tonic firing during learned action sequences, but the significance of this physiology for behavioral performance has not been explored. We hypothesize that CIN pausing is critical to regulate dopamine and glutamate release and MSN excitability, allowing for plasticity necessary for learning. Activation of dopamine type-2 receptors (D2Rs) on CINs drives pauses in firing. Here we show that genetic deletion of D2Rs specifically from CINs by crossing D2-floxed mice with Chat-Cre mice (D2F-ChatCre) inhibits this D2R-mediated pausing. D2F-ChatCre mice show deficits in learning an operant action sequence task and lower breakpoints in a progressive ratio task, suggesting a reduced motivation to work for sucrose reward, but show no deficits in an accelerating rotarod task, indicating that motor skill learning is intact. Additionally, D2F-ChatCre mice perform similar to controls in an operant reversal learning task, indicating normalthat behavioral flexibility, an aspect of cognitive function impaired y is normal, which is impaired in CIN ablation studies. Using in vivo recordings of unit activity in dStr throughout acquisition of the sequence task we found that D2F-ChatCre mice have deficits in sequence encoding, with fewer MSNs encoding action entire action sequences compared to controls., suggesting that Thus, CIN D2 deletion appears to impairs a neural substrate of action chunking. Virally replacing D2Rs in CINs in the dStr, improves action sequence learning, but does not rescue the lower breakpoints, further suggesting that D2Rs on CINs in the dorsal striatum are critical for sequence learning, but not for driving the motivational aspects of the task.

Disclosures: J.H. Chancey: None. D.M. Lovinger: None.

Poster

315. Striatal Physiology

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: E.03. Basal Ganglia

Support: SNSF

Title: Differential impact of specific midbrain to striatal cholinergic interneurons pathways in conditioned behavior

Authors: *K. TAN, Z. LI, A. MERLI, G. RIZZI Univ. of Basel, Basel, Switzerland

Abstract: Cholinergic interneurons (CINs) represent 1 to 2% of the striatal population, yet they play a determining role in learning. Their firing pattern makes them unique; they are spontaneously active, exhibit profound after hyperpolarization activity and respond to salient

stimuli irrespective of the valence, mainly through a brief firing suppression. This pause can be preceded or followed by an acute excitation. Such responses have been shown to mediate the acquisition of the association between a predictive cue and an external stimulus, and as such, the CIN pause response has been proposed to represent a neural correlate of classical conditioning. This response has been shown to be linked to midbrain dopamine (DA) neuron's activity. Specifically, it is absent in DA depleted animals and time-locked to an increase of DA neuronal firing. At the circuit level, midbrain inputs to CINs have been described, however it is still not clear how these inputs impact CIN mediated conditioning. More specifically, the midbrain is composed of several neuronal types including glutamatergic, GABAergic and DAergic cells. Furthermore, DA neurons have been shown to be able to co-release glutamate or GABA. Although a few studies have investigated midbrain to striatal neurons circuitry, the results come from independent and different experimental conditions making the observations hard to reconcile. Here, we aim to systematically dissect the midbrain to CIN pathways and evaluate how each midbrain cell type and related pathway influences CINs firing response in order for them to support conditioned behavior. Our preliminary data suggest that all three neuronal cell types contact CINs but only the glutamatergic and GABAergic inputs (vglut2- and vgat-cre expression) form monosynaptic contacts whereas DA neurons (manipulated trough dat-cre expression) inhibit CINs but via an indirect pathway. Optogenetic manipulation of these pathways in a contextual fear conditioning design differentially affects acquisition of the contextual cues.

Disclosures: K. Tan: None. Z. Li: None. A. Merli: None. G. Rizzi: None.

Poster

315. Striatal Physiology

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Topic: E.03. Basal Ganglia

Support: a NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation

Title: Subcellular functional analysis of direct pathway striatal spiny projection neurons in patch versus matrix compartments of the dorsal striatum

Authors: *E. M. PRAGER, C. CUHNA, M. HARNETT, J. L. PLOTKIN Dept. of Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY

Abstract: The dorsal striatum has the complex computational task of integrating highly convergent excitatory afferents from diverse cortical and subcortical brain regions, in order to guide action selection and motor decision-making. One way the striatum deals with this

challenge is by distributing inputs among specialized neuronal subtypes, including striatal spiny projection neurons (SPNs), which are situated in two different chemically- and anatomicallydefined compartments (referred to as patches and matrix). Differential engagement of patch vs matrix SPNs during specific behaviors has been proposed, but our understanding of the cellular properties that may underlie such differential engagement is limited. This is due to the relative low density of patches within the striatum, and an inability to distinguish compartments in live tissue slices using traditional microscopy. To overcome these limitations, we have crossed Nr4a1-eGFP transgenic mice, in which GFP is preferentially expressed in patches, with Drd1tdTomato mice, which label D1-type dopamine receptor-expressing direct pathway SPNs (dSPNs), allowing comparable SPN types to be functionally compared in patches and matrix. In agreement with previous studies, the soma of dSPNs located in patches were more excitable than those in the matrix, as reflected by higher input resistance and lower somatic current injection required to elicit action potentials. Because patch and matrix SPNs receive different constellations of glutamatergic inputs, all of which synapse on dendrites that are spatially and electrically distant from the soma, we used 2-photon imaging and 2-photon uncaging of glutamate to assess postsynaptic dendritic synaptic integration in an input-unbiased manner. Dendritic spine density and NMDA/AMPA receptor mediated current ratios were similar in dSPNs in patch vs matrix compartments. Postsynaptic integration of spatially and temporally coordinated synaptic inputs was also similar in patch vs matrix dSPNs: there was no difference in the number of neighboring activated spines required to evoke regenerative plateau potentials in distal dendrites, nor was there a difference in the duration of these plateau potentials. Thus, although somatic excitability is higher in SPN patches, postsynaptic dendritic integration of glutamatergic inputs is similar between the two compartments. Despite similarities in synaptic integration, preliminary data suggest that D1-type dopamine receptor modulation of synaptically evoked dendritic plateau potentials may be restricted to dSPNs residing in the matrix. The mechanism underlying this compartment disparity is currently being explored.

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Poster

315. Striatal Physiology
Location: Halls A-C
Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM
Program#/Poster#: 315.14/FF16
Topic: E.03. Basal Ganglia

Support: intramural research program of NIEHS/NIH

Title: Parallel striatal pathways collaboratively control the dynamics and fate of actions

Authors: *C. MENG¹, J. ZHOU², A. PAPANERI², G. CUI² ¹Lab. of Neurobiology/ In Vivo neurobiology group, NIH/NIEHS, Durham, NC; ²NIH/NIEHS, Research Triangle Park, NC

Abstract: The striatum is the central hub of the basal ganglia that integrates glutamatergic inputs from the cortex and the thalamus, and dopaminergic input from the substantia nigra pars compacta. We have recently reported that the two types of striatal projection neurons (SPNs), the D1 dopamine receptor-expressing direct-pathway SPNs and the D2 dopamine receptorexpressing indirect-pathway SPNs, are both activated during action initiation. To further investigate the relationship between these two pathways and their roles in movement control, we have developed a novel spectrally resolved fiber photometry method that allows for simultaneous monitoring of neural activity from two or more molecularly defined groups of neurons. By intrastriatal infusion of a mixture of rAAV vectors expressing Cre-On red calcium indicator jRGECO1a and Cre-Off green calcium indicator GCaMP6f in D1-Cre and A2A-Cre mice, we were able to simultaneously measure the neural activity from direct- and indirect- striatal pathways in freely moving mice. We have found that strong activation in the direct pathway in conjunction with weak activation in the indirect pathway in one hemisphere leads to a 'start and go' type of contraversive movement in the arena, whereas strong activation in both pathways in one hemisphere produces a 'start and stop' type of movement featuring quick initiation of a contraversive movement immediately followed by an abrupt stop. Our results suggest that balance between striatal direct- and indirect-pathways determines the dynamics and fate of actions.

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Poster

315. Striatal Physiology

Location: Halls A-C

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Program#/Poster#: 315.15/FF17

Topic: E.03. Basal Ganglia

Title: Molecular tools for In vivo spectrometry: Viral vectors designed to target neuronal subsets

Authors: *A. PAPANERI, C. MENG, J. ZHOU, C. MAZZONE, G. CUI Neurobio., NIEHS, Research Triangle Park, NC

Abstract: Recent technological and molecular biological advancements have broadened the capabilities for understanding neuronal circuitry, which is a shared pursuit that would provide countless benefits to the scientific and medical communities. Currently, novel optical imaging technologies are being paired with novel foreign gene delivery vectors to improve signal detection sensitivity as well as gene expression efficiency. Additionally, the employment of site-

directed DNA recombination technology and tissue-specific promoters in viral vectors has enabled a refined targeting of neuronal populations with great functionality and flexibility. We use these molecular techniques to produce adeno-associated viruses (AAVs) with fluorescent genetically encoded calcium indicators (GECIs) to visualize the activity of neuronal subsets in behaving mice. Optical fiber photometry is used to record from multiple probe sites within the active mouse brain. Data is thereby collected only from the effected neuronal population and can be analyzed not only for neuronal activity, but also connectivity, kinetics, and degradation, among other measures. Multiple combinations of AAVs with various tissue-specific promoters, recombinases and their recognition sites are possible. By focusing more precisely than tissuespecific promoters, we hope to achieve accuracy and efficiency with even greater specificity. For example, the hSynapsin promoter, known for its exclusive activity in neurons, is often used instead of the more general CAG or Ef1a promoters to target expression of foreign genes to neurons. We now move to the use of promoters of individual proteins associated with neuronal subsets, such as those for dopamine receptor (DAR), pro-opiomelanocortin (POMC) or agoutirelated protein (Agrp) positive neurons. For instance, AAVs expressing Cre recombinase under the control of promoters specific for DARs type I (D1) and/or type II (A2A) can be used in conjunction with AAVs expressing double floxed inverted reporter genes to limit data collection to those specific neurons. We can use this format to study mouse models of Parkinson's Disease (PD). From in vivo recordings in mice, spectral data collected over time can distinguish between normal and degraded neuronal activity, which may correlate with declining motor behavior. This visualization of PD-like disease progression in the brain could be used to evaluate potential treatment methods and prevention strategies in real time. We continue to work to expand this existing molecular toolkit to provide greater flexibility and convenient adaptability for future research studies.

Disclosures: A. Papaneri: None. C. Meng: None. J. Zhou: None. C. Mazzone: None. G. Cui: None.

Poster

315. Striatal Physiology

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 315.16/FF18

Topic: E.03. Basal Ganglia

Support: NIH/NIEHS intramural research program

Title: Simultaneous dual color recording of neural activity from parallel striatal pathways

Authors: ***J. ZHOU**¹, C. MENG², A. PAPANERI⁴, G. CUI³ ¹NIEHS/NIH, Research Triangle Park, NC; ²Lab. of Neurobiology/ In Vivo neurobiology group, NIH/NIEHS, Durham, NC; ³Neurobio. Lab., NIH/NIEHS, RTP, NC; ⁴Neurobio., NIEHS, Research Triangle Park, NC

Abstract: Monitoring the cellular and molecular events in specific types of cells in behaving animals using genetically encoded fluorescent sensors is a powerful approach to decode neural correlates of specific behaviors, and to uncover the mechanisms underlying the psychiatric and neurological disorders. Here we report a novel in vivo method that allows for high speed (50 Hz) continuous recordings of fluorescence signals with high spectral resolution (7.7 nm) and wide spectral range (350-1100 nm) from deep brain structures through a small diameter (125 micron OD) optical fiber in freely moving animals. By applying linear unmixing algorithm on the recorded emission spectra of multiple fluorescent sensors, the new photometry method can be used to simultaneously measure multiple cellular events in a local brain circuit when the animals are performing behavioral tasks. To simultaneously record neural activity from striatal directand indirect-pathway neurons, we infused Cre-On DIO-jRGECO1a (red calcium indicator) and Cre-Off Lox-FAS-GCaMP6f (green calcium indicator) rAAV vectors into the dorsal striatum of D1-Cre and A2A-Cre mice. We found that although the striatal direct and indirect pathways are mostly activated at the same time during voluntary movements, the differences in the magnitude of activation in these two pathways often lead to different behavioral consequences. Using this method, we are studying how dopamine modulates the activity in these two striatal pathways.

Disclosures: J. Zhou: None. C. Meng: None. A. Papaneri: None. G. Cui: None.

Poster

315. Striatal Physiology

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 315.17/FF19

Topic: E.03. Basal Ganglia

Support: Israel Science Foundation (ISF) grant (743/13)

Title: Striatal representation of competing cortical information

Authors: *M. ISRAELASHVILI, I. BAR-GAD Bar Ilan Univ., Ramat Gan, Israel

Abstract: Abstract

The striatum, which is the main input nucleus of the basal ganglia, receives excitatory input from most of the cerebral cortex. The striatum contains feedforward and feedback GABAergic connections encompassing both projection neurons and interneurons, forming an extensive inhibitory network. This unique architecture prompted the hypothesis that the function of the basal ganglia in general and specifically of the striatum is to select a single action out of a

multitude of actions presented by massive convergent cortico-striatal inputs. In this context, the GABAergic network inhibits all the actions presented by the cortex except for a single "selected" action. Currently, there are limited physiological evidences for the functional role of the striatal inhibitory network and for the validation of this hypothesis. In this study we examine the processing of competing cortical input in the striatum in freely behaving rats. We used electrical and optical stimulation of multiple cortical areas in conjunction with somatosensory input while simultaneously performing extracellular recordings from the striatum. Characterization of the neuronal activity of the striatum that occurs during separate as well as simultaneous stimulation of different cortical areas was performed. We observed both synergistic and antagonistic interactions between cortical inputs in the striatum, supporting a complex representation of the cortical activity by the striatum. This remapping of cortical information suggests that the research of cortico-basal ganglia information processing requires the progression to the study of the interaction between multiple sources of input to this pathway.

Disclosures: M. Israelashvili: None. I. Bar-Gad: None.

Poster

315. Striatal Physiology

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 315.18/FF20

Topic: E.03. Basal Ganglia

Support: NIH Grant AA016022

Title: Differences in LTP between dorsolateral and dorsomedial striatum: Induction frequency, D1 receptors, and Trkb receptors

Authors: *V. LEWITUS¹, A. KAISER², R. KEITH³, K. T. BLACKWELL² ¹Sch. of Systems Biol., ²Bioengineering, ³Interdisciplinary Neurosci. Program, George Mason Univ., Fairfax, VA

Abstract: The striatum of the basal ganglia is associated with learning a variety of behaviors such as motor control and habit formation, and has been implicated in habitual drug use. The dorsolateral (DL) striatum receives input from the primary sensorimotor cortex and is involved in procedural learning and habit formation, while the dorsomedial (DM) striatum receives input from the association cortex and is involved in spatial learning and goal-oriented behavior. Previous studies have found that DM neuron spiking is entrained to a higher theta frequency than DL neuron spiking. In addition, previous studies disagree about the requirement for D1 receptors, with some showing that D1 receptors are required for long term potentiation (LTP) in the DM striatum (Hawes et al., 2013) but others showing that D1 receptors are not required in the DL striatum (Park et al., 2014). These two studies used different stimulation paradigms, so it

is possible that the apparent regional difference may instead be attributed to different stimulation protocols, which may recruit different molecular mechanisms. To investigate the extent of regional differences in striatal LTP, we measure whether a larger LTP is produced by a lower theta frequency in the DL striatum, corresponding to *in vivo* theta rhythms. We use extracellular field recording on adult C57B16 mouse brain slices to measure LTP in response to a theta-burst stimulation of either 10.5 Hz or 5 Hz. Our results show that 10.5 Hz but not 5 Hz produces LTP dorsomedially; in contrast, both 10.5 Hz and 5 Hz produce similar LTP amplitudes dorsolaterally. We test LTP induction in both male and female mice and find that male mice exhibit greater LTP than females, independent of estrus status. Using 5 Hz to induce LTP, we demonstrate that blocking D1 receptors with SCH23390 in the DL striatum prevents LTP, suggesting that this molecular mechanism does not differ between striatal regions. This study also investigates the role of BDNF in the DM and DL striatum by repeating LTP induction while blocking TrkB receptors. In conclusion, although no difference between the DM and DL striatum is found in D1 receptor requirement for LTP, a difference is found in temporal sensitivity, suggesting that other molecular mechanisms may differ between the two regions.

Disclosures: V. Lewitus: None. A. Kaiser: None. R. Keith: None. K.T. Blackwell: None.

Poster

315. Striatal Physiology

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Program#/Poster#: 315.19/FF21

Topic: E.03. Basal Ganglia

Support: NIH Grant K99 MH110597

NIH Grant R01 NS078435

NIH Grant F32 NS083369

Title: Fast-spiking interneurons regulate ensemble calcium signaling and striatum-dependent learning

Authors: *S. F. OWEN¹, J. D. BERKE², A. C. KREITZER¹ ¹Gladstone Inst. of Neurolog. Dis., San Francisco, CA; ²UCSF, San Francisco, CA

Abstract: The striatum is the primary input nucleus of the basal ganglia, a set of sub-cortical nuclei that are essential for motor planning and action selection. Parvalbumin-expressing fast-spiking interneurons (FSIs) in the dorsal striatum have been linked to decision making during goal-directed behavior, while defects in striatal FSIs have been associated with movement disorders including tics and dyskinesias. However, their role in learning and network plasticity is less clear. Within the striatum, the primary input nucleus of the basal ganglia, loss of FSI

function has been associated with involuntary movements in animals and Tourette's Syndrome in human patients. Our results indicate that selective ablation of striatal FSIs is not sufficient to impair acute motor performance. Instead, loss of FSI function disrupted striatum-dependent forms of learning. To investigate the mechanism underlying these striatal learning deficits, we combined in vivo optogenetic control of FSIs with Ca2+ imaging of striatal medium spiny projection neurons (MSNs) in freely moving mice. Together, our data support a model in which striatal FSIs facilitate adaptive motor learning through coordination of task-related Ca2+ signaling and plasticity in MSN networks.

Disclosures: S.F. Owen: None. J.D. Berke: None. A.C. Kreitzer: None.

Poster

315. Striatal Physiology

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Topic: E.03. Basal Ganglia

Support: CRCNS program, NIAAA R01 AA016022

CRCNS program, NIDA R01 DA038890

Title: Spatiotemporal GABAergic inhibition regulates cooperative spine calcium signaling in a spiny projection neuron model

Authors: *D. B. DORMAN, K. T. BLACKWELL Krasnow Inst. for Advanced Study, George Mason Univ., Fairfax, VA

Abstract: Spiny projection neurons (SPNs) of the striatum contribute to goal-directed and habit learning by integrating glutamatergic input from cortex and thalamus. SPNs also receive GABAergic input from multiple types of striatal interneurons and SPN collaterals. Experimental work has demonstrated that temporally-aligned glutamatergic activation of 10-20 neighboring spines located distally on SPN dendrites elicits regenerative, NMDA-dependent plateau potentials that resemble the transitions observed *in vivo* from hyperpolarized downstates to depolarized upstates. These results suggest that NMDA plateau potentials may drive state transitions and, due to strong calcium transients accompanying plateau potentials, may control synaptic plasticity in SPNs. Though GABAergic inhibition is important to SPN function *in vivo*, its effect on dendritic and spine calcium dynamics evoked by plateau potentials has not been examined. Here we investigate the effects of spatial and temporal distributions of GABAergic inhibition on distally clustered glutamatergic input in a biologically detailed SPN computational model with sophisticated calcium dynamics. Specifically, we examine the effects of dendritic location and timing of GABA_A synapses (relative to glutamatergic activation of a distal cluster of

spines) on nonlinear spine and dendritic calcium transients. Additionally, as GABA_A currents from neurogliaform synapses exhibit significantly slower kinetics than currents from fast spiking interneurons, low threshold spike interneurons, or SPN collaterals, we investigate the effects of GABA_A kinetics on regulating glutamatergic integration. We find that GABA inhibition can diminish supralinear spine calcium transients evoked by glutamatergic activation of clustered spines when GABA_A synapses are close to the site of glutamatergic activation and within a time window from 50-100 ms before to 25 ms after glutamatergic activation. In contrast, proximal GABA_A synapses did not exhibit an inhibitory effect. Further, we find that slow GABA_A kinetics have a stronger inhibitory effect than the faster GABA_A synapses. These results suggest that distal synapses regulate distally clustered glutamatergic activation and calcium signaling, while the proximally synapsing fast spiking interneurons are unlikely to do so. Together, our results indicate that GABAergic signaling in the striatal network is important for regulating NMDA-plateau potentials and calcium signaling in SPNs.

Disclosures: D.B. Dorman: None. K.T. Blackwell: None.

Poster

315. Striatal Physiology

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 315.21/GG1

Topic: E.03. Basal Ganglia

Title: Coordinated encoding of action velocity by striatal fast-spiking interneurons

Authors: ***M. H. PATTON**¹, B. M. ROBERTS³, M. G. WHITE¹, R. CHEN², B. N. MATHUR¹ ¹Pharmacol., ²Diagnos. Radiology and Nuclear Med., Univ. of Maryland, Baltimore, Baltimore, MD; ³Physiology, Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom

Abstract: The dorsal striatum is critical for action selection. The output of this nucleus is regulated by powerfully inhibitory GABAergic fast-spiking interneurons (FSIs), which comprise roughly 1% of the total population of striatal neurons. By virtue of the presence of electrical synapses between FSIs, modeling studies propose that these neurons synchronize *in vivo*. However, *in vivo* electrophysiological recordings to date suggest that these neurons fire idiosyncratically. To resolve this and to provide insight into how these neurons encode action, we employed *in vivo* Ca²⁺ imaging and optogenetics in mice in conjunction with *ex vivo* whole-cell patch clamp electrophysiology. We find that rapid integration of cortical inputs to FSIs combined with a moderate degree of electrical coupling between FSIs provides a possible substrate for our *in vivo* observation that coordinated FSI activity encodes action velocity. Additionally, optogenetically manipulating FSIs *in vivo* further implicates this population in action control. These results substantiate the critical role of FSI feed-forward inhibitory

governance of striatal output and provide insight into movement disorders, such as Tourette syndrome, that are associated with FSI deficits.

Disclosures: M.H. Patton: None. B.M. Roberts: None. M.G. White: None. R. Chen: None. B.N. Mathur: None.

Poster

315. Striatal Physiology

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Topic: E.03. Basal Ganglia

Support: NSF Grant 1516288 (CRCNS)

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Title: Motor activity modulates slowly oscillating basal ganglia neurons in awake, dopamine depleted mice

Authors: *T. C. WHALEN¹, A. M. WILLARD², K. J. MASTRO³, J. E. RUBIN⁴, A. H. GITTIS²

²Biol. Sci., ¹Carnegie Mellon Univ., Pittsburgh, PA; ⁴Mathematics, ³Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Oscillatory activity in several frequency bands has long been a hallmark of dopamine depletion (DD) and implicated in motor control in the basal ganglia (BG). Previously, oscillations in the delta band (1-4 Hz) have been reported after DD in the anesthetized mouse BG, but were generally attributed to anesthesia-induced cortical slow wave activity permeating into deep brain regions. To explore the oscillatory properties of BG neurons in awake DD mice and observe how they are modulated by motor activity, we injected 6-hydroxydopamine into the medial forebrain bundle, then recorded from mice head-fixed to a wheel on which they could voluntarily walk or run. Using linear multielectrode probes, we recorded single units in the substantia nigra pars reticulata (SNr), external segment of the globus pallidus (GPe) and subthalamic nucleus (STN) of DD and healthy mice. Approximately 30% of all recorded units in each region displayed prominent delta oscillations in DD, whereas no units oscillated in healthy animals. Using short-time autocorrelation measures, we show that bouts of walking correlated with decreased delta band power in oscillating SNr and GPe units, but increased power in STN units. This study shows single unit slow oscillations in several regions of the awake, dopamine

depleted basal ganglia that are modulated by motor activity in a consistent way within a particular BG region. A deeper understanding of how firing patterns in the basal ganglia change after loss of dopamine innervation will help elucidate dopamine's functional role in shaping basal ganglia activity and motor output.

Disclosures: T.C. Whalen: None. A.M. Willard: None. K.J. Mastro: None. J.E. Rubin: None. A.H. Gittis: None.

Poster

315. Striatal Physiology

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Topic: E.03. Basal Ganglia

Support: NIH Grant ZIA AA000407

Title: In vivo and In vitro synaptic plasticity mechanisms in cortico-striatal circuits

Authors: *K. JUCZEWSKI¹, D. M. LOVINGER²

¹Natl. Inst. on Alcohol Abuse and Alcoholism, NIH, Rockville, MD; ²Chief, Lab. Integrative Neurosci, Natl. Inst. on Alcohol Abuse and Alcoholism Rockville Office, Rockville, MD

Abstract: Measurement of local field potentials (LFPs) is a classical physiological technique often used for general characterization of neural activity in brain circuits. LFPs can be used to characterize various physiological phenomena underlying changes in behavior, from spontaneously occurring oscillations to responses evoked by sensory or electrical stimulation. Electrical stimulation applied at various frequency protocols are known to result in long-term plasticity. Although this technique gives a good overview of circuit dynamics, it lacks specificity important for detailed analysis of plasticity mechanisms in specific neural pathways. This specificity is especially difficult to achieve with electrical-stimulation in vivo. In our studies we combined LFP recording with optogenetic afferent stimulation to overcome this problem, taking advantage of the unique precision of this latter technique. First, we compared electrically- and optically-evoked LFPs in the dorsal striatum in brain slices and anesthetized mice to characterize the basic properties using varying stimulus parameters. Next, we compared long-term plasticity changes evoked by low- and high-frequency protocols. Finally, assessed pharmacological effects on plasticity by application of well-characterized pharmacological agents such as AP5, NBQX, TTX, kynurenic acid, alcohol and more. Our results proved that LFPs can be evoked with channel-rhodopsin 2 (ChR2) expression and light stimulation. We also showed similarities and differences between electrically- and optically-evoked LFPs and their dependence on intensity of the stimulation current, stimulation parameters, and topographical organization of the recorded region. Furthermore, we revealed that pharmacological modulation of electrically-induced

plasticity affects electrical and optical LFPs to different extents. In all parts of our project we recorded LFPs evoked by stimulation with a tungsten electrode and recorded with a glass pipette or evoked by light stimulation and recorded with an optrode. Mice expressing ChR2 in cortex were generated by breeding Emx1-Cre mice with AiCOP4 conditional ChR2-expressing mice. We examined plasticity mechanisms specifically in cortico-striatal circuits because these circuits underlie action control and action learning. Furthermore, we focused on dorso-medial and dorso-lateral striatum that receive projections from the prefrontal cortex and motor cortex respectively. These pathways are especially interesting due to their engagement in alcohol-related behaviors.

Disclosures: K. Juczewski: None. D.M. Lovinger: None.

Poster

315. Striatal Physiology

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 315.24/GG4

Topic: E.03. Basal Ganglia

Title: Resting-state fMRI analysis revealed human caudate topology along two principle axes: Medial-lateral and anterior-posterior

Authors: *J. F. O'RAWE, M. KHAN, H.-C. LEUNG Stony Brook Univ., Stony Brook, NY

Abstract: The striatum has been implicated in the gating of motor and cognitive behavior. In particular, the caudate has been implicated in goal-oriented learning and action selection. What computations it performs to produce this type of behavior are largely unknown. Understanding the organizational properties of this region is a critical step. While there is a relatively large animal literature in examining afferent and efferent projections of the caudate, the methodology is intrinsically limited. Neuroimaging methods, however, allow us to tackle some of these limitations via continuous sampling of the brain image space. Previous attempts have been made to utilize human brain imaging data to examine the organization of the caudate, though few have taken full advantage of the continuous nature of the data. We've developed a regression based approach of analyzing functional magnetic imaging (fMRI) data, allowing for the quantification of anatomical gradients in functional connectivity. We examined the caudate spatial topology in two databases of healthy participants: the Cambridge-Buckner subset of the 1000 Functional Connectomes database (n = 198, ages 18-30), and the NKI/Rockland database (n = 189, ages 4-85). Our results supported the previous literature by demonstrating stable gradients of connectivity patterns across the caudate's anterior-posterior (A-P) and medial-lateral (M-L) axis. However, we found that the differential connectivity along the M-L gradient is better described with respect to networks, with default mode network preferring the medial extent of the caudate, and the frontoparietal network preferring the lateral extent of the caudate. We replicated this

result across subsamples within the same and across different databases. Further, stable patterns of caudate topology was evident at the single subject level. Lastly, we examined the effects of aging and found that age predicts the integrity of the M-L organization within the head of the caudate, and that this relationship seems mediated by preferential connectivity from the nucleus accumbens (N.Acc) to the anterior caudate. The systems level differential organization in network connectivity along the M-L axis of the caudate suggests the potential integration of internally and externally oriented information. Further, it seems that connectivity decreases with age.

Disclosures: J.F. O'Rawe: None. M. Khan: None. H. Leung: None.

Poster

315. Striatal Physiology

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 315.25/GG5

Topic: H.01. Animal Cognition and Behavior

Title: Spine density in the nucelus accumbens is differentially changed after rat gambling task with different housing condition

Authors: M. KWAK, W. KIM, B. CHO, W. CAI, *J.-H. KIM Yonsei Univ. Coll Med., Seoul, Korea, Republic of

Abstract: Poor decision-making is closely related to symptoms of various psychiatric disorders. Rat gambling task (rGT) adopts the basic principle of Iowa Gambling Task, which is widely used to assess the decision-making process in humans. Dendritic spine is a key structure for structural plasticity in the brain, and its morphology dynamically changes through the learning process. Here we examined how housing condition and choice preference appeared in rGT contributes to morphological change of dendritic spines in the nucleus accumbens (NAc). Rats were housed as isolated or paired, and trained in a touch screen chamber to learn the relationships between 4 different light signals on the screen and accompanied reward outcomes or punishments set up with different schedules. Once they show a stabilized pattern of preference upon free choice, rats were separated as risk-averse or risk-seeking group according to their preference of choice. A control group of rats were housed in pair and never exposed to rGT training. When we visualized dendritic spines by GFP expression incorporated with adeno-associated virus in the NAc, and conducted quantitative analysis, we observed that rGT alone with pair-housing, whether it turned out to be risk-averse or risk-seeking, did not contribute to show any difference in spine density compared to control group. However, when combined with isolation-housing, rGT showed increased number of total and thin spine density only in risk-seeking compared to control groups. These results indicate that trait (risky choice preference) and environment (isolated housing)

inter-influence to contribute to morphological changes of dendritic spines in the NAc, and may suggest that these changes might underlie maladaptive decision making.

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Poster

315. Striatal Physiology

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 315.26/GG6

Topic: H.01. Animal Cognition and Behavior

Title: Visualization of ERM proteins on dendritic spines in the rat nucleus accumbens

Authors: W. CAI, *W. KIM, M. KWAK, J.-H. KIM Yonsei Univ. Col. Med., Seoul, Korea, Republic of

Abstract: The ezrin-radixin-moesin (ERM) proteins are a family of widely distributed membrane-associated proteins. They have been implicated not only in cell-shape determination but also in signaling pathway. When a threonine residue at C-terminal regulatory domain is phosphorylated, they become activated and crosslink F-actin filaments to plasma membrane. Although it has previously shown that ERM proteins are expressed in neuronal cells in the brain, there are few studies directly visualizing their presence on dendritic spines in vivo. The present study aimed to explore the experimental methods to visually detect phosphorylated ERM proteins on dendritic spines of the medium spiny neurons in the rat NAcc. First, we used immersion weak fixation in aldehyde solution to achieve enhanced immunohistochemical staining of phosphorylated ERM proteins in the NAcc tissues. Second, we labeled medium spiny neurons with eGFP by using adeno-associated virus and examined the presence and distribution of phosphorylated ERM proteins on dendritic spine under confocal microscope. Phosphorylated ERM proteins were found located in dendritic shafts and extra-synaptic sites of dendritic spines. These results, first time directly to our knowledge, visually elucidated subcellular localization of phosphorylated ERM proteins in the NAcc medium spiny neurons, and suggest that we may be able to use these methods as a new tool to examine the functional significance of these proteins in the brain.

Disclosures: W. Cai: None. W. Kim: None. M. Kwak: None. J. Kim: None.

Poster

316. The Control of Reaching Movements I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 316.01/GG7

Topic: E.04. Voluntary Movements

Support: NWO-VICI: 453-11-001

Title: Vestibular input modulates visuomotor feedback responses in reaching

Authors: *L. OOSTWOUD WIJDENES, W. P. MEDENDORP

Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ., Nijmegen, Netherlands

Abstract: Reaching movements are controlled continuously. This implies that information relevant to the task at hand is monitored throughout the movement and that the movement is adjusted if necessary. Visual and mechanical perturbations of the hand and the target elicit task-sensitive movement adjustments at very short latencies. Also vestibular perturbations to an otherwise unperturbed body and environment lead to hand movement adjustments. It is unknown if the vestibular system interacts with the online visuomotor control system for reaching movements. Here we asked if vestibular information modulates the gain (defined as the velocity of the hand to correct for perturbations) of visuomotor feedback responses.

Participants were seated on a vestibular sled that translated them forward or backward with a low or high acceleration profile (1.7 or 3.2 m/s^2 peak acceleration, bell-shaped velocity). During the passive full body translation, they were instructed to move their right index finger in the sagittal plane toward a body-fixed target, which jumped perpendicular to the main movement direction in half of the trials. The jump could occur 100, 200 or 350 ms after trial start. If vestibular information modulates the feedback response, the gain of the response to the early jumps should be higher for the fast than for the slow sled profile because the acceleration is higher at the moment of the jump. The gain of responses to the late jump should be equal for the slow and fast sled profile, as the imposed acceleration is equal at this time.

The first results are in line with our predictions. In response to the target jumps, movements were corrected at very short latencies (~150 ms). The gain of the corrections in response to the early jumps was larger for the high than the low acceleration; the gain was not different for late jumps. Importantly, response gains did not differ between forward and backward passive translation, suggesting that inertial forces on the arm in the main movement direction were not modulating the feedback responses. Critically, the distance of the finger relative to the target at the moment of the early jumps was the same for slow and fast sled profiles, ruling out that the gain effect was due to differences in hand position. These results therefore suggest that vestibular information is integrated in visuomotor feedback control at a very early stage.

Disclosures: L. Oostwoud Wijdenes: None. W.P. Medendorp: None.

Poster

316. The Control of Reaching Movements I

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Program#/Poster#: 316.02/GG8

Topic: E.04. Voluntary Movements

Support: NIH/NINDS R01 NS030853

NIH Training Grant 2T32HD057850

Title: Unique oscillatory entrainment of spike timing after ischemic infarct in rat motor cortex is associated with behavioral outcomes

Authors: *M. D. MURPHY¹, A. R. PACK², C. L. DUNHAM³, S. BARBAY³, D. J. GUGGENMOS³, R. J. NUDO^{3,4}

¹Bioengineering Grad. Program, Univ. of Kansas, Lawrence, KS; ²Neurosci. Doctoral Program, Emory Univ., Atlanta, GA; ³Dept. of Physical Med. and Rehabiliation, ⁴Landon Ctr. on Aging, Univ. of Kansas Med. Ctr., Kansas City, KS

Abstract: Following ischemic infarct in the cerebral cortex, spared neurons fire in noisy bursts that function as a signal for neurite growth. While this activity has been suggested to promote beneficial axonal reinnervation, it may also interfere with behavior by disrupting motor-related neural activity during critical post-infarct periods, when rehabilitation is optimal. Weak, periodic signals, such as neuronal subthreshold membrane voltage oscillations, may be amplified by noisy events via stochastic resonance (SR). In the distribution of inter-spike intervals (ISIs) for a given neuron, SR is evidenced by multiple peaks. Therefore, multi-peak ISI (MP-ISI) may indicate the presence of noisy processes that disrupt normal spike timing in behavior. We investigated whether MP-ISI is associated with behavioral outcomes in rats performing a pellet-retrieval task after an endothelin-1 (ET-1) ischemic infarct of primary motor cortex (M1). Extracellular activity was recorded using micro-wire arrays implanted in ipsilesional premotor cortex (PM) and contralesional M1. Spikes were sorted into clusters according to their waveform and location. ISIs were aggregated separately for spikes in epochs surrounding either unsuccessful or successful grasps. To test whether noisy spiking is associated with behavioral outcomes, we counted and classified the number of spike clusters with MP-ISI during one or both alignment epochs. Spike clusters demonstrated comparable average firing rates and burstiness for both healthy and lesioned rats. While more total spike clusters demonstrated MP-ISI in rats with ET-1 lesions (114 of 157 spike clusters with MP-ISI were from lesioned rats), there was a significant increase in the frequency of MP-ISI in lesioned rats during either only unsuccessful or only successful grasps (57 of 69 spike clusters) compared to spike clusters with MP-ISI in both grasp outcomes (57 of 88 spike clusters; G = 6.44, df = 2, $P[X^2 > G] < 0.05$). Qualitative differences in spike timing patterns indicate that the oscillations emphasized in MP-ISI are unique to

behavioral outcome. These results suggest that noisy spiking processes inherent to recovery from ischemic infarct potentially amplify spike timing entrainment to weak neural oscillations during behavior.

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Poster

316. The Control of Reaching Movements I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 316.03/GG9

Topic: E.04. Voluntary Movements

Title: Sensory prediction and sensory surprise in goal-directed movement

Authors: *G. JURAVLE^{1,2}, F. L. COLINO³, G. BINSTED⁴, A. FARNE⁵

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Abstract: Goal-directed movement affects tactile perceptual processing. Specifically, touch is suppressed during action (see Juravle, Binsted, & Spence, 2016, for a recent review). Here, we set to investigate the reverse influence, that is, whether and how the visually-conveyed tactile qualities of an object to be grasped affect the kinematics of the executed movement. To achieve this purpose, each trial we presented our participants with a cylindrical object and instructed them to prepare to grasp it. Vision was then occluded for a variable delay during which the object could be replaced (or not), depending on the condition for the trial. The return of vision was the go signal for the movement. Participants reached for, grasped, and lifted the object off the table. We used two objects, a smooth cylinder and an irregularly carved one. Furthermore, because we were interested in how sensory priors might affect the movement profile, in two separate experiments (each with N = 16), we manipulated the probability of the object being the same or different at the time of the go signal (by blocks: 100%, 50%, and 80%). In an additional block (80% +), a third unexpected 'spiky' object was presented only twice. Results indicate that the visually-conveyed tactile attributes of an object consistently affect the transport component of the reach-to grasp movement, as a function of their predictability. These findings suggest that perception modulates movement in a reverse fashion to the documented tactile suppression. Taken together, our results point toward sensorimotor integration as a bidirectional process, with the motor prediction being adjusted online during the reaching movement based on available visual input and current sensory priors.

Disclosures: G. Juravle: None. F.L. Colino: None. G. Binsted: None. A. Farne: None. Poster

316. The Control of Reaching Movements I

Location: Halls A-C

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Topic: E.04. Voluntary Movements

Support: National Science Foundation of China

Shanghai Bureau of Science and Technology

Title: Neuronal activity in motor cortex during coherent sequential reach

Authors: *T. WANG, Y. ZHANG, H. CUI Inst. of Neuroscience, CAS, Shanghai City, China

Abstract: While motor cortex has been found to exhibit heterogeneous and dynamic firing patterns related to various movement kinematics (i.e., direction, speed, and distances) during arm movement, sensory or cognitive factors (i.e., serial target presentation) also influence its activities. To examine temporal dynamics of directional selectivity during sequential arm movement, we recorded single-unit activity from the motor cortex while a monkey performed a coherent double reach instructed by memorized cues presented simultaneously. The monkey was seated in front of a touch screen. Trial began with a green circle displayed on the center of the screen, monkey was required to touch the green circle for 300 ms. Then, in 1/3 of trials (singlereach trials), another green circle was presented as a reaching goal for 400 ms (cue period) at one of six vertexes locations of a regular hexagon around the central green circle with side length 15 cm. After the cue was extinguished, the monkey had to keep his hand on the central green circle for a delay period of 600 ms before it was turned off (GO signal), and the monkey was then required to reach to the previously cued location to obtain a reward. In the remaining trials (double-reach trials), a green square and a green triangle were presented simultaneously, with the triangle placed at a location either 120° clockwise (1/3 of trials) or counter-clockwise (1/3 of trials) from the square. After the 600 ms delay, the monkey was required to reach the remembered square location first, and then to reach the remembered triangle location immediately, the monkey was rewarded only if he reached the both targets accurately in the correct order. All the 18 trial types (3×6 , three conditions by six directions) were pseudorandomly interleaved. Among 62 neurons recorded from one monkey to date, 30 of them showed significant directional tuning (one-way ANOVA, p<0.05) during pre-movement (200 ms before first movement onset) or peri-movement (-100-100 ms around first movement onset). While some neurons exhibited differential pre-movement activity for single-reach and double-reach trials with same first reach, most neurons showed similar preparatory activity before GO signal

in the double-reach trials but became tuned for the second reach before the first reach offset. These results suggested that movement preparation rather than execution is a predominant function of motor cortex.

Disclosures: T. Wang: None. Y. Zhang: None. H. Cui: None.

Poster

316. The Control of Reaching Movements I

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Topic: E.04. Voluntary Movements

Support: National Science Foundation of China

Shanghai Bureau of Science and Technology

Title: Neuronal activity in motor cortex during flexible manual interception

Authors: *Y. ZHANG, T. WANG, H. CUI

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Abstract: Although motor cortex traditionally was believed to contain 'upper motor neurons' driving muscle, converging recent evidence suggested that its heterogeneous activity carries information far beyond movement kinematics/kinetics, including large cognitive components and responses to visual and somatosensory inputs. To elucidate dynamic interplay between sensory inflow and motor outflow, we recorded single-neuron activity from motor cortex while macaque monkeys performed a flexible manual interception task. The monkey initiated a trial by positioning a hand at the center of a touch screen for 600ms. Then, a peripheral target (green circle) appeared and whirled along a circular annulus around the center, and the monkey was required to intercept it within 800 ms after the random delay (400 ± 100 ms). Once a peripheral location was touched, the target stopped, and if the angle between target and hand endpoint was less than 15°, the trial was a success and the endpoint was marked for feedback with a red circle (blue if missed). The moving target appeared at a random direction and moved with shuffled five speed conditions: 120° and 240°/s clockwise, 120° and 240°/s counter-clockwise, and 0°/s (control). The arm trajectory was sampled by an electromagnetic tracking system (Polhemus G4). In such a flexible stimulus-response contingency, interceptive reach was launched to the future interception zone rather than the instantaneous stimulus location. Under the incongruent incoming sensory stimuli and impending movements, most neurons we recorded to date exhibited invariant movement tuning for different target motion speeds, suggesting that the motor cortex plays an intimate role in forming motor programs based on sensory inputs and behavioral context.

Disclosures: Y. Zhang: None. T. Wang: None. H. Cui: None.

Poster

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Topic: E.04. Voluntary Movements

Support: NIH NINDS NS035103

NIH NINDS F32 NS093721-01

Title: The functional organization of movement maps in New World titi monkeys

Authors: *M. K. BALDWIN¹, A. C. HALLEY², L. A. KRUBITZER³ ¹Ctr. for Neurosci., Univ. of California Davis, Davis, CA; ²Ctr. for Neurosci., Univ. of California, Davis, Davis, CA; ³UC Davis, Davis, CA

Abstract: The current study is part of an ongoing project in the laboratory exploring and comparing the functional organization and dynamics of cortical motor areas in primates and their close relatives. Recent evidence from our lab using long-train (LT) intracortical microstimulation (ICMS) in macaques and tree shrews (one of the closest living relatives of primates) has shown that movements can be evoked not only from classical frontal and posterior parietal motor regions of the brain, but also from somatosensory cortex (Baldwin et al., 2016; Baldwin et al., 2017). Further, in tree shrews, we have discovered that evoked movements within somatosensory cortex are unaffected when motor cortex is reversibly inactivated (Baldwin et al., 2014). The current study explores the extent and organization of evoked movements using LT-ICMS, and how the different cortical fields from which movements can be evoked are functionally organized using reversible inactivation methods. Titi monkeys were chosen for these experiments because they share many common brain features with macaque monkeys; however, all motor and somatosensory cortical fields are located on the cortical surface and are therefore easily accessible for reversible inactivation using cortical cooling. Our main findings were that evoked movements could be elicited from frontal motor areas, posterior parietal cortex, as well as somatosensory cortical areas. Similar to findings in macaques and tree shrews, these movements were complex in nature involving multiple joints and body parts such as the shoulder + elbow + wrist, the hand + mouth, or the forelimb + hindlimb. Unlike macaques, we found few sites at which movements of single digits could be elicited; however, representations of forelimb movements were extensive. When motor cortex was inactivated by cooling, evoked movements in posterior parietal cortex were diminished, while those in somatosensory cortex were relatively unaffected. Our findings suggest that somatosensory cortex may not only provide feedback for

the execution of complex movements, but could itself be directly involved in the generation of these movements.

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Poster

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Topic: E.04. Voluntary Movements

Support: NIH NS095162

NIH T32LM012203-02

Title: Movement characteristics encoded by single neurons in the cuneate nucleus of an awake behaving rhesus macaque

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Abstract: Located in the dorsal brainstem, the cuneate nucleus (CN) is difficult to access with either acute or chronically implanted recording electrodes. As such, there have been few attempts to record CN single units, virtually none from awake animals. Transmission of afferent signals through CN in anesthetized preparations depends on a number of factors, including the history of afferent input and descending cortical inputs. Thus, the representation of somatosensation in CN during behavior is nearly unknown.

Despite these hurdles, we have successfully implanted multi-electrode arrays in rhesus monkeys. We trained the monkey in three types of tasks using a manipulandum to control a cursor on a screen. In the first, the monkey reached to random targets in the workspace. In the second, the monkey performed a classic center-out task, reaching from a center target to one of four outer targets. On some trials, while the monkey held the handle in the center target, motors in the manipulandum randomly applied a force to the monkey used isometric force on the handle to control the cursor, reaching once again to random targets. Although early implants recorded tens of neurons for ~4-6 weeks, in the most recent animal, we recorded ~20 neurons during a series of experiments spanning six months. Another animal had over 50 units, but with only one recording

session prior to failure. Receptive fields of these neurons were typically were on the proximal arm, with a mix of cutaneous and proprioceptive afferents.

95% of neurons modulated their firing rates significantly during the isometric task, with many well-tuned to the direction of applied force. Virtually the same proportion of neurons responded during the active component of the unloaded, center-out task, with 60% of neurons tuned to movement direction. During these active movements, some neurons appeared to modulate their firing rates 50-100 prior to movement onset, suggesting the influence of descending inputs. Three-quarters of neurons responded to the applied bumps, with approximately one-third having some directional tuning. We are able activate individual muscle receptors (muscle spindles and Golgi tendon organs) selectively through vibration and electrical stimulation. We intend to compare these patterns of activation to the modulation observed during the movement and isometric tasks. Furthermore, by using a combination of motion tracking and musculoskeletal modeling to extract muscle lengths and length changes, we will assess the tuning properties of these neurons in intrinsic, as well as Cartesian coordinates.

Disclosures: C. Versteeg: None. **R. Chowdhury:** None. **T. Tomlinson:** None. **J.M. Rosenow:** None. **L.E. Miller:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: JSPS JP15H03062, JP15K12363

Title: Cortical EEG dynamics during reaching movement under the influence of the mirror illusion

Authors: *K. YAMANAKA Showa Women's Univ., Tokyo, Japan

Abstract: Mirror illusion can be used to induce systematic errors for reaching movement made with the unseen hand behind the mirror. However, it remains unclear the cortical mechanism during the reaching movement under the influence of the mirror illusion. The aim of this study is to investigate the cortical mechanism during the reaching movement under the influence of the mirror illusion. Nine right-handed female volunteers conducted reaching tasks under the influence of the mirror illusion. Participant initially placed right hand in front of, and the left hand behind, a mirror placed vertically in the mid-sagittal plane on the table. She asked to move the right hand to the directions toward or away from the mirror. After 10 s of exposure to the mirror reflection (mirror illusion condition) or to the mirror covered by an opaque sheet (control

condition), participant reached to unseen target situated 15 cm forward with their unseen left hand behind the mirror. We recorded the reaching trajectories made with the tip of index finger of the unseen left hand using by VICON motion capturing system. Surface electroencephalography (EEG) was recorded during the reaching movement in the mirror illusion and control conditions and analyzed by a traditional event-related potential (ERP) and a single-trial-based EEG power and phase dynamics. As a result, during an early stage of the reaching movement, horizontal errors were larger in the mirror illusion condition than in the control condition. These results suggest that, in the mirror illusion condition, participants plan and execute their left hand reaching movements to the direction away or toward from the mirror. Next, positive ERP deflections during reaching movement in the mirror illusion condition were larger than those in the control condition over sensorimotor site opposite to the reaching hand. Theta-band EEG power during reaching movement in the mirror illusion condition sustained longer than in the control condition, while theta-band EEG inter-trial phase-locking in the mirror illusion condition was stronger than those in the control condition. These results suggest that, in the mirror illusion condition, participants are difficult to control their left hand in the late stage of the reaching movement under the influence of the mirror illusion because they become more able to detect their visual-proprioceptive conflict in the late stage of the reaching movement.

Disclosures: K. Yamanaka: None.

Poster

316. The Control of Reaching Movements I

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Support: Piper Health Seed Grant

IGERT APAcT Fellowship

GPSA GRSP

GPSA JumpStart

Title: 3D assessment of upper limb proprioception

Authors: *J. D. KLEIN^{1,2,3}, B. WHITSELL^{1,4}, P. ARTEMIADIS^{1,4}, C. A. BUNEO^{1,5,3} ¹ASU, Tempe, AZ; ²Interdisciplinary Grad. Program in Neurosci., Tempe, AZ; ³Alliance for Person-Centered Accessible Technologies IGERT Program, Tempe, AZ; ⁴IRA A. Fulton Sch. of Engin. Arizona State Univ., Sch. of Engin. of Matter, Transport, and Energy, Tempe, AZ; ⁵Arizona State Univ., Sch. of Biol. and Hlth. Systems Engin., Tempe, AZ

Abstract: Proprioception is the sense of body position, movement, force and effort. Loss of proprioception can affect planning and control of limb and body movements, negatively impacting activities of daily living and quality of life. Assessments employing planar robots have shown that proprioceptive sensitivity directionally-dependent within the horizontal plane (Wilson et al, 2010). Little is known however about proprioceptive sensitivity when tested outside the horizontal plane. To investigate proprioceptive sensitivity in 3D we developed a novel experimental paradigm employing a 7-DoF anthropomorphic robot arm (LWR4+, KUKA Inc.), which enables reliable testing of arm proprioception along arbitrary paths in 3D space, including vertical motion. A participant's right arm was coupled to a trough held by the robot that stabilized the wrist and forearm, allowing for changes in configuration only at the elbow and shoulder. Sensitivity to imposed displacements of the endpoint of the arm were evaluated using a "same-different" task, where participant's hands were moved 1-4cm from a previously visited reference position. The proportion of trials where subjects responded "different" when the stimuli were different ("hit rate"), and where they responded "different" when the stimuli were the same, ("false alarm rate"), were used to calculate d', a measure of sensitivity derived from signal detection theory. Data have been obtained from 76 subjects, each of whom was tested in two directions (34 upward, 36 downward, 21 backward, 19 forward, 18 leftward, and 18 rightward). For all directions sensitivity (d') increased monotonically as the distance from the reference location increased. However, we also found differences in sensitivity between directions at some distances. For example, sensitivity was greater for rightward and leftward displacements than for downward and backward ones at 2cm and 3cm (permutation tests, p<0.05). Sensitivity was also greater for upward and forward displacements than for downward and backward ones at 3cm (permutation tests, p<0.05). These data confirm previous findings that the ability to estimate the position of the limb via proprioception is anisotropic across a 3d workspace, which has important implications for understanding the multisensory planning and control of reaching movements in 3D space.

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Poster

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Fondazione del Monte di Bologna e Ravenna (Italy)

FIRB 2013, RBFR132BKP

Title: Segregated processing of reach depth and direction signals in the macaque area PE

Authors: M. DE VITIS^{1,2}, K. HADJIDIMITRAKIS^{1,3}, R. BREVEGLIERI¹, *A. BOSCO¹, C. GALLETTI¹, P. FATTORI¹

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Abstract: The posterior parietal cortex (PPC) includes several anatomically and physiologically defined areas that combine visual and somatosensory information with motor commands to generate appropriate arm movements in the three-dimensional space. Over the years, the role of several PPC areas in the encoding of arm movements in different directions has been extensively studied, but few works have investigated the processing of reach depth and the relative influence of these two signals in the reach-related activity. Area PE (Brodmann's area 5), located in the rostral part of the PPC, is one of the key nodes of the reaching circuit of the medial PPC and has strong projections to the primary motor cortex. In the present study we examined the relative influence of depth and direction information on neurons of the medial part of area PE and characterized their temporal evolution over the course of a fixate-to-reach task in darkness towards targets placed at different depths and directions. The same task configuration was used in the studies of neighboring superior parietal lobule areas V6A and PEc (Hadjidimitrakis et al., 2014, 2015), thus allowing us to compare direction and depth processing between the three PPC areas. Single unit activity was recorded from two Macaca fascicularis (N= 120) and was quantified within four main epochs: target fixation, reaching preparation, execution, and target holding. We found reach-related cells in PE tuned by target depth (16%) and by direction (12%) and by both of these signals (6%, average percentages across task epochs), and these modulations were differently distributed across the task epochs. The tuning of activity by direction was strong at the beginning of the task, whereas the depth tuning mainly occurred during movement execution. Our study shows that different populations of cells code for reach depth and direction, thus arguing for a segregated processing of depth and direction information in PE. This finding is in contrast with evidence from V6A and PEc where high and intermediate, respectively, degree of convergence of depth and direction signals has been reported. Altogether the results from the three areas suggest a gradual parcellation of spatial information that occurs in a caudal-to-rostral fashion in medial PPC. This coding scheme parallels the increase of somatosensory and the simultaneous decrease of visual processing observed along the caudorostral axis of the medial PPC, and it also correlates with the reference frame transformations from extrinsic to intrinsic coordinates that occur in the same direction.

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Poster

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Topic: E.04. Voluntary Movements

Support: MEXT/AMED SRPBS (BMI)

MEXT KAKENHI 26112004

Title: Relationship between muscle synergies and physical performance in patients with hemiparesis

Authors: *T. KAWASE¹, A. NISHIMURA², A. NISHIMOTO², F. LIU², Y. KIM¹, H. KAMBARA¹, N. YOSHIMURA¹, Y. KOIKE¹

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Abstract: Previous studies have suggested that damage to central nervous system may alter muscle synergies, coordinated activities of multiple muscles that organize modules for motor control. To investigate the alternation following the damage will facilitate more effective design of neurorehabilitation technologies. Most of the studies have focused on muscle synergies of the patients recruited during simple tasks, such as isometric force generation or reaching movements, and contributed significant insight into the research field. We have investigated how the muscle synergies were modulated during a peg transfer task in patients with hemiparesis toward the realization of effective neurorehabilitation systems covering complex movements. In the present study, we investigated relationship between the muscle synergies and physical performance, which was measured by Fugl-Meyer (FM) assessment, in patients with hemiparesis. Thirty-two patients with hemiparesis (FM upper arm score 15-64) picked a peg on 3×3 grid points on a board and placed it out of the board sequentially by using their affected arms. Fourteen able-bodied subjects also performed the same task. We estimated a set of muscle synergies for each subject by applying nonnegative matrix factorization to electromyographic signals measured during the task, and classified the synergies into 14 classes by using a hierarchical clustering method. We investigated which class of the synergies affected the FM upper arm scores by using lasso, which is a sparse regression method that can select relevant variables to the explanation, with binary variables that indicated the synergy was detected in the patient or not as explanatory variables and the FM upper arm score as response variables. The results of the regression showed that the FM upper arm scores could be explained moderately by using only variables for 3 classes of the synergies (correlation between measured and estimated FM upper arm scores 0.73). Two of the classes, which had concentrated activity on anterior deltoid or biceps, were assigned positive weights (i.e. increased the scores), and the

other one, which had co-contraction on shoulder muscles, was assigned a negative weight (i.e. decreased the scores). Post-hoc logistic regression with the FM upper arm score as an explanatory variable showed that probabilities of detection of the synergies in the patients significantly depended on the FM upper arm scores (p < 0.05).

These results suggested that the extracted muscle synergies had relationship with physical performance in the patients. The results may be useful for development of new neurorehabilitation technologies.

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Poster

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Topic: E.04. Voluntary Movements

Support: NIH Grant 5T32HD055180-08

Title: Neurobehavioral effects of sensory loss and augmented feedback

Authors: *J. T. JOHNSON¹, L. A. WHEATON²

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Abstract: Motor planning and control are influenced by the availability and reliability of sensory feedback. When proprioceptive sensory feedback is deficient or unavailable, compensatory processes may increase function, but may come at the price of increased cognitive load or decreased quality of movement. It is unclear whether augmented feedback, such as vibrotactile feedback, can ameliorate the effects of these sensory changes. By assessing neural and behavioral activity during a well-characterized motor task with sensory reduction and augmentation, the role of augmented sensory feedback for motor control can be assessed. Electroencephalography (EEG) and kinematic data were gathered while persons (n=12) with sound limbs using a prosthetic limb simulator performed a repetitive reach and grasp task involving medium, small, and large discs. The participants were divided into two groups, one with full vision, and one with a barrier that occluded their vision during the reach and return phases of a reach-grasp- transport-return task. Subjects in both groups performed the task in two conditions, with or without vibrotactile feedback. We hypothesized that vibrotactile feedback would reduce visuomotor cortical network activation with accompanying improvements in efficiency of motor behavior. Neural results for the vibrotactile condition show decreased activation of the visuomotor cortical network in the full vision group, while the occluded group

showed decreased activation of the frontoparietal network. Kinematic results showed no significant effect of vibrotactile feedback on transport velocity. There was a significant effect on aperture, but only for the large disc. In conclusion, the effect of vibrotactile feedback supports our hypothesis regarding neural activity, yet kinematic results appear minimal. This may demonstrate the limitations of artificial sensory feedback for improving motor behavior independent of sensory adaptations following sensory loss.

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Poster

316. The Control of Reaching Movements I

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: E.04. Voluntary Movements

Support: Canada Research Chair

Title: Involvement of the posterior parietal cortex in online control of reaching

Authors: *L. MIKULA^{1,2}, L. PISELLA¹, G. BLOHM³, A. Z. KHAN²

¹Equipe ImpAct, Ctr. de Recherche en Neurosciences de Lyon, Inserm U1028, CNRS UMR5292, Bron, France; ²Ecole d'optometrie, Univ. de Montreal, Montreal, QC, Canada; ³Ctr. for Neurosci. Studies, Queen's Univ., Kingston, ON, Canada

Abstract: Optic ataxia is a consequence of brain damage to the posterior parietal cortex (PPC) resulting in errors during visually-guided arm movements. Because the PPC is known to be involved in multisensory integration processes, optic ataxia has been proposed to originate from a deficit of transforming sensory information into an accurate reaching movement. Optic ataxia patients are paradoxically more accurate when they point toward remembered targets indicating that they do not benefit from on-line motor control (lack of hand automatic pilot). Accurate reaching relies on a comparison between hand and target positions before and during movement execution. In the case of a visual target, this comparison can be cross-modal (when only proprioceptive information about the hand is available) or visual-to-visual (when visual information is also available for hand location). In order to investigate the role of the PPC in these hand-target comparisons at different phases of the movement we asked a patient with unilateral optic ataxia to reach to remembered peripheral positions of visual targets while maintaining fixation under three different conditions: without vision of the hand (noV), with vision of the hand only before the movement (startV) or during the entire reach (fullV). Six neurologically intact control participants also took part in this experiment. Overall, reaching errors of the patient were reduced by 38% in the fullV as compared to the noV condition. However, such an improvement was not found in the startV condition, suggesting that the optic

ataxia patient benefitted from hand visual information only at the end of the movement. This finding suggests that the optic ataxia patient can perform correct reach movements only when proximal visual-to-visual comparison is possible between hand and target locations. Together, these results indicate that the PPC is involved specifically when the visual target location has to be compared with hand location informed from proprioception or from internal models. In addition, visual information of the hand at start does not improve the ongoing dynamic prediction of hand location. This could also be due to inability to process visuo-visual comparisons at further distances due to impairments of visual localization in peripheral vision.

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Poster

316. The Control of Reaching Movements I

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Topic: E.04. Voluntary Movements

Title: What is the best methods to determine reaction times

Authors: *J. B. SMEETS, E. BRENNER

Human Movement Sci., Vrije Univ. Amsterdam, Amsterdam, Netherlands

Abstract: Reaction times can be measured and analysed in many different ways. By comparing several options we show that the method that one chooses influences both the judged reaction time and, more importantly, conclusions about how the reaction time depends on the circumstances under study. We compared simple auditory reaction times in two conditions: one in which the response amplitude was constrained and one in which it was not. One might expect that adding constraints will increase reaction times. Indeed, when the reaction time was defined as the time that was needed to release a micro-switch (i.e. to raise the finger by 0.5 mm) it was longer when the amplitude was constrained than when it was not. However, constraining the amplitude of the response makes the response less vigorous, so determining the reaction time on the basis of a fixed displacement, even if it is a very small displacement, could make the reaction time appear to be shorter for a more vigorous response, even if the moment of movement onset is unaffected. We therefore analysed the data in various alternative ways. Reaction times determined in what appeared to be the most reliable ways were not systematically longer for the more constrained movements. The most reliable methods use extrapolation of the change in the average force that the finger exerts on the surface or of the change in the finger's average acceleration to estimate the reaction time.

Disclosures: J.B. Smeets: None. E. Brenner: None.

Poster

316. The Control of Reaching Movements I

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Program#/Poster#: 316.15/GG21

Topic: E.04. Voluntary Movements

Title: Reaching for water: A novel cortex dependent forelimb task for mice

Authors: *C. BONARDI, G. GALINANES, D. HUBER Fundamental Neurosci., Univ. of Geneva, Geneve, Switzerland

Abstract: Cutting edge genetic and optical tools to study neural circuits in the mouse only gain their full potential in combination with well-controlled behavioral paradigms. In rodent motor research, the "reach-to-grasp" behavior has proven to be a powerful paradigm because it closely resembles that of primates and it has been well characterized in the context of stroke and Parkinson's disease. However, the classical reaching for food pellets in rodents also has some technical shortcomings and its use under head-fixed conditions is rather limited. Here, we explore a novel version of this paradigm in which mice are trained to reach for water droplets instead of food pellets. We found that freely moving mice immediately engage in this water droplet reaching task and become fully proficient after 5 training sessions, performing hundreds of reaching trials per hour. Importantly, the "reach-to-grasp" kinematics for water droplets follow the same sequence described for classical pellet reaching.

Inspired by the classical "center-out" reaching task in primates, we next investigated whether mice were able to perform the task under head-restrained conditions and towards different target locations. We found that head-restrained mice can rapidly learn to locate, reach out and grab water drops presented in different target locations around their snout. Interestingly, not the whiskers, but the olfactory system is principally used for target localization. Optogenetic inactivation of the motor cortex halted the initiation, as well as the execution of ongoing reaching movements.

Taken together, reaching for water has the potential to become a universal and flexible behavioral platform for systems neuroscience research in mice.

Disclosures: C. Bonardi: None. G. Galinanes: None. D. Huber: None.

Poster

316. The Control of Reaching Movements I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 316.16/GG22

Topic: E.04. Voluntary Movements

Support: Wellcome Trust DBT India Alliance Early Career Fellowship

Ramanujan Fellowship, Govt. of India

Department of Science and Technology, Govt. of India

Title: The influence of action reinforcement on perceptual decision-making

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Abstract: There is increasing appreciation that perceptual decision-making is based not just on features of the stimulus, but also actions we associate with that stimulus. This occurs because sensory predictions generated as action consequences are combined with sensory input during decision-making. Congruent with this, we recently showed that perceptual decisions were better when sensory feedback and predictions were aligned, even when the predictions were modified through learning. Importantly, instability in the modified predictions caused the perceptual system to transition to "natural" predictions for maintaining enhanced perceptual judgments. Why does the perceptual system go back to the default natural predictions? We hypothesized that this is because actions that generate these predictions have been reinforced through everyday experience. Thus reinforcing a different set of actions should create a new "natural" and cause the perceptual system to rely on those predictions. To test this, two groups of subjects adapted to a 10-degree visuomotor rotation via feedback about motor errors and task success. Following adaptation, subjects continued to experience the rotation while receiving feedback about only error (Group 1, non-reinforcement) or only success (Group 2, reinforcement). Subsequently, all subjects adapted to an additional 10-degree rotation, but stabilization of this learning was prevented by a concomitant tone discrimination task. Subjects then performed a perceptual task in which they searched for and reported the color of a predefined visual target that moved on a screen among other distractors while they also moved their unseen hand. Importantly, target motion was congruent with the hand, or rotated by 10 or 20 degrees relative to hand motion. We noted that accuracy of the color report in both groups was initially better for targets that were rotated by 20 degrees. However, for Group 1 subjects, accuracy gradually became better for targets whose motion was aligned with the hand, while for Group 2 subjects, accuracy became better for targets whose motion was rotated by 10 degrees relative to the hand. Thus,

reinforcement of the 10-degree adapted action caused subjects to rely on predictions derived from those actions for perceptual decision-making, but the lack thereof caused increased reliance on the default natural predictions. While this reaffirms that the perceptual system flexibly incorporates the most stable sensory predictions for perceptual judgements, these results suggest that this "stability" is defined by movement success. Thus, perceptual judgements are biased by sensory predictions derived from our most successful actions.

Disclosures: N. Kumar: None. P.K. Mutha: None.

Poster

316. The Control of Reaching Movements I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 316.17/GG23

Topic: E.04. Voluntary Movements

Title: Circle drawing as an objective indicator of handedness

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Abstract: Whether a person is right- or left-handed can currently be established only by using subjective handedness questionnaires. However, recent findings of motor control research open an opportunity to develop objective indicators of handedness. It has been shown that in righthanded subjects, control of interaction torque at the shoulder and elbow is accomplished better by the dominant arm compared with the nondominant arm. This causes differences in the shape of the hand trajectories between the arms. In particular, our bimanual study of circle drawing suggested that the nondominant arm stronger deforms circular shapes han the dominant arm. This points to a possibility to use circle drawing as an indicator of handedness. To explore this hypothesis, we assessed quality of unimanual circle drawing in both arms of three groups of subjects, right-handed, left-handed, and ambidextrous. Arm dominance was assessed with two handedness questionnaires. Subjects were presented a template of a circle of 20 cm diameter and asked to repetitively draw a similar circle with the fingertip of each arm on a horizontal table. The circle drawing was performed at a comfortable speed and as fast as possible. The fingertip motion did not leave any visible traces. The table height was individually adjusted to restrict motion of the arm in the horizontal plane of the shoulder. Movements of the arm were recorded. Two trials of 20 sec duration were performed in each speed condition. The quality of circle drawing was assessed by computing the aspect ratio of each circle and averaging this number across the circles in each trial. Right-handed subjects produced more circular shapes compared with their left arm. This difference was more pronounced in fast movements. Left-handed subjects had no significant differences between arms in movements of either speed, although the trend in the fast condition was that the left arm movements were more circular. Ambidextrous

subjects produced more circular trajectories in right arm movements in both speed conditions. An additional finding was that during fast movements, the dominant hand of left-handed subjects distorted the circular shape substantially more than the dominant hand of right-handed subjects. These results extend our understanding of the influence of arm dominance on arm movement trajectories and control. They also indicate that the circle drawing test could potentially be used for objective assessment of handedness.

Disclosures: N. Dounskaia: None.

Poster

316. The Control of Reaching Movements I

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Program#/Poster#: 316.18/GG24

Topic: E.04. Voluntary Movements

Support: NSERC PGS D3-489507-2016

Title: Co-activation uses early antagonist response in upper limb postural control

Authors: *C. M. SALIBA¹, M. J. RAINBOW¹, W. S. SELBIE³, K. J. DELUZIO¹, S. H. SCOTT²

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Abstract: The objective of this study was to determine the influence of co-activation on the response to a physical perturbation during a postural control task. We examined the effect of preperturbation muscle activity on the motor response of muscles stretched (agonist muscles) and shortened (antagonist muscles) by the perturbation. Subjects interacted with a KINARM End-Point robot (BKIN Technologies, Kingston, ON, Canada). The subjects moved the endpoint handle to the center of a target and a perturbation pulse (50 ms duration) applied a force to flex or extend the elbow. The perturbation was applied after a random hold time of 1 - 4 s. Trials were successful if the subject returned the handle to the target within 500 ms of perturbation onset and stayed inside the target for 1000 ms. Surface electromyograph (EMG) signals were recorded for muscles spanning the elbow. Background loads were used to increase the baseline activation of agonist muscles to high and low levels. Trials were also performed with agonist and antagonist muscles co-activated. Co-activation targeted baseline muscle activity equal to the levels achieved by the background loads using visual feedback of the EMG signal. Subjects completed the task with greater success when co-activating than when resisting background loads with similar agonist activation. Co-activation reduced overshooting and oscillations when recovering from the perturbation and returning to the target. Agonist muscles exhibited a short latency stretch response and gain scaling when activated through both co-activation and
background loads. Under the co-activation condition, antagonist muscles displayed a burst of EMG activity starting 80 - 100 ms post-perturbation. This early antagonist response was not observed in the background load condition, when the antagonist muscles were not pre-activated. Differences in hand position between the background load and co-activation conditions did not occur fast enough post-perturbation to be attributed to changes in intrinsic muscle properties. The response of the agonist muscles to the perturbation was similar in both the background load and co-activation conditions and did not explain the behavioral differences observed when co-activating. The early antagonist response occurred before reversal of the hand position, while the antagonist muscles were still shortening; thus, the response was not caused by a stretch reflex. This demonstrated an anticipatory response that provided an early braking mechanism to reduce overshoot and oscillations in the recovery from the perturbation, likely improving task performance.

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Poster

316. The Control of Reaching Movements I

Location: Halls A-C

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Topic: E.04. Voluntary Movements

Support: JSPS KAKENHI Grant JP15K12597

Nagaoka University of Technology Presidential Research Grant

Title: Fitts' law explanation based on human arm dynamics

Authors: *M. TAKEDA, I. NAMBU, Y. WADA Nagaoka Univ. of Technol., Nagaoka, Niigata, Japan

Abstract: During human arm reaching movements, a universal phenomenon occurs in which the end-point accuracy decreases as movement speed increases. More than half a century ago, Fitts conducted a pioneering research on this speed-accuracy trade-off by formulating the relationship between movement amplitude, duration, and accuracy. This relationship is generally known as Fitts' law, which describes the duration *D* of arm movement as a function of the amplitude *A* and target width *W*. When the target width *W* is considered to be an allowable error of the hand endpoint (i.e., the end-point accuracy), Fitts' law represents the speed-accuracy trade-off. In

previous studies, this law has been applied under various conditions (i.e., using different experimental tasks, devices, and subjects). Most, if not all, studies support Fitts' law. Thus, Fitts' law has been widely accepted as a practical evaluation tool for human performance modeling and human-computer interfacing. However, since Fitts' law is an empirically derived model, its theoretical background has not been sufficiently elucidated. To understand the mechanism of the human speed-accuracy trade-off, several studies have sought to improve Fitts' law by generating new models related to the central nervous system or musculoskeletal system. However, conventional model was based on kinematic theory and dynamics was not taken into consideration. Human arm movements are achieved by transforming motor commands through dynamics. Thus, speed-accuracy trade-off characteristics can be explained by the joint torque produced by motor commands. Here, we investigated whether the speed-accuracy trade-off described by Fitts' law can be better explained by joint torque, which is directly controlled by motor commands. To examine the relationships between the movement speed, end-point accuracy, and joint torque, we performed temporally and spatially constrained experiments. A positive correlation was observed between the integrated absolute torque and end-point error, which is a measure of end-point accuracy for the trial-averaged values of each experimental condition. These findings suggest the possibility that the speed-accuracy trade-off described by Fitts' law can be explained by human arm dynamics and its parameters.

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Poster

316. The Control of Reaching Movements I

Location: Halls A-C

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Topic: E.04. Voluntary Movements

Support: Grant-in-Aid for JSPS Research Fellow

Title: Competitive game influences risk-sensitivity in motor decision-making

Authors: *K. OTA^{1,3}, K. TAKIYAMA²

¹Fac. of Engin., Tokyo Univ. of Agr. and Technol., Tokyo, Japan; ²Dept. of Engin., Tokyo Univ. of Agr. and Technol., Koganei-Shi, Japan; ³Japan Society for Promotion of Sci. Res. Fellow, Tokyo, Japan

Abstract: Athletes are required to make a decision about where to throw a ball or where to kick a ball. Recent studies have shown that humans tend to be risk-seeking in motor decision tasks (Wu et al., PNAS, 2009). The risk-seeking strategy is robust; it is difficult to improve the strategy even after 9 days practice of 2250 trials (Ota et al., Sci Rep, 2016). However, it is unclear whether the risk-seeking strategy can be improved. Here we investigated the robust risk-

seeking strategy in motor decision task can be influenced by a virtual opponent in a competitive game because players unintendedly imitate opponent's behavior in a competitive game (Naber et al., PNAS, 2013). Twelve participants performed both a one-player reaching game to maximize total rewards and a competitive reaching game to obtain higher total rewards than an opponent. In each trial, the participants obtained higher rewards if their endpoint of a reaching movement was located closer to a reference line (300 mm from an initial position). However, no rewards were assigned if the endpoint was over the reference line. The participants required to make a decision about where to aim for under risk of no rewards. The participants first performed the one-player game for 5 blocks and then performed the competitive game for 12 blocks. Each block consisted of 10 trials. In the competitive game, the participants confronted either optimal or risk-averse opponent. Optimal opponent set its mean endpoint to be the each participant's optimal mean endpoint that maximized the expected rewards under the existence of their own motor variance. Risk-averse opponent set its mean endpoint farther away from the reference line than the optimal mean endpoint. Consistent with the previous studies, the risk-seeking strategy was adopted in the one-player game. The observed mean endpoint ($279 \pm 7 \text{ mm}$) was significantly closer to the reference line than the optimal mean endpoint $(272 \pm 7 \text{ mm})$ in the last block of the one-player game. However, compared with this block, the observed mean endpoint significantly decreased in the first block of the competitive game (270 ± 7 mm), suggesting that the participant's aiming point approached their optimal endpoint. Furthermore, when the opponent player was optimal, the observed mean endpoint gradually increased from middle to end of the competitive game. In contrast, it plateaued when the opponent player was risk-averse. These results indicate that the presence of the opponent player influences risk-sensitivity in motor decision-making.

Disclosures: K. Ota: None. K. Takiyama: None.

Poster

316. The Control of Reaching Movements I

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Topic: E.04. Voluntary Movements

Support: USC Viterbi Graduate School Fellowship

NIH R01-HD-065438

Title: Control of arm reaching movements as a trade-off between movement variability and subquadratic effort

Authors: *V. BARRADAS PATINO¹, C. WANG², E. BURDET³, N. SCHWEIGHOFER² ¹Biomed. Engin., ²USC, Los Angeles, CA; ³Imperial Col. London, London, United Kingdom **Abstract:** Our previous study suggested that human arm reaching movements without visual feedback are planned as a trade-off between effort and movement variability arising from signal-dependent and constant motor noise (Wang et al., 2016). Modelling effort as a quadratic function of the motor command can predict qualitative differences between durations of movements in different conditions, but does not predict well the exact movement duration, nor decision making and movement speed in a variety of tasks (Shadmehr et al., 2016). The systematic prediction error of movement duration suggests the hypothesis that effort corresponds to a sub-quadratic function of the motor command. We thus test through simulation of planar arm movements considering a two link arm model with signal-dependent and constant noise how using $|u|^n$ with 1 < n < 2 as effort predicts motion features quantitatively. Cubic splines were used to parameterize the motor command and thus reduce parameter space dimension and speed up optimization. Prediction of arm reaching movement duration is improved with n > 1. This suggests that the central nervous system considers a sub-quadratic effort cost as well as variability due to signal dependent and constant noise to plan movement.

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Poster

316. The Control of Reaching Movements I

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Topic: E.04. Voluntary Movements

Support: MIUR PRIN 2015 (ModuLimb)

Title: EMG-controlled force field generation: Incorporating muscle geometry and muscle activation dynamics

Authors: *N. LOTTI, V. SANGUINETI

Dept. of Informatics, Bioengineering, Robotics and Systems Engin., Univ. of Genoa, Genova, Italy

Abstract: Muscle activity (EMG) has been often used as a control signal to operate a prosthesis. In isometric conditions, EMG activity may be assumed to reflect the force generated by that muscle. Based on this notion, myocontrollers can be used to predict in real-time the forces generated by individual muscles during isometric tasks. This approach has been used to virtually 'remove' individual muscles and to study the resulting adaptation phenomena. Similar approaches have been used to assess motor system modularity. These applications use a proportional myocontroller in conjunction with an isometric force control task, with the limb placed at a fixed location. Extension of this approach to actual movements, using a kind of EMG-controlled force field generation, would enable a deeper insight into adaptive and modular properties of the motor system. To do so would require to appropriately account for (i) the nonlinear dependence of muscle length on arm configuration and (ii) the muscle activation dynamics, which depends non-linearly on muscle length and its rate of change. As a step toward this direction, here we compare three different models of muscle geometry, characterised respectively by (i) constant moment arms; (ii) moment arms described by polynomial functions of the joint angles with fixed coefficients; and (iii) a polynomial model fitted to individual subjects' data. As regards activation dynamics, we also compare a linear model with a Hill-type muscle model, with realistic models of contractile and tendon elements. We focused on planar arm movements while grasping a robot manipulandum. We designed a motor task which alternates reaching movements between seven targets evenly distributed within the workspace, and isometric force steps while the hand is kept fixed within each of the spatial targets. Once a spatial target is reached, subjects had to generate 25 N force steps in twelve directions. We recorded movement kinematics, endpoint force and the activity of seven muscles. Using a constrained optimization procedure, we estimated muscle geometry and muscle activation parameters from these data, for each of the model variants. Fitting performance was assessed in terms of vector correlation, rotation and scaling between observed and predicted endpoint force trajectories. The different model variants exhibited acceptable reconstruction performance in both isometric and dynamic conditions. This suggests that myoelectrically-controlled force field generation in different areas of the workspace and during movements is a feasible way to probe motor system adaptation and possibly to deliver personalized forms of robot-mediated therapy.

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Poster

316. The Control of Reaching Movements I

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Topic: E.04. Voluntary Movements

Support: UIUC Research Board Grant

UIUC CHAD Pilot Research Grant

Title: Whole body muscle activity in quasi-static force efforts

Authors: N. SPEIDEL¹, P. CHEMBRAMMEL², R. MCNISH⁴, S. N. MCKEEMAN³, *C. LOPEZ-ORTIZ⁵

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Abstract: In the study of human motor control, the synergistic and antagonistic interactions between muscle groups play a large role in the movement and force exertion of the limbs. Determination of the degree to which different muscles are activated during a given action can provide significant insight into how the central nervous system organizes combinations of muscles to perform various tasks. In this poster, we report on the tests on eight participants with no known neurological disorders. All the procedures were approved by the local IRB and informed consent was obtained from all participants before enrolling in the study. The activity of 32 different muscles is compared across 16 different quasi-static force effort tasks to create a mapping between combinations of muscular activation and intended action by the user. Persistent homology, and two different statistical analysis methods, viz. principal component analysis and non-negative matrix factorization (NMF) are applied to the 32 electromyography signals and the six-degree-of-freedom force data to determine the muscles synergies that play the largest role for a given effort. The number of muscle groups in NMF is determined by the modified Akaike information criterion (AICc). The results of the three analysis methods are compared to better understand the mechanisms at play for synergistic muscle activations.

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Poster

316. The Control of Reaching Movements I

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Program#/Poster#: 316.24/GG30

Topic: E.04. Voluntary Movements

Title: Motor-evoked pain increases force variability in chronic jaw pain

Authors: *W.-E. WANG, A. ROY, S. COOMBES Dept. of Applied Physiol. and Kinesiology, Univ. of Florida, Gainesville, FL

Abstract: Conventional approaches to studying motor variability in jaw function use experimental pain models to elicit pain via the delivery of capsaicin injections to the jaw. Evidence using this approach shows that increase in jaw pain is associated with increases in variability of jaw function. However, it is not clear whether individuals with chronic jaw pain also show increased motor variability of jaw force control. The purpose of this study was to investigate the effect of motor-evoked clinical pain on force production in chronic jaw pain. Seventeen participants with a history of jaw pain (> 6 months) and 19 pain-free healthy controls performed visually guided isometric jaw contractions at 2% and 15% of their maximum

voluntary contraction. Self-reported pain ratings (i.e. pre-task and post-task ratings) using a visual analog scale (VAS) were collected on a trial by trial basis. Psychological factors were measured using clinical questionnaires. The findings showed that the self-reported pain and force variability were higher in the jaw pain group relative to controls at both force levels. In the jaw pain group, the self-reported pain was higher at the high force level than at the low force level, whereas normalized force variability was greater at the low force level than the high force level. At the low force level, a multiple linear regression analysis found that disability days, pain intensity, disability score, anxiety, depression, and pre-post pain rating change explained 64.6% of the variance in motor variability. At the high force level, 45.6% of the variance in motor variability was explained by pain duration, pain intensity, anxiety, and the pre-task pain rating. Our observations demonstrate that motor-evoked clinical jaw pain is associated with increased motor variability, and this increase in variability can be predicted based on measures of self-reported pain and psychological measures.

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Poster

316. The Control of Reaching Movements I

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Topic: E.04. Voluntary Movements

Support: NIH-R01-HD087089

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Title: Probing motor priorities with electrical manipulation of neural noise scaling

Authors: F. LUNARDINI¹, D. STERNAD², *C. J. HASSON³

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Abstract: A hallmark of human movement is smoothness. Notably, movement trajectories are not only smooth but are maximally smooth, i.e. they follow minimum-jerk profiles. This phenomenon has been observed in a wide range of human actions and has been interpreted as evidence that the human neuromotor system prioritizes smoothness in motor planning. Conversely, it has been suggested that smoothness may not be a neuromotor priority per se but a consequence of multiplicative neural noise. A minimum-jerk movement minimizes neural command amplitudes, which in turn minimizes multiplicative noise, and therefore minimizes

task performance variance. Since both alternative theories, i.e., that smoothness is a by-product, or, is prioritized in of itself, lead to the same motor outcome - a maximally smooth movement, it has proved challenging to falsify either theory experimentally. To this end, we electrically manipulate the scaling relation of neuromotor noise and observe how the nervous system responds. Previous research showed under isometric conditions that applying a train of electrical pulses to a muscle decreases the coefficient of variation of force fluctuations (Jones et al., J Neurophysiol, 2002), a consequence of a partial reversal of motor unit recruitment. In our paradigm, we: 1) magnified this effect by applying a noisy train of stimulation pulses with an amplitude inversely related to arm acceleration, making large motor commands relatively less noisy than small ones, and 2) applied this acceleration-modulated electrical noise while humans performed an as-fast-as-possible accuracy-constrained elbow flexion movement with their right arm. We hypothesized that if smoothness is a by-product of end-point variance minimization, then when higher accelerations are made less noisy, humans should increase the jerkiness of their movements, allowing them to move faster for a given end-point variance. Conversely, if movement smoothness is a priority, subjects should maintain maximally smooth (minimum-jerk) movements, forfeiting the opportunity to move faster. In our experiment, subjects practiced under four randomized conditions (80 trials each): 1) no added noise, 2) acceleration-modulated electrical noise, 3) constant electrical noise, and 4) a sham condition with noise applied to the non-involved arm. Early results suggest that subjects do increase the jerkiness of their movement profiles when the scaling of their neural noise is electrically altered to make high accelerations less variable. Within a relatively short time, it appears that the nervous system can modify its control strategy to take advantage of its current noise properties.

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Poster

316. The Control of Reaching Movements I

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Topic: E.04. Voluntary Movements

Support: Matthieu Casteran was supported by a postdoctoral grant of the Conseil Régional de Bourgogne

Title: Sifts in strategic time points of a complex motor task with aging

Authors: *E. THOMAS¹, P. HILT², P. MANCKOUNDIA³, F. MOUREY¹, M. CASTERAN⁴ ¹Univ. De Bourgogne, Dijon Cedex, France; ²Italian Inst. of Technol., Ferrara, Italy; ³Service de Médicine Interne Geriatrie, Hosp. of Champmaillot, Dijon, France; ⁴Univ. de Lorraine, Lorraine, France

Abstract: In this study we compared several temporal characteristics in the whole body pointing movements of ageing and young subjects performing whole body pointing (WBP). As a movement used in daily life that involves significant inclination of the heavy trunk segment, it is important to understand for considerations of equilibrium. We examined two strategic time points during the task - first, the crossover point where the velocity of the Centre of Mass (CoM) in the vertical dimension outstripped the velocity in the anteriorposterior dimension and secondly, the time to peak of the COM velocity profile. Both time points occurred earlier in ageing subjects. The crossover point also showed adjustments with target distance in aging subjects while this was not observed in younger subjects. Studies with a model showed that this temporal adjustment with target distance fit into an optimal control strategy that emphasized stability rather than absolute work as an optimal control strategy. The lack of significant differences in movement duration eliminated this as a cause for the differences observed between the two groups. Comparisons of the head and angular excursions did not reveal any significant differences between the old and young subjects, hence indicating that the observed temporal shifts in the WBP aging subjects took place through modest adjustments of a common movement strategy.

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Poster

317. Motor Learning and Recovery

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 317.01/GG33

Topic: E.04. Voluntary Movements

Title: Searching for the genetic components of long-term memory for operant self-learning in *Drosophila*

Authors: *B. BREMBS, W. SUN Univ. Regensburg, Regensburg, Germany

Abstract: In contrast to many other forms of learning, the genetic components of operant selflearning, a kind of motor-learning, are still poorly understood. So far, only Protein Kinase C (PKC) and FoxP have been identified as necessary for operant self-learning. It is also unknown whether the training procedure used in *Drosophila* is capable of supporting long-term (i.e., 24h) memory. Here we tested mutants for the gene *radish*, coding for a RAP-like GTPase activating protein. We found that these mutants do not show any decrement in operant self-learning, but improved operant world-learning, where the flies do not need to show any motor-learning, but color-learning is sufficient. We also tested *rover* and *sitter* flies, alleles of the *foraging* gene, a member of the protein kinase G (PKG) family. We found that PKG does not seem to be involved in operant self-learning, either. We also replicated a difference in heat sensitivity between these two alleles that had been described previously. Both PKG variants as well as a different wild type strain (wild type Berlin) showed savings of the operant memory after a brief (60s) reminder training 24h after the initial training.

In conclusion, operant self-learning appears to be independent of both RAP-like GTPase activating proteins (*radish*) and PKG (*foraging*). Long-term memory of operant self-learning is also independent of PKG activity.

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Poster

317. Motor Learning and Recovery

Location: Halls A-C

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Program#/Poster#: 317.02/HH1

Topic: E.04. Voluntary Movements

Support: R01 NS094384

Title: Cortical map plasticity as a function of vagus nerve stimulation intensity paired with motor training

Authors: *R. A. MORRISON, T. DANAPHONGSE, S. SARKER, J. MONG, H. ZHANG, D. HULSEY, K. ADCOCK, S. A. HAYS, M. P. KILGARD, R. L. RENNAKER Univ. of Texas At Dallas, Richardson, TX

Abstract: Vagus nerve stimulation (VNS) paired with motor training enhances plasticity of movement representations in the motor cortex. A previous study demonstrated that there is an inverted-U relationship between stimulation intensity and VNS-dependent enhancement of plasticity in the auditory cortex after VNS-tone pairing. Low intensity (0.2 mA) and high intensity (1.6 mA) VNS drive significantly less cortical plasticity than moderate intensity (0.4mA or 0.8 mA) VNS. We sought to determine whether VNS current intensity similarly altered the degree of plasticity in the motor cortex when paired with motor training. To do so, we investigated the effects of a range of VNS intensities paired with forelimb training on reorganization of map representations in the motor cortex. Rats were trained on a motor task in which they pressed a lever twice in rapid succession to receive a food reward. Once proficient, rats were implanted with a vagus nerve stimulation cuff. After a week of recovery, rats were randomized to one of three groups: 0.4mA VNS, 1.6mA VNS, or sham stimulation. All groups returned for five days of training on the motor task, during which VNS at the appropriate intensity was repeatedly paired with forelimb movement. After the five days of VNS paired behavioral training, intracortical microstimulation (ICMS) was used to investigate map representations in the motor cortex. Preliminary data indicates that rats receiving 0.4mA VNS

display a significant increase in proximal forelimb representation in the motor cortex compared to 1.6mA and sham groups. Rats receiving 1.6 mA VNS fail to show an expansion in proximal forelimb representation compared to the sham group. These findings are consistent with the existence of an inverted-U effect on VNS-dependent plasticity in the motor cortex and may provide insight into optimization of VNS parameters for clinical implementation in motor disorders.

Disclosures: R.A. Morrison: None. **T. Danaphongse:** None. **S. Sarker:** None. **J. Mong:** None. **H. Zhang:** None. **D. Hulsey:** None. **K. Adcock:** None. **S.A. Hays:** None. **M.P. Kilgard:** F. Consulting Fees (e.g., advisory boards); MicroTransponder, Inc. **R.L. Rennaker:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vulintus, Inc..

Poster

317. Motor Learning and Recovery

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 317.03/HH2

Topic: E.04. Voluntary Movements

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National Science Foundation Graduate Research Fellowship (DBS)

American Heart Association Post-doctoral Fellowship (AY)

Title: Optogenetic stimulation leads to connectivity changes across sensorimotor cortex in nonhuman primates

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Abstract: Brain stimulation is believed to modulate the excitability of neural circuits and increase the likelihood of neuroplasticity. The local effects of stimulation are an active area of investigation; however, the effects on network connectivity remain largely unexplored. Here, we tracked changes in network connectivity across sensorimotor cortex in response to stimulation. We used a large-scale optogenetic interface that enables stimulation of excitatory neurons (AAV5-CamKIIa-C1V1-EYFP) with simultaneous recording of surface potentials (μ ECoG) from primary somatosensory (S1) and motor (M1) cortices. In two macaque monkeys we explored connectivity dynamics using a simple stimulation protocol; we delivered 5 ms laser

light pulses at a frequency of 5 Hz at one or two cortical sites.

We quantified functional connectivity between S1 and M1 by measuring how neural activity propagates through the network. Optogenetic stimulation evokes a direct response in the stimulated area followed by a delayed (3-6 ms), indirect response in the other area. We calculated the inter-area connectivity by measuring the relative amplitude of the direct and indirect evoked responses and by computing the coherence of spontaneous activity between the stimulation site and the other area.

In general, stimulation strengthened the connectivity between S1 and M1. After 50 minutes of stimulation, there was a significant increase in the relative amplitude of evoked responses and in inter-area coherence at the stimulation frequency. To capture the dynamics of these changes, we tracked each of these measures across time. There was a trend of increasing connectivity with continued stimulation.

In addition to the trend of increased inter-area connectivity, stimulation led to heterogeneous changes across the network at a finer scale. To assess these changes, we measured the pairwise coherence during spontaneous activity. Stimulation evokes a network-wide pattern of activity, so the coherence during stimulation is different than the baseline coherence. This difference, which we call *input coherence*, predicted changes in coherence after stimulation, consistent with a Hebbian plasticity model. To further test this model, we delivered more complicated patterns of optical stimulation. Although there were more variable effects on the network, the *input coherence* continued to predict changes in connectivity.

Our results show that stimulation induces changes within and between cortical areas. Regardless of the complexity of the stimulation, connectivity across the network follows the coherence driven by the evoked activity, which is consistent with Hebbian learning.

Disclosures: D.B. Silversmith: None. **A. Yazdan-Shahmorad:** None. **V. Kharazia:** None. **P. Sabes:** A. Employment/Salary (full or part-time):; Neuralink Corp..

Poster

317. Motor Learning and Recovery

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Topic: E.04. Voluntary Movements

Support: NIH P20 GM103446

Title: Effects of chronic antidepressant use on neurophysiological responses to tDCS post-stroke

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Abstract: Transcranial direct current stimulation (tDCS) has gained interest as an adjunct tool to promote motor recovery after stroke. While evidence has shown that tDCS can improve motor performance, there is more limited reporting on the neurophysiological brain changes induced by tDCS post-stroke in the absence of a motor task. Moreover, very few studies of persons with stroke consider the potential effects of medications on the efficacy of brain stimulation. Recently, it has been shown that selective serotonin reuptake inhibitors (SSRIs) alter the effects of tDCS in healthy individuals. Stroke survivors are often prescribed SSRI antidepressants either for the treatment of post-stroke depression or to help promote motor recovery. However, SSRIs may alter cortical excitability differently in healthy vs. stroke-affected brains, or in the acute vs. chronic administration of these drugs. Therefore, we aimed to investigate the neurophysiological effects of anodal tDCS in stroke survivors on vs. off antidepressants. Chronic, unilateral stroke survivors participated in the study, and were assigned to the control or antidepressant group based on their medications. Subjects received real and sham anodal tDCS over the lesioned hemisphere primary motor cortex (M1) over two sessions held at least one week apart. In each session, bilateral motor cortical excitability measurements were collected via transcranial magnetic stimulation before and after tDCS. We compared baseline excitability and responses to tDCS between groups. Results showed that at baseline, cortical silent period durations from the non-lesioned (NL) hemisphere were shorter in the antidepressant group than the control group, indicating less intracortical inhibition. Unexpectedly, motor evoked potential (MEP) amplitudes from the NL hemisphere increased in the antidepressant group after real tDCS, compared to either sham tDCS, or to the control group. All other comparisons were not significant. Given our results, we speculate that anodal tDCS over the lesioned M1 in stroke subjects taking antidepressants may have preferentially affected the excitability of transcallosal neurons, which in turn resulted in an increase in MEP amplitudes through complex inhibitory mechanisms in these subjects and not those not taking antidepressants. In summary, anodal tDCS over the lesioned M1 of stroke survivors seems to have complex effects on cortical excitability, with antidepressant medications and/or the presence of depression, as important factors. Further investigation is warranted and future tDCS studies in stroke should consider examining antidepressant medications as covariates.

Disclosures: X. Li: None. S.M. Morton: None.

Poster

317. Motor Learning and Recovery

Location: Halls A-C

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Topic: E.04. Voluntary Movements

Support: NIH Grant R01AR069176-01A1

Title: Female sex hormones modulate the response to low-frequency rTMS in the human motor cortex

Authors: *L. M. ROGERS^{1,2}, Y. Y. DHAHER^{1,2,3}

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Abstract: Background: The importance of sex-specific research has been demonstrated in a growing number of studies but has yet to translate to rehabilitation therapy. Recently, adjuvant therapies aimed at creating short-term neuroplastic change to balance motor cortical excitability after stroke have been particularly effective at diminishing impairment and improving motor function, but few studies have considered the impact of fluctuating sex hormones on these interventions. Objective: Accordingly, the goal of this study was to evaluate the modulatory effect of sex hormones on cortical excitability and the response to repetitive noninvasive brain stimulation in healthy, normally cycling women. Our goal is the lay the foundation for understanding how cyclic fluctuations may impact the treatment of a chronic neurological disease such as stroke. Methods: Serum estradiol and progesterone levels were measured in six healthy female subjects (average age 31.3) every other day for two months. Three healthy males (average age 21) served as a comparison group. All subjects underwent sessions of repetitive Transcranial Magnetic Stimulation (rTMS), with corticomotor excitability tested before and after. Results: We found significantly enhanced rTMS-induced inhibition when serum estradiol concentrations were rising and progesterone concentrations remained low. In contrast, the effect on corticomotor excitability was reversed just after peak estrogen (post-estrogen peak) where the normally down-regulatory rTMS protocol resulted in significant facilitation despite serum estradiol levels remaining high. All six women exhibited enhanced inhibition when estradiol was rising; in all but one woman, excitability increased by at least 30% at post-estrogen peak. Conclusions: These preliminary findings suggest female sex hormones influence the effect of rTMS on corticomotor excitability, and that the nature of this influence varies across the menstrual cycle. More broadly, this study highlights the importance of sex-specific science in rehabilitation research.

Disclosures: L.M. Rogers: None. Y.Y. Dhaher: None.

Poster

317. Motor Learning and Recovery

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Program#/Poster#: 317.06/HH5

Topic: E.04. Voluntary Movements

Support: NIH Grant NS094384

Title: Vagus nerve stimulation dependent enhancement of motor cortex plasticity requires noradrenergic innervation

Authors: *D. HULSEY¹, M. SHEDD¹, J. MONG¹, R. L. RENNAKER², S. A. HAYS², M. P. KILGARD³

¹Behavioral and Brain Sci., ²Bioengineering, Univ. of Texas at Dallas, Richardson, TX; ³Behavioral and Brain Sci., Univ. of Texas At Dallas, Richardson, TX

Abstract: Pairing forelimb movements with vagus nerve stimulation (VNS) drives robust plasticity within primary motor cortex (M1). VNS activates cholinergic circuits, which are required for VNS-depended enhancement of plasticity. However, there may be multiple neuromodulatory mechanisms required for VNS-dependent enhancement of plasticity. Norepinephrine regulates plasticity, and the noradrenergic locus coeruleus is driven vigorously by VNS. However, the role of norepinephrine in VNS-dependent enhancement of plasticity is unknown. We hypothesize that noradrenergic innervation of M1 and/or basal forebrain is necessary for M1 plasticity associated with VNS pairing. To test this, we trained rats on a skilled lever press task emphasizing use of the proximal forelimb. After demonstrating proficiency on the task, rats received M1 injections of vehicle or DBH-Saporin to selectively deplete norepinephrine in motor cortex, and underwent implantation of a stimulating cuff electrode on the vagus nerve. Sham and NE-lesioned rats resumed training one week after surgery. After returning to pre-surgical performance, both groups received 10 sessions of training with VNS paired on successful trials. Intracortical microstimulation was performed to derive M1 maps within 24 hours of the final training session. Initial data suggests that sham lesioned animals who receive VNS pairing with successful trials show a robust expansion of proximal forelimb movements represented in M1. Noradrenergic lesion of M1 blocks this VNS-dependent expansion of proximal forelimb representation, indicating that cortical norepinephrine innervation is necessary for VNS driven plasticity. Ongoing experiments will determine whether noradrenergic input to the central cholinergic systems is required for VNS-dependent enhancement of plasticity.

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Poster

317. Motor Learning and Recovery

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 317.07/HH6

Topic: E.04. Voluntary Movements

Title: The effect of volitional movement on paired associative stimulation-induced plasticity

Authors: *A. O. ALOKAILY^{1,3}, M. YAROSSI⁴, S. V. ADAMOVICH² ¹Biomed. Engin., ²Dept of Biomed. Engin., New Jersey Inst. of Technol., Newark, NJ; ³Dept. of Biomed. Technol., King Saud Univ., Riyadh, Saudi Arabia; ⁴Biomed. Engin., Rutgers Univ., Newark, NJ

Abstract: Paired associative stimulation (PAS) is a recognized technique to induce neuro-plastic changes in the human motor cortex (M1). PAS modulates cortical excitability by pairing peripheral nerve stimulation with transcranial magnetic stimulation (TMS) of M1 in a timingdependent manner to induce Hebbian-like plasticity. To date, PAS is most often performed with the subject at rest. A key question about how to combine PAS and voluntary movement is the timing of stimuli with respect to muscle activation onset. Here, we investigate the effect of PAS on M1 excitability when the stimuli are delivered during preparation or execution of a voluntary movement. Thirteen healthy right-handed subjects (age: 26±3), (9 Male and 4 Female) participated in the experiment. Prior to each session, twenty trials were conducted without stimulation to compute the mean reaction time (RT) for timing of stimulation during PAS. Subjects were instructed to extend their right hand fingers activating the extensor digitorum communis (EDC) in response to a visual cue. Each PAS session consisted of 240 pairs applied at a rate of 0.2 Hz. All subjects completed the following four PAS sessions: Triggering the TMS during: 1) movement preparation (RT-50ms), 2) movement onset (RT), 3) movement execution (RT+50ms), and 4) conventional PAS at rest. TMS induced motor evoked potentials (MEPs) were measured in the muscles of interest prior to and following the intervention to asses changes in corticospinal excitability. The MEPs amplitude was used for statistical analysis. PAS triggered during voluntary contraction (RT+50) or at rest increased excitability, while PAS delivered at the same inter-stimulus interval during movement preparation (RT-50) decreased excitability. Additionally, these changes were significant only for the targeted EDC muscle and not the flexor digitorum superficialis (FDS) indicating a muscle specific effect. Unlike most of PAS studies, our focus was on hand extensors for its rehabilitation importance. The results of this preliminary investigation suggest that the direction PAS induced plasticity was dependent on the order of stimulation and voluntary movement onset. These findings have important implications for the incorporation of PAS into neuromotor rehabilitation training. Future investigations will explore the underlying neurophysiological mechanisms, and possible clinical applications.

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Poster

317. Motor Learning and Recovery

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Topic: E.04. Voluntary Movements

Support: NSF #1406750

NSF #1361549

Title: Voluntary control of residual antagonist muscles in transtibial amputees: Coactivation, reciprocal activation, and residual muscle plasticity

Authors: *S. HUANG, H. HUANG

Joint Dept. of Biomed. Engin., North Carolina State Univ., Raleigh, NC

Abstract: Electromyography signals recorded from residual muscles of transtibial amputees are a potential neural control source for feedforward myoelectric control of powered prostheses. However, the ability for transtibial amputees to activate their residual antagonistic muscles to reach coordinates in a two-dimensional (2-D) control input space via proportional myoelectric control has not been examined. Understanding the reachable control input space of the residual muscles is required in order to utilize the amputee user's residual muscles for myoelectric control of a powered prosthesis. Peripheral damage to residual muscles results in changes to mechanical properties of the muscle, damage to muscle sensory receptors, and disruption of afferent pathways from the muscle. Disruption of afferent pathways likely affects residual muscle recruitment patterns. The purpose of this study is to examine the voluntary residual muscle activation patterns of transtibial amputees when mapping out a reachable 2-D control input space that requires both coactivation and reciprocal activation of residual antagonistic muscles to maximize the control input space. We asked ten transtibial amputee subjects to move a computer cursor to define a reachable control input space, where the cursor's 2-D position was directly proportional to independent continuous myoelectric control signals from the residual lateral gastrocnemius and residual tibialis anterior. Our results show that the reachable control input space varied widely across amputee subjects ranging from 52% to 81% of the maximum possible control input space (see Figure). The amputee subjects used different strategies to expand their reachable control input space depending on their ability to perform coactivation and reciprocal activation using their residual muscles. Future development of powered prostheses using proportional myoelectric control should evaluate the amputee user's reachable control input space in order to design a controller that can align/adapt to the current state of the amputee user's residual muscle function.



Disclosures: S. Huang: None. H. Huang: None.

Poster

317. Motor Learning and Recovery

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Topic: E.04. Voluntary Movements

Support: College of Human Sciences Untenured Seed Grant from Iowa State University

Title: Music training differentially modulates motor cortical activity in healthy young adults

Authors: *P. IZBICKI, S. ANDERSON, E. STEGEMOLLER Neurosci. & Kinesiology, Iowa State Univ., Ames, IA

Abstract: Previous studies have demonstrated that music training may induce neuroplastic effects in the brain, especially in the motor cortex. Furthermore, research using transcranial magnetic stimulation (TMS) in healthy young adults has revealed that listening to music can modulate global motor cortical excitability. However, no research has shown if music training affects how motor cortical excitability is modulated by music listening. Thus, the aim of this study is to determine the effects of listening to music on motor cortical activity in healthy young adults with formal music training. Single pulse and paired pulse short interval intracortical inhibition (SICI) TMS were applied during three conditions: no music, relaxing music, and activating music. Motor evoked potentials (MEPs) were recorded from the first dorsal interossieous muscle using bipolar surface electromyography (Delsys). MEP amplitude was obtained and averaged across each condition. Participants were divided into groups of 1) formal music training < 5 years and 2) formal music training > 5 years. A 2 x 3 repeated measures ANOVA (using gender as a covariate) was completed comparing MEP amplitude between music conditions and music training groups. There was a statistically significant difference (p=0.043) in global motor cortical excitability between music listening conditions with no group effect. However, there was a near statistical difference (p=0.054) in intracortical inhibition between groups. The group with >5 years of training had decreased intracortical inhibition during music conditions as compared to the group with <5 years of training. This suggests that while listening

to music, increased music training has no effect on global motor cortical excitability but has an effect on intracortical inhibition. These results will further elucidate the mechanism by which music affects the motor cortex with implications in both music education and music therapy.

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Poster

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Topic: E.04. Voluntary Movements

Support: NIH R01 EY025349

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Title: Volitional control of inhibitory neuron subtypes

Authors: *A. MITANI¹, T. KOMIYAMA²

¹UCSD, LA Jolla, CA; ²Ctr. for Neural Circuits and Behavior Rm 304, Univ. of California San Diego, La Jolla, CA

Abstract: Animals have the ability to adapt their behaviors to new environmental demands. A dramatic example is found in closed-loop neural feedback tasks in which animals can modulate the activity of specific neuron(s) that are arbitrarily selected by the experimenters. However, brain circuits consist of diverse cell types and it is unknown how different cell types adapt to new demands. Here, we examined the adaptability of three subtypes of inhibitory neurons (INs) in a closed-loop task with two-photon calcium imaging with mice expressing GCaMP6f in parvalbumin (PV), somatostatin (SOM), and vasoactive intestinal peptide (VIP)-expressing INs. The same two nearby neurons were targeted over multiple sessions, and the mice were rewarded when the activity of the positive target neuron (N+) exceeds that of the negative target neuron (N-) beyond a set threshold. With all subtypes, mice learned to increase the reward rate over several days, but the strategies differed across cell types. When PV-INs were targeted, the activity of N- decreased, but SOM- or VIP-INs targeting led the activity of N+ to increase, with a mild decrease in N- activity in SOM-INs. These results extend the notion of adaptability of

individual neurons to INs and highlight the versatility of neural circuits in adapting to new demands in a cell-type specific manner.

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Poster

317. Motor Learning and Recovery

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Topic: E.04. Voluntary Movements

Support: Arkansas Department of Higher Education

Title: An ERP study of homeostatic plasticity in human motor cortex following artificial syndactyly

Authors: *S. M. LONG, M. M. GARDNER, M. A. GANNON, N. A. PARKS Psychological Sci., Univ. of Arkansas, Fayetteville, AR

Abstract: Examining topographical reorganization in human sensorimotor cortex following amputation and stroke has been instrumental in understanding the neural mechanisms of longterm plasticity and cortical reorganization. An equally vital component to understanding representational plasticity is the delineation of the short-term homeostatic mechanisms involved in the functional reorganization of motor cortex, which are proposed to drive these long-term adaptations. Here, we investigated these mechanisms of homeostatic plasticity in human motor cortex by measuring cortical motor event-related potentials (ERPs) during an artificial syndactyly paradigm. To simulate the digit movement restriction of syndactyly, the index and middle finger of the dominant hand were temporarily fused together with a topical skin adhesive. This fusion constrained the movement of the two digits, necessitating movement together as a single digit. Immediately following artificial syndactyly, baseline motor potentials were recorded in two bimanual motor response tasks: a cued choice response task and a cued go/no-go task. Participants were then given motor dexterity practice under the conditions of artificial syndactyly through several tasks (ex. Purdue pegboard). Motor potentials were again recorded following practice using the same bimanual motor response tasks. Participants returned for a second session where they completed the same procedures and tasks but without artificial syndactyly. Preliminary analyses of motor potentials elicited by the dominant hand indicate that artificial syndactyly resulted in reduced amplitude of early-phase lateralized motor potentials, followed by increased amplitude in the late-phase of the motor potential. These findings may reflect early functional homeostatic adaptations in the motor cortex in response to movement constraints induced by artificial syndactyly.

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Poster

317. Motor Learning and Recovery

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Topic: E.04. Voluntary Movements

Support: American Heart Association (14CRP18150008)

Title: Influence of age, motor cortex stimulation, and motor training on neuroplasticity

Authors: *C. L. MASSIE, A. BERCOVITZ, B. STAMPER, S. MCGUIRE, K. JEFFERS Occup. Therapy, Indiana Univ., Indianapolis, IN

Abstract: Age-related neuronal changes that impact motor function can lead to a decrease in effective motor control. Repetitive transcranial magnetic stimulation (rTMS) has the potential to modulate neuronal plasticity in healthy aging adults, which may yield changes in motor control. However, little research exists on the influence of age with motor practice or motor practice plus rTMS. The purpose of this study was to examine how rTMS alone, motor practice alone, and rTMS paired with motor practice influenced neuroplasticity and motor skills in healthy adults. Methods: Thirteen subjects participated in the three different intervention protocols, one per visit separated by a minimum of 48 hours. The interventions included 30 bouts of 6 seconds of wrist extension during the motor practice only, 30 trains of subthreshold 10 Hz stimulation during the rTMS only, and a combination of the two during the motor practice and rTMS intervention. To measure the effect of the interventions, the Box and Blocks Test (BBT), wrist extension force steadiness at 20%, and TMS outcomes (cortical excitability and short-interval intracortical inhibition) were assessed immediately before and after the intervention. Change scores for the TMS measures were calculated to measure neurophysiological changes from baseline and data were analyzed using an ANOVA. Results: The BBT data demonstrated a significant main effect for time for the right upper extremity (p=0.02), but not for the left upper extremity (p>0.05). During the force steadiness testing at 20% of max force, there was a main effect for time (p=0.025) but no difference between conditions. A significant interaction was observed in both TMS outcomes across muscles, conditions, and subjects. The combined intervention (motor practice plus rTMS) differentially targeted the extensors versus the flexors. Conclusions: This study highlights trends that rTMS coupled with motor practice has the potential to target active muscle groups (extensors in this study), while inhibiting inactive antagonist muscle groups (flexors). This finding adds to the understanding that pairing non-invasive brain stimulation with motor practice can have differential effects. This is an area of interest that should continue to be explored given the potential clinical implications of this approach.

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Poster

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Topic: E.04. Voluntary Movements

Support: NIMH IRP (ZIAMH002893)

Title: Task and region specific changes in resting state fMRI induced by short-term motor sequence and visuospatial learning

Authors: *C. THOMAS¹, A. STEEL¹, A. TREFLER¹, G. CHEN², C. I. BAKER¹ ¹Lab. of Brain and Cognition, ²Scientific and Statistical Computing Core, NIMH, Bethesda, MD

Abstract: While prior studies have reported changes in resting-state functional connectivity (rsFC) after learning, the specificity of such effects to particular tasks and regions has not been firmly established. Further, the extent to which any apparent training-related changes in rsFC are influenced by factors such as time-of-day are unclear. Here, we address these issues using a within-subject design involving 19 healthy adults over four experimental visits. Each visit included two scan sessions, at 1000 and 1400 hours (AM/PM). On visits 2 and 3 between the AM and PM scans, participants trained for 90 minutes on either a visuospatial-learning (VSL) or a left-lateralized motor sequence-learning (MSL) task respectively. On visits 1 and 4 (control visits), participants received no training allowing us to model diurnal changes in rsFC. We examined training-specific changes in rsFC in anatomically and functionally-defined task-related networks using seed-based correlation analysis with the bilateral hippocampi (HIPP) and motor cortices (MC), given their involvement in VSL and MSL, respectively. Both HIPP and MC networks showed diurnal fluctuations in connectivity. After controlling for diurnal fluctuations, the effect of VSL on HIPP was evidenced by a training-related decrease in connectivity between the hippocampi and sensorimotor, premotor, and lateral prefrontal cortex, but an increase in connectivity with anterior medial place area and putamen. In contrast, MSL evoked an increase in connectivity between MC and temporal and occipitotemporal cortices, but a decrease in connectivity between MC and the caudate nucleus. Better performance in MSL was positively correlated with an increase in connectivity between MC and putamen and cerebellum and these effects were lateralized to the trained cerebral and cerebellar hemispheres. Finally, we explored the impact of time-of-day and training on large scale resting-state networks. Time-of-day related fluctuations in rsFC in multiple networks were observed. However, short-term motor training strongly modulated the inter-network rsFC with a decrease in connectivity between dorsal motor network to posterior default mode network as well as connectivity between the somatosensory

network and right dorsal attention network. These results suggest that task- and region-specific effects of training can be detected using rsFC, but also underscore the importance of controlling for potential confounds in functional connectivity based analyses.

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Poster

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Topic: E.04. Voluntary Movements

Support: MEXT/JSPS KAKENHI Grant Number 16K16421

Title: Voluntary rehabilitation for promoting motor paralysis recovery in intracerebral hemorrhage model rats

Authors: *C. SATO, S. KOEDA, K. SUMIGAWA, M. MIKAMI, K. AKAHIRA, J. YAMADA Hlth. Sci., Hirosaki Univercity, Hirosaki city, Japan

Abstract: Following intracerebral hemorrhage (ICH), the enormous burden of secondary insults causes a deterioration of quality of life and health in surviving patients. Given the fact that the viability of severe stroke patients has increased with technological progress, the importance of post-stroke treatment becomes increasingly prominent. Motor paralysis in particular is one of the severe aftereffects that deteriorates not only bodily function but also mental health. Because recovery from motor paralysis requires long-term rehabilitation, establishment of new therapies is indispensable. Exercise is recognized to be beneficial for promoting recovery of motor function after stroke in general. However, the most effective kinds of exercise for the rehabilitation of motor paralysis are still unknown. Recently, the notion that motor function recovery is promoted by patients' individual psychological factors such as motivation has attracted much attention, but the mechanism of this effect is still unclear. In this study, the purpose was to compare the effects of forced and voluntary exercise for motor function recovery after collagenase-induced striatal ICH in rats. Male SD rats were placed on a stereotactic apparatus and injected with collagenase (200 U/mL) into the right striatum to induce ICH under deep anesthesia. ICH rats were randomly divided into forced exercise (F-Ex. group, n=7), voluntary exercise (V-Ex. group, n=8) and non-exercise (Non-Ex. group, n=10). The F-Ex. group was trained with forced treadmill exercise and the V-Ex. group was trained with voluntary wheel running. The rehabilitation period was 4-14 days after the surgery. Motor functions were assessed using the motor deficit score (MDS) and its subdivisions on days 0-15 in all groups. The animals in all groups were paralyzed severely at 1 day after surgery throughout the period.

Paralytic symptoms continued in the Non-Ex. rats for 2 weeks after the surgery. Both of the trained groups had higher scores in all subdivisions and higher total MDS score than the non-Ex. group. The recovery of the V-Ex. group was faster than that of the F-Ex. group. These data suggest that exercise promotes the recovery of motor function, and that exercise with motivation may be more effective than forced exercise.

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Poster

317. Motor Learning and Recovery

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 317.15/HH14

Topic: E.04. Voluntary Movements

Support: SPU Grant H2728B1

Title: Influence of nerve regeneration on anterior cruciate ligament injury healing process in a rat model

Authors: *N. KANEMURA¹, T. KOKUBUN¹, Y. MORISHITA², K. MURATA³, A. NAKAJIMA³, K. MATSUI⁴, K. ONITSUKA⁵, S. FUJIWARA³

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Abstract: [Purpose]Anterior cruciate ligament (ACL) injuries are one of the most common knee trauma during sports activity. The injured ACL is less able to control knee movement. The abnormal knee movement can also damage the soft tissue. These results lead to the instability, loss of motor and sensory functions of the knee joints. Although ACL is thought not to heal spontaneously, we clarified that the ligament heals when normalizing the joint movement(Kokubun 2016).The purpose of this study was to evaluate neurogenesis during the process of ACL healing. [Methods]Adult Wistar male rats (11 weeks old) were randomly assigned to three groups, ACL transection (ACL-T; n=12), ACL transection and controlling abnormal joint movement (CAJM: n=12), Sham op (Sham; n=12). ACLs were harvested for PCR array analysis, histological analysis at 1, 2 and 4 weeks after surgery. Expression of 84 genes was analyzed using RT² PCR profiler PCR array analysis (Neurogenesis; Qiagen). These mRNA expressions were normalized respect to multiple internal housekeeping genes and analyzed using $\Delta\Delta$ CT method. We evaluated the histologic sections at 4 weeks groups using immunofluorescence methods for primary antibodies Protein Gene Product (PGP 9.5), growth associated protein 43 (GAP43), nerve growth factor (NGF), substance P. Secondary antibodies were used Dylight488 and Cy3. [Results] With cutoff more than two-fold change, Up-regulated gene expressions in CAJM were 20 at 1week, 44 at 2 weeks, 26 at 4 weeks, respectively. Up-regulated gene expressions in ACL-T were 11 at 1week,47 at 2 weeks, 22 at 4 weeks, respectively. In CAJM, PGP 9.5, NGF, GAP 43, positive nerves were observed in the bone attachment part of the ACL, the ligament surface, and the synovial membrane. But ligament regressed and neural elements were not observed in ACL-T. [Discussion] We observed to healing ligament at 2 weeks in ruptured ACL in a rat model in our previous study. Normalization of abnormal joint movement increases the expression of synapse formation, neuronal differentiation, growth factor transcription, neural plastic factor, and decreases that of apoptosis factor. Normal movement of the knee joint alters in biomechanical microenvironment changes of the joint. It was suggested that abnormal joint movement continued for a long period of time, the expression of related factors such as nerve regeneration and axonal outgrowth decreased, adversely affecting nerve regeneration. These findings might have important consequences for neural plasticity and regeneration in the neuromuscular system in the knee deficiency.

Disclosures: N. Kanemura: A. Employment/Salary (full or part-time):; Saitama Prefectural 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.; principal investigator. T. Kokubun: None. Y. Morishita: None. K. Murata: None. A. Nakajima: None. K. Matsui: None. K. Onitsuka: None. S. Fujiwara: None.

Poster

317. Motor Learning and Recovery

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Program#/Poster#: 317.16/HH15

Topic: E.04. Voluntary Movements

Support: DFG RE 2740/3-1

Title: Implicit learning in a visuomotor skill task mimics the SRTT

Authors: ***J. REIS**¹, M. ULBRICH², M. CURADO³, B. FRITSCH⁴ ²Neurol., ¹Univ. of Freiburg, Freiburg, Germany; ³Neurol., Univ. Med. Ctr. Freiburg, Freiburg, Germany; ⁴Univ. of Freiburg/ Neurocenter, Freiburg, Germany

Abstract: Motor skill learning contains implicit and explicit memory components that contribute to overall learning. The serial reaction time task is well known for its measures of sequence specific implicit learning, behaviorally expressed as increased reaction times when exposed to blocks of trials that differ from those containing a sequence. Here we probed, whether implicit

learning on a sequential isometric pinch force task would be observable as changes in reaaction time or as other performance measures, namelly target error, movement time, motor skill measure (adopted from Reis et al 2009) and movement smoothness. 46 healthy subjects performed the SRTT-SVIPT version on two consecutive days. Subjects were divided in 4 different groups (2 main experimental conditions, 2 control groups). In the main experiment subjects practiced either 11 random blocks OR 1 random block followed by 8 sequence containing blocks interleaved with 2 additional random blocks to assess sequence specific implicit learning.

In the sequence condition, subjects improved motor skill more than in the random condition. However, greater sequence specific learning was only observed in the reaction times but not in any of the motor execution variables.Interestingly, sujects anchored the beginning of the sequence implicitly to the first item in the sequence, since this was the only target that did not show reaction time prolongation when switched to teh random blocks.

These data suggest that during learning of an implicit version of the SVIPT, sequence specific learning is manifested in reaction times, but two particular item-associations are stored in parallel, one for the start of the sequence and one for the item-item-association.

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Poster

317. Motor Learning and Recovery

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Program#/Poster#: 317.17/HH16

Topic: E.04. Voluntary Movements

Title: Modulation of the sodium potassium ATPase function and expression by transcranial direct current stimulation of the right sensorimotor cortex

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Abstract: Transcranial direct current stimulation (tDCS) is a noninvasive technique used in various therapeutic applications. Studies have shown that tDCS affects neuronal excitability. However, biological effects of tDCS on the underlying tissues remain unknown. The role Na⁺ K⁺ ATPase in the elicitation and maintenance of membrane potentials is established. In the present study, we investigated the effects of tDCS on Na⁺ K⁺ ATPase expression and modulation. Our findings show that the expression of Na⁺ K⁺ ATPase α 1 is significantly increased in sensorimotor cortex after single session cathode stimulation, single session cathode tDCS, and repeated cathode tDCS. Expression of Na⁺ K⁺ ATPase α 1 is significantly reduced in mice treated with single anode tDCS only. Atp1a1 mRNA expression is increased in mice treated with single

session cathode stimulation of the exposed right sensorimotor cortex. Expression of Na⁺ K⁺ ATPase α 3 is significantly increased in exposed sensorimotor cortex after single session of cathode stimulation, and repeated cathode tDCS. Expression of Na⁺ K⁺ ATPase al phosphoserine 943 is significantly increased in exposed sensorimotor cortex after single session of anode stimulation, and anode tDCS. Expression of Na⁺ K⁺ ATPase α1 phospho-serine 943 is significantly reduced in exposed sensorimotor cortex after single session of cathode stimulation and single session of cathode tDCS. Expression of phospho-Darpp-32 is reduced after single session of anode tDCS, but was increased after repeated anode tDCS. Expression of phospho-Darpp-32 is increased after single session cathode tDCS, but no significant changes seen after repeated session of cathode stimulation. Expression of Na⁺ K⁺ ATPase α1 phospho-serine 23 is increased in exposed sensorimotor cortex after single session anode stimulation, single session of anode tDCS, and repeated anode tDCS. Expression of Na⁺ K⁺ ATPase al phospho-serine 23 is reduced after single session of cathode tDCS. Expression of Na⁺ K⁺ ATPase β2 is increased in exposed sensorimotor cortex after single session of anode stimulation, and single session of anode tDCS. These results reveal that tDCS not only causes changes affecting neuronal excitability, but also affects molecular targets involved in the establishment of the membrane potentials. These molecular changes could play the underlying mechanism mediating the long term neurophysiological and functional changes caused by tDCS.

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Poster

317. Motor Learning and Recovery

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Topic: E.04. Voluntary Movements

Support: PHRC French Ministry of Health NCT00875654

RESSTORE European Grant H2020 RCB 2015-A01938-41

Title: Effect of mesenchymal stem cells on motor recovery in subacute stroke

Authors: ***A. JAILLARD**¹, T. A. ZEFFIRO², A. MOISAN³, M. BARBIEUX-GUILLOT⁴, I. WIKI FAVRE⁵, K. GARAMBOIS⁵, W. VADOT⁶, S. MARCEL⁷, M. J. G. HOMMEL⁸, O. DETANTE⁵

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Hospitalier de la Région d'Annecy, Annecy, France; ⁷Neurol., Ctr. Hospitalier Chambery, Chambery, France; ⁸AGEIS, Univ. Grenoble Alpes, Grenobe, France

Abstract: Purpose. Cell-based restorative therapies are emerging as promising treatments for stroke. We studied the effects of autologous, bone-marrow derived, mesenchymal stem-cell (MSC) therapy on motor recovery following ischemic stroke.

Materials & Methods. Thirty-one stroke patients (mean age 52 ± 10 years; 20 males; 17 left hemisphere lesions) were enrolled as a part of the ISIS (Intravenous Stem cells after Ischemic Stroke) study, receiving intravenously delivered MSCs, standard medical care, physical therapy and occupational therapy. Study details are provided at:

<u>https://clinicaltrials.gov/ct2/show/NCT00875654?term=ISIS+stroke+stem+cells&rank=1</u>. MSC therapy was randomly administered after baseline assessments and patients were followed longitudinally for two years. The motor Fugl-Meyer Scale (FMS) was used as the main motor performance outcome measure. Lesion location and extent were estimated using 1mm³ T1-weighted brain images. Longitudinal mixed effects linear regression models were used to explore effects of time and treatment on the motor FMS.

Results. All stroke lesions involved the middle cerebral artery territory and most were large (mean volume 102.8 ± 63.4 ml). The mean motor-FMS for both treated and untreated patients increased from 35.4 + 28.1 at one month to 49.1 ± 29.8 at six months and 53.9 ± 30.9 at two years. Sixteen patients received MSC treatment with a mean delay of 33.6 ± 6.1 days and injected doses of 158.2 ± 111.2 million cells. No adverse treatment effects were observed. MSC treatment was associated with better motor performance at all times after treatment (p<0.05) (Figure 1).

Conclusions. This randomized control trial demonstrates that intravenous MSC treatment, given in the subacute period after ischemic stroke, can facilitate clinically significant motor recovery. Confirmation of these results in a larger sample of 400 patients using allogenic stem-cell treatment (RESSTORE http://www.resstore.eu/) is currently underway.





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Poster

318. Neuroethology: Development and Anatomy

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: F.01. Neuroethology

Support: Odyssey Internship Program at the University of Chicago to S.G.

Bill and Melinda Gates Foundation to A.B.

Title: Examining the proportion of brain devoted to various structures across certain mammalian orders

Authors: W. TOMITA¹, S. GRETA³, A. BURRE⁴, D. ROSTAMIAN², K. UNO², N. SCHOTTLER², *W. E. GRISHAM²

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Abstract: Proportional scaling allows brains of vastly different size ranges to be compared by determining the relative amount of a brain that is dedicated to a given structure. Examining these relative proportions also provides a sense of the differential demand for the functions of a structure across different species and orders. We focused on primates and carnivores in comparison to representatives from 18 other orders and examined the proportional relationship of the hippocampus, striatum, thalamus, cerebellum, and neocortex as a function of the log brain size.

We used images from 61 species from 20 different orders, including 10 Primates and 17 Carnivores from the Comparative Mammalian Brain Collections

(<u>http://neurosciencelibrary.org/</u>). Histological details can be obtained from <u>http://neurosciencelibrary.org/explore/histoprocedures.html</u>. The volume of the whole brain as well as the volume of hippocampus, striatum, thalamus, cerebellum, and neocortex was determined via ImageJ. Cross-sectional areas were summed and multiplied by the sampling interval.

The proportion of the brain devoted to hippocampus, striatum, and thalamus was inversely related to the overall log brain volume. The hippocampus was proportionately smaller in both primates and carnivores relative to the remaining orders collectively. The striatum was proportionately smaller in carnivores compared to either primates or to the other orders collectively, and the striatal proportion did not change as a function of log brain size in

carnivores. The thalamus was proportionately smaller in primates relative to carnivores and the other orders considered collectively.

The neocortex and cerebellum showed different patterns. The proportion of brain devoted to neocortex was directly related to the log brain volume. The neocortex was proportionately larger in primates and carnivores than it was in the other orders collectively, but there was a lot of variation among species. The proportion of the brain devoted to the cerebellum showed no significant relationship with log brain volume. The cerebellum was proportionately smaller in primates relative to other orders collectively, but primates and carnivores did not differ. Species also varied enormously in the proportion of brain devoted to the cerebellum. Both the cerebral cortex and the cerebellum develop late in ontogeny. The proportional data reveal a high degree of variability in these regions relative to other regions. So a rule of late = variable may be suggested. Late developing structures may present more opportunity for various

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evolutionary pressures to sculpt them, which leads to more variability in their final forms.

Poster

318. Neuroethology: Development and Anatomy

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 318.02/HH19

Topic: A.10. Development and Evolution

Support: EY025422

Title: The auditory system of two pinniped species

Authors: *J. KRUEGER¹, E. C. TURNER², E. K. SAWYER², J. H. KAAS¹ ¹Psychology, ²Vanderbilt Univ., Nashville, TN

Abstract: Pinnipeds (sea lions, seals, and walruses) are carnivorous mammals who have adapted to a life on land and at sea. Very little is known about their central nervous system but recent studies have begun to characterize their sensory systems. Their ape-size brains are marked by an extensive arrangement of sulci and gyri and thus far appear to be similar in their organization to other carnivores such as cats. Here, we extend past anatomical studies from our lab of the northern sea elephant (*Mirounga angustirostris*) and the California sea lion (*Zalophus californianus*) investigating their somatosensory (Sawyer et al, 2016) and their visual (Turner et al, 2017) networks to include the auditory system. Utilizing Nissl substance, cytochrome oxidase, and vesicular glutamate transporters (VGluT 1 and 2), we identified the auditory brainstem, midbrain, and thalamic nuclei in addition to primary auditory cortex. We found that the cytoarchitectural organization of the cochlear nucleus (CN), the superior olivary complex

(SOC), the inferior colliculus (IC), the medial geniculate nucleus (MGN), and the core auditory region (A1) appeared to be similar between the elephant seal (n=1) and sea lion (n=1), with the former featuring slightly larger nuclei across the auditory system. Cytochrome oxidase stained sections of the elephant seal for example revealed a dorsal and ventral division of the CN, at least two subnuclei (the medial and the lateral divisions respectively) of the SOC, a clear division between the dense central nucleus and the dorsal cortex of the IC, the basic MGN subdivisions, and a strong layer IV band in auditory cortex. Similar observations were made with Nissl and VGluT2 in elephant seal and appeared to also hold true in the sea lion brain. Several auditory regions of these two pinniped species exhibited strong similarities with the auditory system of cats making cats an ideal reference for nuclei identification. Furthermore, some architectural similarities were also observed in ferrets and dogs. Thus, these results may provide additional insight in the evolution of large brains supporting a conservation of a set of auditory nuclei across species.

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Poster

318. Neuroethology: Development and Anatomy

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Topic: F.01. Neuroethology

Support: ANR-15-CE32-0007-01 LOCOGAZE

Title: Quantifying the spinal locomotor network-driven oculomotor behavior and its developmental adaptation in larval and adult frog

Authors: *J. BACQUE-CAZENAVE¹, F. LAMBERT¹, M. CAYREL¹, G. COURTAND¹, M. BERANECK², D. COMBES¹ ¹Univ. de Bordeaux INCIA - CNRS UMR 5287, Bordeaux Cedex, France; ²Univ. Paris Descartes, CNPP CNRS UMR8119, Paris, France

Abstract: Body movements require compensatory eye adjustments to maintain a stable visual field. Our previous data from *in vitro* experiments on *Xenopus laevis* revealed that during locomotion, this gaze stabilization is achieved not only by classical visuovestibular reflex feedback but also by feedforward signaling from the spinal locomotor central pattern generator itself. If the spino-extraocular pathway that conveys this efference copy of the locomotor pattern starts to be well known in larval and juvenile Xenopus, the resulting oculomotor behavior remained poorly investigated. This study aims to provide a fine *in vivo* quantification of compensatory eye movements produced by this efference copy before metamorphosis, during larval undulatory swimming, and after metamorphosis, during limb-based swimming. In both

developmental stages, swimming sequences were video-recorded at 500fps in semi-intact preparations lacking visuovestibular sensory inputs, allowing to track eye and tail or limb joints angular positions frame-by-frame. This provides a quantification of amplitude and time relations of the locomotor-induced oculomotor behavior. In larvae, tail undulations produce conjugated eye movements which amplitude (up to 30°) increased with tail movement amplitude (up to 60°) but not with tail swimming frequency (5-15Hz), allowing the animal to maintain a constant gain (eye/tail amplitude ratio, ~0.5) despite the swimming intensity. Eye and tail movement amplitude peaks occurred in phase but with a 10ms lag explainable by the synaptic delay in the spinoextraocular pathway previously estimated from our in vitro experiments. Moreover, those compensatory conjugated eye movements are relevantly modulated during swimming trajectory changes. The locomotor-induced oculomotor behavior seemed to become more robust during larval development. In the froglet, eye movement recordings showed a combination of convergent and retraction eye movements in phase with bilateral synchronous limb extension. Angular eye movement amplitude was much lower (up to 5°) than in larvae. The fine quantification of the locomotor-driven oculomotor behavior in larval and adult xenopus suggests a concomitant adaptation of the feed-forward spino-extraocular command with the maturation of vestibulo-ocular reflexes before and after metamorphosis. This study will bring important information to investigate 1/ interaction between the spino-ocular command and the vestibuloocular reflexes; 2/ cellular basis of spino-extrocular efference copy signaling.

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Poster

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Topic: F.01. Neuroethology

Support: NSF Grant 1257923

Title: What lies below: A visual tour through Tritonia's brain

Authors: *W. N. FROST¹, M. BRITTON¹, A. FERRIER², J. WANG¹, N. WANG², C. J. BRANDON¹ ¹Dept Cell Biol. and Anat., The Chicago Med. Sch., North Chicago, IL; ²Dept. Biol., Lake Forest Col., Lake Forest, IL

Abstract: Gastropod mollusks have been extremely productive model systems for studies of the neural basis of behavior and learning. Key to this success has been the unusually large size and relatively low numbers of their neurons, and the fact that the neuronal somata are conveniently

organized in typical invertebrate fashion around a central neuropil, facing the experimenter, where they can be readily accessed with sharp electrodes. Inevitably, most recording efforts focus on the minority of neurons visible on the ganglion surface. However, many functionally important neurons lie below the surface layer, invisible and therefore largely unstudied. Our lab uses fast VSDs to optically record the action potentials of up to 200 neurons simultaneously. Because shifting the focal plane of the imaging lens allows recording from neurons at different depths, this work would benefit from a better understanding of how the neurons in the imaged ganglia are physically organized in the layers below the surface (e.g., their sizes, depths, and the number of layers). With this aim, we conducted a serial-section light microscopy study of Tritonia's cerebropleural and pedal ganglia. Ganglia dissected from adult Tritonia were fixed, embedded in epoxy, sectioned either vertically or horizontally at 2 or 5 micron intervals, and stained with Toluidine Blue. After photographing each section, the resulting images were rendered into movies to allow high resolution travel through each ganglion, top-to-bottom and side-to-side. Typical for invertebrate ganglia, *Tritonia's* neuronal somata surround a central neuropil, with each soma sending a single axon to either make local connections or to join axon bundles heading into nerves bound for other ganglia or the periphery. Resolution was sufficient to track many axons from their soma origin to their point of exit from the ganglion. Neurons were readily distinguishable by their large nuclei, which occupied 70-90% of the soma diameter. For the pedal as well as the cerebropeural ganglia, the layers of neurons lying between the sheath and the neuropil ranged from 1 to 8, depending on soma size. In the pedal ganglion, neuron somata ranged from 14 - 280 um in diameter, with the smallest neurons typically located closest to the neuropil. A complete section-by-section count for the pedal ganglion tallied 1885 total neurons. The largest of these had axons up to 55 um in diameter - significantly larger than the somata of the smallest neurons. This study represents the most detailed serial-section atlas of gastropod ganglia of which we are aware, and provides a clear view of the neuronal population under electrophysiological study in this well-established model system.

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Poster

318. Neuroethology: Development and Anatomy

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Support: NSF IOS 143602

NIH R21 GM120464

Title: Using gene expression to define the architecture of the zebra finch arcopallium

Authors: *T. M. KASER¹, M. WIRTHLIN², P. V. LOVELL³, C. V. MELLO³ ¹Behavioral Neurosci., Oregon Hlth. and Sci. Univ., Portland, OR; ²Computat. Biol. Dept., Carnegie Mellon Univ., Pittsburgh, PA; ³Dept. of Behavioral Neurosci., Oregon Hlth. and Sci. Univ. Sch. of Med., Portland, OR

Abstract: The arcopallium (Arco), a major pallial subdivision of the avian telencephalon, receives projections from several other forebrain subdivisions, originates descending projections that target sub-pallial areas, and is a major output structure through which cortical-like circuits modulate sensory and motor systems. While an exact correspondence to mammalian structures has not been not fully established, some portions of the Arco are thought to correspond to pyramidal projection neurons in cortical layers 5/6, while others may correspond to pallial portions of the amygdala. In spite of its importance, relatively little is known about the internal organization of this brain area. Here we have conducted a gene expression analysis to define the molecular organization of the Arco in a songbird species, the zebra finch, using in situ hybridization for a set of Arco expressed transcripts (mostly derived from the zebra finch gene expression atlas, ZEBrA: www.zebrafinchatlas.org). We found that some transcripts (e.g. C1QL3, ETV1, PCP4, FEZF2) are expressed in broad patterns that help define the borders of the Arco even when they cannot be easily visualized by cytoarchitectonics in Nissl-stained sections. Other transcripts (e.g. SCUBE1, PLPP4/PPAPDC1A, SEMA6A, CBLN2, CCK, NECAB2, ZBTB20, CYP19A1, PLS3, KCNQ5, MGP, CNTN4, SNCA, PVALB, CRHR2, ANXA6) are expressed in patterns that are more discrete, allowing us to segment the Arco into at least five major domains, and further divide them into additional subdomains (e.g. lateral vs. medial dorsal, dorsal vs. ventral medial). We have also identified markers of the song nucleus robustus arcopallialis (RA), although the majority of these do not differentiate between RA and the lateral core of Arco, suggesting a possible evolutionary origin for this nucleus. While several identified Arco domains and sub-domains appear to correspond to Nissl-defined and/or previously described areas, others have not been previously reported, and/or cannot be easily visualized by Nissl. These results represent a first systematic segmentation of the Arco based on molecular criteria for the zebra finch, and provide a basis for future comparative analyses with other avian, reptilian, and mammalian brain organizations.

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Poster

318. Neuroethology: Development and Anatomy

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Topic: F.01. Neuroethology

Support: BELSPO SSTC PAI P7/17

NIH NS35467

Title: Perineuronal nets development correlates with developmental and adult neuroplasticity related to song learning in the songbird brain

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Abstract: Perineuronal nets (PNN) are aggregations of extracellular matrix components surrounding the soma of specific neurons, mainly GABAergic interneurons expressing the calcium binding protein parvalbumin (PV+). In mammals, the development of PNN limits synaptogenesis around PV+ neurons and PNNs have been validated as a marker characterizing the end of critical periods for visual system development. In songbirds, song learning is limited to critical periods during ontogeny in closed-ended learners such as zebra finches and to specific phases of the annual cycle in open-ended learners such as canaries that are able to modify their song seasonally. Developmental and adult sensitive periods for song learning are associated with neuroplasticity in song control nuclei, including major morphological changes reflecting neurogenesis and synaptic reorganization The hormonal control of developmental and seasonal neuroplasticity is relatively well documented in songbirds but little is known about its possible regulation by PNN. Interspecific comparisons indicate however that more PNN are present in the song control nuclei of closed-ended compared to open-ended learners suggesting a relationship with song plasticity. To explore the expression of PNN throughout development, we used male and female zebra finch brains collected at different key ages in the song learning process (10, 20, 30, 40, 50, 60, 90, 120 days post-hatch, dph) and we quantified the expression of PNN and their colocalization around PV+ interneurons. The number of PNN and the % of PNN surrounding PV+ interneurons increased progressively during developmental song learning in 3 song control nuclei (HVC, RA and Area X). Females who never sing in this species had fewer PNN than males in HVC and RA, two song control nuclei involved in song production, and their number never increased with age so that they became different from males for all ages after 50 dph. Two separate experiments used adult male and female canaries treated with a subcutaneous Silastic implant filled with testosterone or left empty in control subjects to mimic what happens in the spring when the seasonal plasticity of the song ends and the song crystalizes. Testosterone significantly increased the number of PNN in these forebrain song control nuclei in both sexes. Together these data demonstrate that an increased expression of PNN in the songbird brain correlates with the end of sensitive periods for song plasticity and might thus limit further synaptic reorganization at the end of these periods of behavioral and neural plasticity.

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Poster

318. Neuroethology: Development and Anatomy

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Topic: F.01. Neuroethology

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Title: Developmental reconfiguration of the Drosophila ecdysis neural circuit

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Abstract: Shedding the exoskeleton to permit further growth is an essential and recurrent step in the development of all insects and requires at each stage the execution of a stereotyped behavioral sequence, called an ecdysis sequence. The motor patterns-and body parts-required for the ecdysis sequence can vary dramatically from one developmental stage to the next, as they do in the fruit fly, Drosophila melanogaster, which undergoes a complete change in body plan at metamorphosis. Despite their profound behavioral differences, the ecdysis sequences of the larval, pupal, and adult fruitfly are all orchestrated by the release of a common, conserved set of neurohormones. The identity of some of the neurons that release these hormones change during development, indicating some malleability in the circuitry underlying ecdysis, but little is known about what changes occur in this circuitry downstream of the key hormones. To investigate changes in the downstream ecdysis circuitry, we have adapted several methods that allow us to track the development of neurons that express the receptors for the ecdysial hormones ETH, CCAP, and Bursicon. These methods include the previously published G-Trace (Evans et al., 2009, Nature Methods: DOI:10.1038/NMETH.1356) and Cre-Dog (Tang et al., 2015, Nat. Neurosci: doi:10.1038/nn.4081) methods, as well as a novel, Cre-recombinase-based method that we call Cre-Trace. Each of these systems relies on the expression of two different markers, one that is directly driven by the transcription factor Gal4, and identifies neurons that are currently expressing the receptor gene, and one that becomes immutably expressed in the neurons that express the receptor gene in response to activation of a recombinase. The latter marker records the complete expression "history" of the the receptor gene, i.e. all neurons that have expressed it—past and present. We have applied the Cre-Trace, which utilizes a split Cre molecule, to track the known changes in expression of Bursicon over development and find that it reports changes similar to those found by other methods. Application of Cre-Dog, which employs a GFPdependent split Cre molecule, suggests a previously undocumented change in CCAP expression in adult abdominal ganglia neurons. We are currently using the G-Trace method to map changes
in the expression of the Bursicon and ETH receptors. Preliminary evidence suggests broad developmental changes in the patterns of expression of both genes.

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Poster

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Support: HHMI

Title: Systematic identification of ocellar ganglion interneurons and their projections in the brain of *Drosophila melanogaster*

Authors: *J. GOLDAMMER¹, G. M. RUBIN², K. ITO³

¹HHMI: Janelia Res. Campus, Ashburn, VA; ²Howard Hughes Med. Inst. Janelia Farm Res. Campus, Ashburn, VA; ³Dept. of Zoology, Univ. of Cologne, Cologne, Germany

Abstract: Many flying insects possess a secondary visual system besides compound eyes - the so-called ocelli. The ocelli are involved for example in measuring ambient light levels to detect contrast between the sky and land for stabilizing the flight course (e.g. Mizunami 1994). The fruit fly Drosophila melanogaster has three ocelli located on top of the head, two lateral ocelli in the posterior and one medial ocellus in the anterior. Photoreceptors of each ocellus converge into a tripartite neuropil structure, the ocellar ganglion (OCG), which is attached to the dorsal part of the fly brain. As yet, we do not know how neurons in the OCG convey visual information to the brain. We screened several thousand GAL4 expression driver lines of Drosophila to identify and describe the morphology of neurons which innervate the OCG. By using the split-GAL4 intersectional strategy (Luan et al. 2006, Pfeiffer et al. 2010), we were able to generate stable fly lines whose expression patterns are specific to OCG interneurons. We also used the multi-color flip-out technique to provide detailed information about single cell morphology. Our screening revealed three major classes of OCG neurons: 1. Local interneurons, innervating only distinct OCG neuropil portions to for a patchwork of slightly overlapping fibers. 2. Ocellar output neurons consisting of four subgroups. A large axon type with branches in one or two OCG neuropils and terminals in the posterior slope. A cell type innervating all three OCG neuropils and terminals bilaterally in the posterior lateral protocerebrum. A cell type class innervating two OCG neuropils and terminals in the central posterior brain. A descending interneuron type innervating a lateral OCG neuropil and terminals in the contralateral posterior slope and the wing neuropil of the ventral nerve chord. 3. Ocellar input neurons consisting of three subtypes. Two

types with branches in the posterior brain and terminals in one or two OCG neuropils, and a cell type with numerous branches in several brain regions and terminals in all OCG neuropils. These OCG interneuron-specific driver lines can further be tested with optogenetic stimulation or silencing experiments to facilitate better understanding of their functions in visual processing.

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Poster

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Topic: F.01. Neuroethology

Support: German Research Society (RTG 1960)

Title: Anatomy and function of distinct groups of sensory neurons in the femoral chordotonal organ of *Drosophila*

Authors: A. S. CHOCKLEY, S. RATICAN, V. GODESBERG, A. BÜSCHGES, *T. BOCKEMÜHL

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Abstract: Most terrestrial animals rely on legged locomotion (i.e., walking) for many behaviors such as foraging, courtship, and escape. To fit this behavioral variability, walking must be adaptable to internal and external perturbations, such as general motor output variability, unforeseen obstacles, and variations in the walking surface. Therefore, sensory signals must continuously encode and transmit the state of the extremities for animals to produce sensible locomotor output. The motor output required for locomotion in general relies on not only inherently rhythmic neuronal networks (central pattern generators) but also sensory signal-based modulation and adaptation of these networks. Insects have six ambulatory legs that must be coordinated during walking to reliably transport them through their environment. Insect legs contain many sensory organs that measure various aspects of walking, like tactile stimuli, the angular position of leg segments, and load. Among these structures, the femoral chordotonal organ (fCO), an internal proprioceptor in the proximal femur, has been well-studied. It contains dozens to hundreds of neurons, depending on the species; studies have shown that, in addition to detecting substrate vibrations, the fCO provides important information about the leg's state during walking by measuring the position, velocity, and acceleration of the femorotibial leg joint. In Drosophila, it is unknown whether these movement variables are measured by subgroups of fCO neurons. To address this and to characterize the internal organization of the Drosophila fCO in greater detail, we investigated the morphology and function of fCO neurons using ten Gal4 driver lines that sparsely label fCO primary sensory neurons. We found that the distribution of

these neurons within the fCO as well as their central projection patterns varied between Gal4 lines. We then optogenetically activated these neurons in quiescent flies while monitoring tibia position to test for influences on muscle activity. Notably, some Gal4 lines generated tibial flexion, some tibial extension, and some produced no response. Finally, we inhibited these fCO neurons optogenetically in the intact fly in a free-walking paradigm. While the effects on walking were mostly mild, there were clear qualitative differences between the various Gal4 lines, suggesting that these subsets of neurons encode different movement parameters of the femorotibial joint. Our findings add to the understanding of the functional structure of the dipteran fCO and the role of information about various movement parameters during walking.

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Poster

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Topic: F.01. Neuroethology

Title: Non-visual functions of opsins in Drosophila larval mechanosensors

Authors: *D. GIRALDO-SANCHEZ¹, D. ZANINI¹, B. R. H. GEURTEN¹, M. ANDRÉS^{1,2}, M. C. GÖPFERT¹

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Abstract: Evidence is accumulating that opsins can sense more than light. In Drosophila, opsins were recently implicated in larval temperature preference behavior and in hearing in adult flies. Here, we report that Drosophila larvae also require visual opsins for locomotion, and show that the proprioceptors that control locomotion express these opsins. Opsin mutant larvae had reduced muscle contraction amplitude, frequency of peristaltic contractions, locomotion speeds and crawling agility. A genomic rescue of the respective opsin gene restored normal locomotion. Opsin-dependent locomotion defects closely resembled the locomotion deficits of mutants whose chordotonal neurons are impaired. Promoter-fusions revealed that opsins are expressed in the serially arranged, proprioceptive chordotonal neurons in the larval body wall. Opsin expression was confirmed with antibodies, and chordotonal neurons seemed to be the only neurons that express opsins outside the larval eye. This strongly supports the idea that light-independent opsin functions evolutionarily predated their use as photoreceptor proteins.

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Poster

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Title: Generation and utilization of sensory signals encoding force decreases in insect legs

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Abstract: Information from sense organs that detect forces is integral to the control of posture and locomotion. Furthermore, some mechanoreceptors, such as FA1 Meissner's corpuscles of the feet, discharge when forces decrease. In insects, signals of force decrements occur in campaniform sensilla, receptors that detect forces as cuticular strains. However, the specific mechanisms underlying encoding of force decrements and their potential functions in walking have been not been determined. Sensitivity to decreasing forces is not apparently reflected in sensory membrane encoding properties but instead appears dependent upon mechanical properties of the cuticle. In the present experiments, we are characterizing sensory discharges to decreasing forces in stick insects and cockroaches to 1) gain insight into how they are generated and 2) delineate the extent of their effects in walking. Tests use extracellular recording (CS Groups 3, 4 and 6, leg muscles) and application of bending forces to legs as 1) ramp and hold functions and white noise stimuli and 2) joint torques calculated by inverse dynamics from ground reaction forces in freely moving animals. In experiments to date, sensory firing to decreasing forces was invariably phasic and strongly dependent upon the level of load: discharges were minimal/did not occur after large initial load offsets but vigorously reflected the rate of change of force during complete unloading of the leg. Current experiments indicate that these characteristics are also reflected in the motor discharges and activation of leg muscles as synergists elicited by campaniform sensillum stimulation and in discharges recorded during walking in cockroaches. In addition, tests using bending forces applied as ramp and hold functions have shown that sensory firing to decreasing force is strongly dependent upon the duration of the hold phase. We are currently evaluating the contributions of viscoelastic

properties of the cuticle and muscle tensions to this effect. Thus, the information about decreasing forces is strongly context dependent: receptors do not unequivocally encode the magnitude of force decline but are specifically tuned to detect rapid decreases in forces as they approach zero. The resultant sensory bursts can act as cues in detecting leg slipping prior to large changes in joint position and facilitate the onset of swing or compensatory leg lifting. These properties permit detection of critical signals of unloading without the computational cost required to continuously monitor body weight and also provide a specific mechanism for 'emergent' coordination of walking.

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Poster

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Support: Ellen Miller Casey Award

Title: Potential dopaminergic modulation rescues acoustic startle responses after lesions of the telencephalon in goldfish

Authors: A. N. OPALKA¹, N. FISCHER², C. A. ANZULEWICZ¹, *R. F. WALDECK¹ ¹Biology/ Neurosci. Program, Univ. of Scranton, Scranton, PA; ²Col. of Osteo. Med., Western Univ. of Hlth. Sci., Pomona, CA

Abstract: Goldfish react to a vibratory stimulus with a two-stage, C-shaped turn in the opposite direction of the stimulus known as the acoustic startle response. This response is mediated by Mauthner-cells (M-cells) located in the brainstem, which signal motor neurons in the spinal cord. Acoustic input stimulates the eighth cranial nerve and triggers this response. In previous studies, dopamine was found to enhance the eighth cranial nerve activity suggesting a dopaminergic pathway innervating the M-cell (Kumar and Faber, 1999). Further investigations revealed that full ablation of the goldfish telencephalon significantly decreased the likelihood of a complete startle response (Collins and Waldeck, 2006); however, the precise identification of any upstream influence or modulation of the M-cell in the brainstem has yet to be determined. Preliminary data found similar decreased mean startle angles (MSA) of the acoustic startle response between two different groups of goldfish that received medial telencephalon lesions or immersions of a dopamine D1 receptor (D1R) antagonist (Fischer et al., 2016). In this study, medial portions of the telencephalon were lesioned, and fish were then immersed in a D1R agonist to determine a rescue effect of the acoustic startle response after injury. Acoustic startle

responses were tested and recorded for three consecutive pre-testing days, a post-testing day to determine effect of the surgery, and three post-testing days after a D1R agonist immersion. Statistical analysis revealed no significant difference between the ratio of final post-testing day MSA after D1R dopamine administration to pre-testing average MSA. These findings suggest a rescue effect and possible dopaminergic modulation from the telencephalon to the Mauthner-cell circuitry. Future directions hope to reveal the precise dopamine involvement in this modified startle response and the neuroanatomical projections from the telencephalon to the brain stem.

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Poster

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Title: Comparative study of movement behavior and spiking patterns of the electrosensory nerves in glass catfish under sinusoidal electrical stimulation

Authors: *Y. ADACHI¹, K. TATENO²

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Abstract: Weakly electric fish use the electroreceptors for communication, prey detection, or predator detection. Glass catfish (Kryptopterus bicirrhis) is not electrogenic, but electroreceptive. Nerve impulses in their electroreceptors are modulated in response to electrical stimulation. A few report indicates that the sinusoidal electrical stimulation induces avoidance movements of glass catfish. It is not clear how electrical stimulation was coded as neural activity and induced avoidance movements. We investigated non-linear characteristics of electrosensory nerves of glass catfish to sinusoidal electrical stimulation and its associated behavioral responses. We first observed fish behavior during electric sinusoidal stimulation. Behavior responses of glass catfish to a weak sinusoidal electrical stimulation (250 nA, 500 nA, and 1000 nA) were observed in a circular aquarium located in a dark room. The sinusoidal stimulation current (1 - 100 Hz) was applied to the aquarium for 60 s. The electrical stimulation induced avoidance movements depending on the stimulation frequency and its amplitude. For 250 nA, 1 Hz of the stimulation evoked avoidance behavior. For 500 nA and 1000 nA stimulation, the glass catfish avoided the electrodes in the frequency of 1 - 40Hz, but not 100 Hz. Second, we observed spiking property of

glass catfish (5-7 cm long) were placed in an experimental chamber in which water of 20.3 ± 0.4 C was filled. The stimulation electrodes were placed near the anterior and posterior tips of the fish. Stimulation frequency were 1 - 100 Hz. Stimulation amplitudes were 400 - 2000 nA. Nerve impulses were recorded in an ampullary canal. Spontaneous nerve impulses showed periodic beating. The electrical stimulation altered impulse patterns in the low frequency range of 40 Hz or less. The impulse pattern alternations were also dependent on the amplitude. Nonlinear analysis classified the impulse patterns into 5 groups: periodic beating, quasiperiodic beating, bursting, transition, and unclassified. For the weak stimulation, the quasiperiodic beating or bursting patterns were predominantly observed below 40 Hz. In the low frequency range, the periodic beating patterns of the electrosensory nerves were altered to non-periodic beating or bursting and avoidance movements were observed. Those results indicate that the impulse pattern alternations of the electrosensory nerves were associated with avoidance movements.

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Poster

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Title: Activation profiles of Purkinje neurons during optomotor adaptation in larval zebrafish

Authors: *S. NARAYANAN, V. THIRUMALAI Natl. Ctr. For Biol. Sci., Bangalore, India

Abstract: It is believed that the cerebellum acquires and stores models of sensorimotor transformations. How these models are acquired and modified by learning is not clearly understood. Using the larval zebrafish as a model, we are working towards understanding how the cerebellar circuitry refines sensorimotor transformations to minimize errors in motor output.

We use a custom designed closed-loop behavior setup to engage larval zebrafish in an optomotor adaptation task. In this task, head-restrained zebrafish larvae are placed in a virtual environment that provides optic flow. Tail movements made by the fish are detected in real time and transformed to forward displacement in the virtual environment. When feedback gain of this closed-loop environment is changed, the fish modulate motor output to compensate for sensorimotor mismatches introduced by the gain change. Using this behavioral paradigm, we have currently identified two distinct modes of motor adaptation that occur at different timescales. A fast mechanism, that enables fish to correct for gain changes within a swim bout and a slow mechanism that builds up over several bouts. After adapting to the new gain, fish showed consistent changes in bout velocity, duration and inter-bout interval, indicating that they had acquired novel sensorimotor transformations. To investigate the neural basis of these sensorimotor transformations, we imaged activity in specific cerebellar cell types during motor adaptation. We find both motor and sensory components in the activity profiles of Purkinje cells. We are now looking at how these activity profiles are modulated with respect to the specific kinematic changes observed during motor adaptation. Preliminary analysis shows distinct gain related activity in Purkinje cells.

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Poster

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Topic: F.01. Neuroethology

Title: The weakly electric fish Apternotus displays stochastic resonance

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Abstract: Stochastic resonance (SR) is a property of periodic systems in which non-detected signals become detectable with the addition of white noise. Using either direct play-back of its electric organ discharge (EOD) or a sine wave with $f=EOD \pm f$ (where $0 \le f \le 8$), we used the Jamming Avoidance Reflex (JAR) of *Apternotus sp.* to determine if SR is a property of the neural circuit generating the JAR. A program developed in the LabView environment i) digitized the fish's EOD, ii) identified threshold transitions to calculate the CoV, iii) varied the output playback amplitude to the fish, iv) added white noise on demand, v) varied the Vrms of the noise signal when added to the output EOD signal, and vi) recorded and saved to file, the instantaneous EOD frequency. A playback that did not change the coefficient of variation (CoV) of the interpulse interval (IPI) of the EOD was considered subthreshold. Changes in the CoV after

adding varying amounts of noise to a playback or sine wave stimulation revealed the property of Stochastic Resonance, that is, the CoV changed, often by an order of magnitude. The CoV rose more with a smaller amplitude white noise than a greater amplitude when added in a subthreshold playback. In addition to the demonstration of SR, various filterings of the noise spectrum revealed important frequency ranges necessary to eliciting SR.

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Topic: F.01. Neuroethology

Title: How a soft surface releases its grasp: Radular opening in Aplysia californica

Authors: *C. E. KEHL¹, D. M. NEUSTADTER³, S. C. LU, 44118², H. J. CHIEL⁴ ¹Biol., ²Case Western Reserve Univ., Cleveland, OH; ³Calore Med. LTD, Or Akiva, Israel; ⁴Case Western Res. Univ., Cleveland, OH

Abstract: Biomechanics is the study of how the physical body both creates and constrains behaviors. Understanding biomechanics provides a necessary link to neural control, as it clarifies the constraints upon and opportunities available to the body. The biomechanics of soft bodied creatures, such as worms, slugs and squids have few limits to their degrees of freedom, making them particularly challenging to model. These systems can also serve as the inspiration for softbodied robots, which could navigate complex terrains or grasp delicate objects. The feeding grasper of *Aplysia californica* is a model system for understanding neural control. The grasping surface is a thin, finely toothed cartilaginous sheet that undergoes conformational changes as it opens and closes. The opening of this grasping surface has been attributed to the action of the I7 muscle, but experiments in our lab have shown only small effects from removing this muscle from intact behaving animals. In contrast, we have discovered fine muscular fibers that adhere to the radular surface. When activated, these fibers produce openings in reduced preparations. Lesions of these fibers greatly impede the opening of the grasper in vivo. We have created a 3D model based on high resolution MRI data, which simulates both intact graspers and those with sub-radular fiber lesions. This model validates the role of the sub-radular fibers in radular opening. Understanding the motor control of the feeding grasper is likely to have implications for understanding the function and dysfunction of other soft structures, such as tongues and the digestive system.

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Poster

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AFOSR FA9550-14-1-0303

Title: Axonal dynamics during thermal block in unmyelinated axons

Authors: *M. GANGULY¹, M. W. JENKINS², E. D. JANSEN¹, H. J. CHIEL³ ¹Vanderbilt Univ., Nashville, TN; ²Case Western Reserve Univ., Cleveland, OH; ³Case Western Res. Univ., Cleveland, OH

Abstract: Elevated temperatures have been shown to cause propagation block of action potentials in both unmyelinated invertebrate and mammalian axons. Early work suggested that the block was due to potassium currents increasing in speed and "overwhelming" sodium currents. An exact mechanism of the block and the dependence of the block characteristics on axon diameters has not been studied. Recently, through analytical methods, application of infrared laser (IR) light and water bath experiments to Aplysia californica unmyelinated axons, and application of IR to shrew unmyelinated axons, it was shown that small-diameter axons can be blocked at temperatures lower than that required to block large-diameter axons. We explore the mechanism and scaling of axonal block using computational modeling. The computational model consists of an unmyelinated axon that is divided into three regions of different lengths. The first region, R1, is maintained at control temperature of 6.3 °C; the second region, R2, is maintained at a temperature of 25 °C, and the third region, R3, is once again maintained at the control temperature of 6.3 °C. The length of R1 is fixed, and an action potential is initiated at R1 that travels through R2 and R3. The length of R2 is the minimum length of axon required (Lthresh) to produce a block of action potential propagation for a specific axon diameter. The variation of L_{thresh} is shown to be directly proportional to the square root of axon diameter, as shown before through analytical and experimental studies. To confirm the role of potassium ion channels in this observation, dynamic conductance clamps were used along the axon so that the conductance of the various regions of the axon were modulated to mimic the membrane properties that were observed when potassium channels were present in those regions. It was observed through dynamic clamp studies that the traveling action potential wave experiences significant weakening starting at some distance proximal to the onset of the heated region R2. The extent of this distance is a function of the square root of the axon diameter, based on observations of the

spatial extent of the block currents. These studies are likely to provide guidance for the development of novel tools for control of neural activity using thermal modalities such as IR laser, conductive heating, RF or focused ultrasound.

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Title: Mechanisms of motor neuronal recruitment for load response in feeding Aplysia

Authors: *J. P. GILL, H. LU, D. N. LYTTLE, H. J. CHIEL Dept. of Biol., Case Western Reserve Univ., Cleveland, OH

Abstract: Animals can flexibly and robustly respond to changing conditions in their environment. Understanding the neural mechanisms of flexibility could be useful. In the marine mollusk Aplysia, previous studies have shown a complex response to mechanical loading during feeding (Hurwitz and Susswein 1992). Animals recruit a stronger muscular response to small mechanical loads to generate more force. If mechanical loads are large enough to injure the animal, it may cut and release food. We seek to understand the neural control of this behavior. Previously we have shown that, as animals switch from grasping food (biting) to swallowing, activity increases in specific motor neurons that aid in retraction (Lu et al. 2015). Furthermore, in a reduced preparation capable of feeding behaviors (the suspended buccal mass; McManus et al. 2012), we have preliminary results suggesting that a small increase in mechanical load during swallowing may increase recruitment of these motor neurons, whereas a large increase may actually suppress activity in these neurons. What mechanism controls recruitment of the retractor motor neurons in adaptive responses to load? We have observed in feeding motor patterns a reciprocal relationship between the activity of large units on buccal nerves 3 and 2, corresponding to multiaction neurons B4/B5 and retractor motor neurons, respectively. B4/B5 have inhibitory connections to these motor neurons (Gardner 1993), and they can act as proprioceptors that respond to muscle stretching (Jahan-Parwar et al. 1983). Here we study a hypothesized role of B4/B5 in regulating retractor muscle activation under load. Such a mechanism may allow animals to flexibly adjust motor output in response to mechanical load and may suggest general principles for nervous systems in many other animals.

Disclosures: J.P. Gill: None. H. Lu: None. D.N. Lyttle: None. H.J. Chiel: None.

Poster

318. Neuroethology: Development and Anatomy

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Topic: F.01. Neuroethology

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GAANN Fellowship P200A150316

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Title: Methodology for analyzing the performance of muscles of *Aplysia* californica as actuators for biohybrid devices

Authors: *F. R. YOUNG¹, V. A. WEBSTER-WOOD¹, O. AKKUS¹, U. GURKAN¹, H. J. CHIEL², R. D. QUINN¹

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Abstract: A biohybrid robot relies on actuation to navigate areas of interest, allowing it to carry out specific tasks in an open environment. Such biohybrid robots often utilize cells from mammalian or avian sources to provide organic actuation. However, these cells require precise environmental conditions to function. To develop biohybrid robots for use in challenging environmental applications, more robust tissue is needed. To this end, muscle tissue from *Aplysia californica*, which can survive challenging environments, has been used as a robust actuator for biohybrid robots.

Muscles from the feeding apparatus of *Aplysia californica* have been selected as possible actuators. This allows the natural circuitry of the neuromuscular system, which has been well studied, to control the muscle. It is important to characterize the capabilities of the tissue constructs or native muscle tissues that provide propulsive force. Specifically, the I2 muscle has been isolated and characterized using a cantilever force characterization system. The muscle is attached between a flexible cantilever and a rigid base, such that the contraction of the muscle deflects the cantilever and the force can be calculated.

Using this platform, the force capabilities and fatigue properties of the muscle have been assessed under different stimulation protocols. These protocols are based on three ways of actuating the muscle using direct electrical stimulation, nerve stimulation, and neural circuit stimulation, because neuromodulation of muscle force through circuitry has been well documented in *Aplysia*. The cantilever force characterization platform was used to compare these

protocols. Stimulation via the ganglia, and thus via the natural neural circuitry, resulted in significantly higher force output than either direct electrical stimulation or nerve stimulation (p<0.05), with a mean force of 0.1 N. Additionally, direct electrical stimulation of the muscle resulted in the fastest rate of fatigue. In the future, the force and fatigue capabilities of the extrinsic muscles will be investigated to develop biohybrid devices capable of steering.

Disclosures: F.R. Young: None. V.A. Webster-Wood: None. O. Akkus: None. U. Gurkan: None. H.J. Chiel: None. R.D. Quinn: None.

Poster

318. Neuroethology: Development and Anatomy

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Program#/Poster#: 318.20/II1

Topic: F.01. Neuroethology

Title: Combining temporal structure with quantified bursting character aids in ENG classification

Authors: *J. P. SASSE, J. GILL, P. MYERS, M. CULLINS, H. LU, J. M. MCMANUS, H. J. CHIEL

Biol., Case Western Reserve Univ., Cleveland, OH

Abstract: Flexible and robust neural pathways are ubiquitous in animals. Behavioral flexibility is necessary to survive in unpredictable and changing environments. Previous work has demonstrated that variability in feeding behavior in the marine mollusk Aplysia californica can be useful to the animal - in general, motor components relevant to feeding show higher variability within animals, even as they vary less across different animals. (Cullins et al. Current Biology 2015). In this research, we have begun to investigate how to determine common patterns within this variability as a step towards automated analysis of neural recordings for behavioral prediction using a Bayesian network based neural net. Custom Mathematica software was written to carefully map the specific order of activation of motor neuronal bursts extracellularly recorded from the key protractor muscle (I2) and motor nerves (buccal nerves 2 and 3 and the radular nerve) that control the feeding apparatus (the buccal mass) of Aplysia californica during both biting and swallowing behaviors. Results of initial studies showed there is some conserved structure within individual animals and across different animals. Associating basic statistics for activity in each recording channel (i.e., duration of bursting, mean instantaneous firing frequency, and integrated rectified amplitude) with maps of conserved structure led to classification accuracy of 78% (n = 1000, std. error = 0.002 ± 0.001). This combination improved upon the success rate using only basic statistics (72%, n = 1000, std. error = $0.002 \pm$ 0.001) and only structural maps (70%, n = 1000, std. error = 0.002 ± 0.001) considerably. We plan to construct a neural net and to train it using the previously described structural and channel

information. Should this procedure lead to accurate behavioral classification, it will be of general interest as, in theory, the technique will be implementable within a wide range of biological signal analysis settings.

Disclosures: J.P. Sasse: None. J. Gill: None. P. Myers: None. M. Cullins: None. H. Lu: None. J.M. McManus: None. H.J. Chiel: None.

Poster

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Topic: F.01. Neuroethology

Support: NSF

The Hartwell Foundation

Title: Neural dynamics of a feeding pattern-generating circuit in the marine mollusk *Aplysia* californica

Authors: ***J. YANG**¹, H. LU⁵, N. KODAMA², T. FENG³, R. FERNANDEZ GALAN⁴, H. J. CHIEL⁶

¹Dept. of Biol., ²Dept. of Neurosciences, ⁴Dept Neurosci, ³Case Western Reserve Univ., Cleveland, OH; ⁵Biol., ⁶Case Western Res. Univ., Cleveland, OH

Abstract: Because of its large, identified neurons, the buccal ganglion of Aplysia californica is a model system for understanding the neural basis of feeding behavior, which provides insights into motivated behavior and multi-functionality. We are monitoring in real time the activity of multiple key neurons in the neural circuit that controls feeding behavior. To this end, we are combining nerve recordings of the circuit's motor output with recordings of cell bodies of neurons in the neural circuit using a two-dimensional, high-density (100 um pitch) microelectrode array (MEA, 120 electrodes). The majority of previous studies have focused on recordings from single neurons, or small groups of neurons. However, many interesting aspects of neural dynamics can only be understood by looking at large populations of neurons and their relationships to each other. We are using the MEA and at the same time recording from buccal nerves 2 and 3, the radular nerve and the I2 muscle to identify motor neurons on the array during motor patterns, and distinguish them from interneurons. We have successfully recorded from large identified neurons in the ganglion while recording one to one extracellular action potentials in the nerves. At the same time, we can monitor the extracellular potentials and current densities throughout the ganglion. Our preliminary data suggests this approach will provide deeper insights into a pattern-generating circuit.

Disclosures: J. Yang: None. H. Lu: None. N. Kodama: None. T. Feng: None. R. Fernandez Galan: None. H.J. Chiel: None.

Poster

319. Birdsong: From Neurogenesis to Genetics and Epigenetics

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Topic: F.01. Neuroethology

Support: NSERC Discovery Grant 326869

Title: Global DNA methylation patterns in the brain of the black-capped chickadee (Poecile atricapillus)

Authors: S. D. AITKEN¹, C. A. BLACKMAN², I. C. WEAVER³, *L. S. PHILLMORE¹ ¹Psychology and Neurosci., ²Biol., ³Psychology & Neurosci., Dalhousie Univ., Halifax, NS, Canada

Abstract: Like other songbirds, black-capped chickadees (*Poecile atricapillus*) have specialized neural regions for song production, perception, and learning. In addition, they are well-studied for their spatial memory and its associated neural underpinning, the hippcampus. Previous research has typically focused on short-term neural changes in perceptual regions in response to acoustic stimuli; however, long-term changes in these regions have not been investigated to the same degree. DNA methylation is one of several persistent epigenetic mechanisms that change the accessibility of DNA to be transcribed; increased levels of DNA methylation block transcription and can affect behavioural memory formation and maintenance. We were interested in whether global patterns of DNA methylation in various regions (caudomedial nidopallium NCM, caudomedial mesopallium CMM, HVC, and hippocampus) differed between male and female chickadees. We were also interested in whether learning ability on an operant discrimination task was related to levels of methylation in perceptual regions. Using 5-Methylcytosine (5mC; a marker of DNA methylation) and NeuN-a neuronal marker, we labeled tissue from male and female chickadees that had participated in a discrimination paradigm. Preliminary results show there are no differences in levels of DNA methylation between males and females in perceptual regions, but there is evidence of lateralization of methylation between hemispheres in CMM. This study lays the groundwork for investigating acquired and heritable changes in DNA methylation at regulatory sites of genes involved in song perception and learning.

Disclosures: S.D. Aitken: None. C.A. Blackman: None. I.C. Weaver: None. L.S. Phillmore: None.

Poster

319. Birdsong: From Neurogenesis to Genetics and Epigenetics

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Topic: F.01. Neuroethology

Support: Japanese Government MEXT scholarship

JSPS KAKENHI 16H01261

Title: Interspecific divergence of cis- and trans-regulation for gene expression associated with species-specific learned vocalization

Authors: *W. HONGDI, A. SAWAI, S. HAYASE, K. WADA Hokkaido Univ., Sapporo, Japan

Abstract: Birdsong is acquired through vocal learning with species-specific constraints. Over 3,500 songbird species produce their unique songs using evolutionally conserved song circuits across species. Interspecies variation of gene expression in the song circuits is considered a crucial regulatory factor for species-specific vocal learning. Previous studies showed that cisand/or trans-regulatory changes during evolution contribute to interspecific gene expression patterns. However, it remains unknown how the regulatory mechanism of interspecific gene expression affects species-specific learned vocal pattern. Here we show that gene expression in the song circuits is regulated by both interspecific divergence of cis- and trans-regulation, which are also associated with species-specific song pattern. Whole transcriptome analysis revealed 1,730 and 1,620 genes in RA and HVC, respectively, as differentially expressed genes between zebra finch (ZF) and owl finch (OF). Using species-specific SNPs between ZF and OF, 403 and 505 genes were differentially regulated with allelic imbalance bias in their F1 hybrid's RA and HVC, respectively. Analysis of regulatory mechanisms showed that both cis- and transregulations play important roles in interspecies different expression in the motor circuits nuclei. Furthermore, cis- and trans-regulated genes are significantly enriched in some cluster of genes that were significantly correlated with acoustic and sequence features of their learned song. These results suggest a potential contribution of both cis- and trans-mechanisms in the regulation of species-specific gene expression and song patterns.

Disclosures: W. Hongdi: None. A. Sawai: None. S. Hayase: None. K. Wada: None.

Poster

319. Birdsong: From Neurogenesis to Genetics and Epigenetics

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Topic: F.01. Neuroethology

Support: HHMI

Title: Enhancing vocal learning: Gene regulatory specializations in the song circuit

Authors: *L. CANTIN¹, M. WIRTHLIN², J. CAHILL¹, G. GEDMAN¹, A. R. PFENNING², E. D. JARVIS^{1,3,4}

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Abstract: Vocal learning is a rare trait shared by a small number of distantly related species, such as songbirds, parrots, hummingbirds, cetaceans, bats, elephants, pinnipeds and humans. Similarities in behavior, development, learning and brain circuit structure are found across vocal learning species examined thus far, likely as a result of convergent evolution. In the vocal learning birds and humans, using high throughput transcriptome analyses our groups previously found convergent gene expression specializations (up- or down-regulation) of 10-100s of genes in song regions of birds and speech brain regions of humans relative to adjacent motor learning pathways. Here we begin to ask what are the molecular causes of the specialized gene expression. We performed H3K27ac ChIP-seq experiments on the song production nucleus of songbird (Taeniopygia guttata) RA (robust nucleus of the arcopallium) and the adjacent motor region and comparative genomic experiments across species to identify non-coding regulatory regions of the genes specialized in vocal learning brain regions. H3K27ac is generally associated with active enhancer DNA. Using native ChIP-seq, we identified several hundred H3K27ac peaks with significantly differential activity between chromatin of neurons in songbird RA and the adjacent motor regions, including a subset associated with specialized genes identified in RNA-seq transcriptome experiments. Aligned genomes of avian vocal learning species and their closest vocal non-learning relatives revealed many vocal learning specific accelerated regions in close proximity to specialized genes. We will next combine these data sets to determine whether accelerated regions have differential H3K27ac peaks between RA and adjacent motor areas. These findings suggest that epigenomic regulation at the level of enhancers with accelerated evolution could provide a basis for the molecular specializations associated with vocal learning brain regions. These experiments will help to identify the genetic mechanism in which vocal learning evolves and will benefit research for understanding vocal communication disorders.

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Poster

319. Birdsong: From Neurogenesis to Genetics and Epigenetics

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UCLA Council on Research Grant

Title: Targeting activity-dependent chromatin remodeling pathways to rescue deficits in learned vocal communication

Authors: *C. M. AAMODT¹, C. FUENTES MARTINEZ², J. T. WEISS¹, S. A. WHITE¹ ¹Neurosci. Interdepartmental Ph.D Program, ²Dept. of Biol. Chem., UCLA, Los Angeles, CA

Abstract: In striatal regions activated during learned vocalization, the transcriptional profiles of zebra finches and humans are more similar to each other than to more closely related non-vocal learning species. This makes the zebra finch essential for discovering drugs that treat communication deficits. Humans with learned vocalization deficits benefit from speech therapy, suggesting that pharmacologically targeting transcriptional machinery activated by experience could enhance the efficacy of environmental interventions. To investigate activity-dependent changes underlying vocal learning in the basal ganglia song nucleus Area X, we mined gene expression data generated via weighted gene co-expression network analysis and identified several candidate transcriptional regulators that are highly correlated to singing. Upregulation of the microRNA-128 (miR-128) host gene and downregulation of many miR-128 targets associated with human communication deficits were strongly correlated to singing. One target associated with auditory learning in rodents, histone deacetylase 3 (HDAC3), dynamically changed with singing at the protein level. Decreased HDAC3 (and other singing-induced changes in transcriptional regulators) leads to an increase in epigenetic marks associated with histone variant exchange. In line with this, we found a positive correlation between histone variant H3.3 protein and singing in adult birds.

Ginsenoside Rh2, a biologically active compound isolated from ginseng, has been shown to increase miR-128, and downstream pathways related to epigenetic modification in in vitro or peripheral systems. Ongoing experiments using oral administration of ginsenoside Rh2 to

juvenile birds learning their songs are aimed at determining whether or not this drug has an effect in vivo on song control neurons and song learning. Preliminary behavioral analysis suggests that activity-dependent chromatin remodeling underlying learned vocalization may be a promising therapeutic target for treating communication deficits in humans.

Disclosures: C.M. Aamodt: None. **C. Fuentes Martinez:** None. **J.T. Weiss:** None. **S.A. White:** None.

Poster

319. Birdsong: From Neurogenesis to Genetics and Epigenetics

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Topic: F.01. Neuroethology

Support: R03 DC 014753

Title: Epigenetic mechanisms enable song specific auditory memories in songbirds

Authors: *M. L. PHAN, M. M. GERGUES, S. MAHIDADIA, R. BERNABE, J. JIMÉNEZ CASTILLO, D. S. VICARIO, K. M. BIESZCZAD Psychology, Rutgers Univ. Dept. of Psychology, Piscataway, NJ

Abstract: How do naturally salient sounds like conspecific vocal communication signals have unique access to memory encoding and storage? Long term memory (LTM) for auditory cues depends on neural mechanisms that rely on gene expression for auditory plasticity to encode specific sounds. Because gene expression is regulated by epigenetic mechanisms, we used a selective inhibitor of histone deacetylase 3 enzyme (HDAC3i) to determine whether histone acetylation may gate experience with conspecific vocalizations into long-term memory, following earlier work showing that rats treated with HDAC3i acquire sound-specific memory for the characteristic features of a synthetic but behaviorally-significant pure tone sound (Bieszczad et al., 2015). We now report that HDAC3i in the adult male songbirds enables the transformation of a limited experience with a conspecific song into LTM for that song in the caudomedial nidopallium (NCM). NCM is analogous to superficial layers of mammalian A1 or secondary cortex, and known to function in song discrimination and memory. NCM plasticity appears as stimulus-specific adaptation: evoked responses to familiar (remembered) sounds adapt more slowly than to novel (or forgotten) sounds. Thus, adaptation rate (slope of decrease in response amplitude as a function of song repetition number) between pre-exposed familiar (F) vs. completely novel (N) songs provides a measure of the neuronal strength of memory for familiar songs. Normally, >200 repetitions (200X) of a single conspecific song can produce LTM in adult male zebra finches (revealed in NCM neuronal plasticity >20h later). Here, exposure to only 20 repetitions (20X) of 8 novel zebra finch songs was sufficient to induce

neuronal memory to the 8 previously exposed F songs compared to 8 never-before-heard N songs when the limited exposure was followed by systemic injection of HDAC3i (n=8), but not of vehicle (n=6). Furthermore, we established a link between HDAC3 inhibition and song-specific memory enabled via gene expression. NCMs dissected from birds sacrificed 30-minutes after HDAC3i or vehicle injection reveal HDAC3i induced increases in *zenk* (aka *egr-1*) expression. Strikingly, the effects of HDAC3i on NCM electrophysiology and transcription were lateralized: only left NCM exhibited neuronal memory and increased *zenk* expression in HDAC3i-treated birds relative to vehicle-treated birds. Therefore, HDAC3i transforms sub-threshold auditory experiences into LTM by inducing a lateralized reorganization of the representations of specific, salient sounds, which may naturally gate privileged entry of conspecific vocalizations into long-term memory stores.

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Poster

319. Birdsong: From Neurogenesis to Genetics and Epigenetics

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Support: NSF Grant 143602

NIH R24-GM120464

Title: Genomics and transcriptomics of ion channel genes in the vocal control system of the zebra finch

Authors: *S. R. FRIEDRICH¹, C. R. OLSON², C. V. MELLO¹ ¹Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR; ²Dept. of Physiol., Midwestern Univ., Glendale, AZ

Abstract: Vocal learners acquire complex vocalizations through the imitation of tutors. Studies in zebra finches, a vocal learner songbird species, have revealed significant insights into vocal learning behavior, as well as the anatomical and functional organization of underlying circuitry. A great advantage of this model organism is that we have at our disposal a high-quality genome, brain cDNA/EST libraries, and extensive in situ hybridization and transcriptome datasets of brain gene expression. Because ion channels are essential in determining neuronal firing properties and conducting currents that initiate and propagate action potentials, we have been rigorously examining families of genes that encode ion channels. As a follow-up to our previous survey of potassium channels, we have now performed thorough analyses of sodium, calcium,

and chloride channels, as well as their accessory subunits. We first used genomics analysis to determine which ion channel-related genes are present in the zebra finch genome, making use of best reciprocal alignments and synteny to verify orthology to mammalian genes. We next used in situ hybridization and microarray (cDNA- and oligonucleotide-based) analyses to examine their expression within five key nuclei of the song system -- HVC, RA, and nXIIts in the direct vocalmotor pathway, and Area X and LMAN in the anterior learning-related pathway. We found that numerous members of these families are differentially regulated within several vocal nuclei in comparison to adjacent areas, and that many represent exquisite positive or negative markers of these nuclei (e.g. SCN3B, CACNB4, CACNA2D1.) From these expression patterns, inferences can be made about likely molecular determinants of excitability for each nucleus. Each nucleus is characterized by a diverse set of differentially expressed genes from each family, and there are no cases of unidirectional regulation across all nuclei for any examined channel, suggesting that each nucleus relies on a unique profile of ion channel genes. Of note, several calcium channels are positive markers for both HVC and Area X, suggesting shared upregulation that may be related to properties of the anterior forebrain pathway. These ion channel expression profiles provide insights into nucleus-specific versus shared determinants of firing properties in key vocal nuclei, and offer compelling targets for future manipulation studies.

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Poster

319. Birdsong: From Neurogenesis to Genetics and Epigenetics

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Title: Development of a more efficient approach for generating germline transgenic songbirds

Authors: *M. T. BIEGLER^{1,2}, A. KEYTE^{3,2}, K. M. JUNG⁴, Y. H. PARK⁴, J. Y. HAN⁴, E. D. JARVIS^{3,2,1}

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Abstract: The lack of efficient approaches for development of transgenic songbirds represents an obstacle in assessing the molecular basis of vocal learning. Lentiviral mediated germline transgene expression has been achieved in songbirds, but this approach only allows random knock-in of genes and requires manipulating hundreds of embryos to obtain at least one successful germline transgenic. Recent technical improvements in isolation of avian primordial germ cells (PGC), avian embryonic manipulation, and general CRISPR-Cas9 genome editing have theoretically made transgenic development in songbirds more efficient. Here, we adapted methods used in Galliform birds to attempt to isolate zebra finch PGCs and integrate transgenes into them. Separately, we have successfully used Cas9 targeting of guide RNAs to manipulate the genome of the zebra finch. We are employing these techniques to develop CRISPR-Cas9 expressing chimeras, some of which are currently being assessed for germline transmission. We hope to use Cas9 transgenic offspring to induce targeted knockout of candidate genes through both local viral injections and subsequent embryonic manipulations for global disruption.

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Poster

319. Birdsong: From Neurogenesis to Genetics and Epigenetics

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Support: NIH R24 GM092842

NIH R21 GM120464

Title: Update and applications of the zebra finch expression brain atlas (ZEBrA; www.zebrafinchatlas.org)

Authors: *C. V. MELLO, P. V. LOVELL

Dept. of Behavioral Neurosci., Oregon Hlth. and Sci. Univ. Sch. of Med., Portland, OR

Abstract: We present here an annual update to the Zebra finch Expression Brain Atlas (ZEBrA; www.zebrafinchatlas.org). ZEBrA is a publicly accessible online resource for investigating the brain distribution of genes involved in the physiology, development, and maintenance of functional circuits in the brain of songbirds. It consists of an in situ hybridization database for transcripts expressed in the brain of adult male zebra finches (T. guttata), a songbird species and the major model organism for studying vocal learning. Its major features and recent updates include: (1) The In situ database - an expanding collection of high-resolution (0.46 μ m/pixel) digital images presented along with annotated drawings from a reference Histological Atlas. We have recently added ~150 image sets, so that ZEBrA currently houses more than 2,000 images (>100 GB) corresponding to ~660 brain expressed genes, including markers of all major nuclei that comprise the song system. (2) A reference Histological Atlas Browser - A set of 18 annotated drawings prepared in registration with Nissl- and Myelin-stained images of sagittal brain sections derived from the Karten/Mitra atlas. (3) A Neuroanatomical Marker Search Tool -

A search engine that allows users to retrieve a list of genes that are markers of a given structure, or of multiple structures. Markers of specific song nuclei are now highlighted in the homepage to facilitate access and use of the database. (4) Homepage vignettes that highlight and provide fast access to genes of great interest to songbird biology (e.g. song system markers), human speech and language disorders (e.g. FOXP2 and related targets), human neurological diseases, and membership in gene families (e.g. glutamate or GABA receptors). Recent uses of ZEBrA include: (1) utilizing the in situ database for validation and establishing cut-off criteria for microarray studies of the song system; (2) performing quantitative analysis of regional gene expression patterns, in search of molecular signatures of specific avian brain areas; (3) obtaining evidence of heterogeneity of cellular patterns of gene expression as a basis for cellular phenotyping within vocal control nuclei; (4) utilizing the ZEBrA platform and its embedded features as a resource that can be adopted by individual labs for cataloguing, storing and accessing high resolution brain gene expression data; (5) obtaining molecular data to characterize the internal organization of major avian brain subdivisions (e.g. arcopallium, mesopallium, hippocampus).

Disclosures: C.V. Mello: None. P.V. Lovell: None.

Poster

319. Birdsong: From Neurogenesis to Genetics and Epigenetics

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NIH-R21_GM120464

Title: Molecular specializations of the tracheosyringeal portion of the hypoglossal nucleus (nXIIts) in a vocal learning songbird, the zebra finch (Taeiniopygia guttata)

Authors: *N. HUIZINGA¹, P. V. LOVELL², C. V. MELLO²

¹Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR; ²Dept. of Behavioral Neurosci., Oregon Hlth. and Sci. Univ. Sch. of Med., Portland, OR

Abstract: The ability to imitate novel and complex vocal sounds (vocal learning) has been linked to the ability of forebrain circuits to modulate vocal and respiratory centers in the brainstem. Direct connections between forebrain and brainstem vocal areas have been found in all three vocal learning bird groups (songbirds, hummingbirds, parrots), as well as in humans where they comprise a projection from the laryngeal motor cortex to nucleus ambiguous in the brainstem. In contrast, such projections are absent in non-vocal learning organisms, including

avian orders like galliformes (chicken, quail) and columbiformes (pigeon, dove), as well as mammals like rodents and non-human primates. In birds, while motor neurons within the tracheosyringeal portion of the hypoglossal nucleus (nXIIts) innervate the syrinx, the vocal organ, a direct projection from forebrain nucleus robustus arcopallialis (RA) to nXIIts is only present in vocal learners. This RA-to-nXIIts projection allows for forebrain circuits to modulate vocal output, and is thought to be critical for the production of learned vocalizations. Given the importance of this projection, and the temporal and structural complexity of learned, compared to innate vocalizations, we hypothesized that songbird nXIIts might express unique properties that are essential for learned song, and absent in non-vocal learning birds. To test these hypotheses, we examined publically available microarray datasets (45K Agilent oligo array) from previous studies that compared nXIIts to the adjacent non-vocal supraspinal motor neurons (SSp) in order to identify molecular markers of nXIIts. We performed parallel analyses on the nXIIts/SSp of zebra finches and two non-vocal learning species (rock dove, quail), and subtracted the resulting marker lists to identify nXIIts markers unique to finches. Whereas numerous nXIIts transcripts were expressed in combinations that do not reflect the occurrence of vocal learning, a set of ~600 specializations were unique to zebra finch nXIIts, including several genes identified in previous screens (e.g. ROBO1, PVALB). Bioinformatics analyses reveal enrichment in genes related to axon guidance, glutamate receptor binding, and ion channel activity. In sum, our study reveals that the main avian vocal-motor output nucleus, common to both vocal learners and non-learners, expresses a large set of genes that are specific to vocal learners, and thus represent novel candidate modulators of learned vocalizations.

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Poster

319. Birdsong: From Neurogenesis to Genetics and Epigenetics

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 319.10/II12

Topic: F.01. Neuroethology

Support: NSF-IOS-143602

NIH-R21_GM120464

Title: Molecular specializations of vocal nuclei in zebra finches (Taeniopygia guttata) associated with neurotransmitter receptors, neuropeptides, and axonal guidance cues

Authors: *P. V. LOVELL, C. V. MELLO Dept. of Behavioral Neurosci., Oregon Hlth. and Sci. Univ. Sch. of Med., Portland, OR

Abstract: Vocal learning, the ability of animals to acquire vocalizations through imitation, is a rare behavioral trait. Besides occurring in humans and possibly in a few mammalian groups, it is prevalent in songbirds, where it is subserved by a set of brain nuclei that control the vocal and respiratory organs. The connectivity and physiology of these vocal nuclei have been extensively studied in zebra finches (T. guttata). Previous efforts using cDNA and oligo microarrays to study adult finches have identified large sets of differentially expressed transcripts in nuclei HVC, RA, and nXIIts of the direct vocal-motor pathway (Lovell et al., 2008; Hara et al., 2012, Pfenning et al., 2015; Friedrich et al., 2016), and in striatal Area X (Hilliard et al., 2014) within the anterior pathway for vocal learning and plasticity. Bioinformatics analyses provided initial evidence for the differential regulation of genes of relevance to neuronal excitability and plasticity (e.g. ion channels, transmitter receptors, neuropeptides), as well as neuronal connectivity (e.g. axon guidance cues). We now report an in-depth and systematic examination of these gene families based on microarray analyses, as well as in situ hybridization data from the ZEBrA online database (www.zebrafinchatlas.org) and/or from previous published studies. We compared the expression patterns across major nuclei in both the vocal-motor and anterior pathways for ~200 genes in the above categories. Our results indicate that multiple members of these families are differentially expressed in various vocal nuclei, in comparison with the adjacent areas and in contrast to other gene family members. Factors like membership in a particular gene family, the receptor class with regards to the neurotransmitter type (e.g. excitatory vs. inhibitory vs. modulatory), or axon guidance category (e.g. ligand vs. receptor) did not predict which vocal nucleus showed differential regulation. While the data generally suggest that each vocal nucleus possesses a unique molecular profile, some general trends were noteworthy, namely most glutamate receptor types were down-regulated, whereas serotonin receptors were up-regulated. In sum, this is the first time that the expression of this large cohort of genes of relevance to the development, maintenance and physiology of the vocal control system in finches is evaluated in a systematic fashion. Our observations provide novel insights into molecular specializations of vocal control circuits in finches, identify candidate pathways related to patterns of connection between vocal nuclei, and point to candidate target genes for further mechanistic and regulatory studies.

Disclosures: P.V. Lovell: None. C.V. Mello: None.

Poster

319. Birdsong: From Neurogenesis to Genetics and Epigenetics

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 319.11/II13

Topic: F.01. Neuroethology

Title: Experience dependent changes in immediate early gene expression in the auditory forebrain in response to conspecific song in female canaries (Serinus canaria)

Authors: *C. M. HAAKENSON¹, F. N. MADISON², G. F. BALL³ ¹Neurosci. and Cognitive Sci. Program, Dept. of Psychology, ²Dept. of Psychology, ³Univ. of Maryland, College Park, MD

Abstract: Female songbirds are thought to make mate choices based on aspects of male song quality. Male canaries (Serinus canaria) produce songs with "special" or "sexy" syllables composed of fast, frequency-modulated syllables that are especially attractive to females. These "special" syllables have been shown to be highly salient to female listeners and elicit high rates of sexual displays and enhanced immediate early gene (IEG) expression in the caudal mesopallium (CMM) and nidocaudal mesopallium (NCM), two major auditory areas important in processing conspecific song. The purpose of this study was to examine the effects of experience on activity in the auditory forebrain in female canaries. In this study, female canaries were placed on long days (14L:10D) for 6 weeks (photostimulated) then pair housed in sound attenuated chambers. After a brief acclimation period, females were exposed to either song with "special" syllables or song without "special" syllables for 14 days. After transfer to individual housing and one day of silence, birds were exposed to one of the aforementioned stimuli or silence for 90 minutes then sacrificed. Immunohistochemisty for the immediate early gene ZENK was used to detect neural activation in NCM and CMM. Preliminary data suggests that ZENK expression in both the CMM and NCM was higher in females chronically exposed to song containing "special" syllables, while ZENK expression was attenuated in females chronically exposed to song without special syllables. Follicle volume tended to be larger in the females who were exposed to the song with special syllables. This change in the forebrain ZENK response following exposure to preferred song may serve as a mechanism for increasing the likelihood of mating in environments with an abundance of high-quality mate options.

Disclosures: C.M. Haakenson: None. F.N. Madison: None. G.F. Ball: None.

Poster

319. Birdsong: From Neurogenesis to Genetics and Epigenetics

Location: Halls A-C

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Topic: F.01. Neuroethology

Support: JSPS KAKENHI 23115701

JSPS KAKENHI 16H01261

Title: Nature via nurture of vocal learning in hybrid songbirds

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Abstract: Both nature and nurture influence learning process. Like human speech, birdsong is a learned vocalization regulated by both genetic and environmental factors. Songbird juveniles learn their songs, which are species-specifically constrained but individually unique, during the critical period of vocal learning. However, it is not well understood how genetic and environmental instructions modulate the vocal learning process. We examined song development in hybrid songbirds between zebra finch and owl finch, which possess species-specific traits at acoustics and sequence of syllables composed in the songs. The hybrid songbirds exhibit a wide variability of individual differences of song phenotypes under the tutoring environments of both parental species song playbacks. Half population of hybrid birds acquired intermediated patterns of both parental species. In contrast, the other population of hybrids acquired only either one of parental species songs. The individual variability of song was emerged from initiation of song production as the biased acoustic features. Intriguingly, even under the tutoring environment with single-species song playback, a population of hybrid birds developed the songs with nontutored parental species features, indicating the predisposition of learning intentions. Furthermore, the trend of predisposed-learning intention of song was differently biased among families. These results demonstrate nature via nurture for development of the individual uniqueness of vocal learning processes.

Disclosures: K. Wada: None. A. Sawai: None.

Poster

319. Birdsong: From Neurogenesis to Genetics and Epigenetics

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 319.13/II15

Topic: F.01. Neuroethology

Title: Transcriptional clustering reveals molecular convergence between human and songbird vocal learning circuits

Authors: *S. ANNALDASULA¹, M. WIRTHLIN², G. GEDMAN³, E. D. JARVIS⁴, A. R. PFENNING²

¹Computat. Biol., ²Computat. Biol. Dept., Carnegie Mellon Univ., Pittsburgh, PA; ³Rockefeller Univ., New York, NY; ⁴Duke Univ. Hosp., Durham, NC

Abstract: Vocal learning is a complex trait evolved in a handful of lineages in mammals and birds, including humans and songbirds. Though phylogenetically distant, the brain circuits for vocal learning in these two groups display remarkable convergence in terms of their anatomy,

physiology, and molecular specializations. Previously, we discovered that multiple human and zebra finch vocal circuit regions that serve analogous functions also display significant convergence in transcriptional correlation, including songbird RA and Area X, and human Laryngeal Motor Cortex and Anterior Striatum, respectively. These transcriptional similarities between analogous brain regions were discovered by comparing the correlation of overall expression between regions. We now improve and expand upon these results by taking a reverse, 'gene-centric' approach. We developed computational algorithms to cluster the complete set of 10,783 human genes according to their co-expression across 3,681 human brain regions, and statistically relate the similarity of cluster transcriptional specialization across human and songbird anatomical regions. This set allowed us to identify sets of transcriptional modules that could support the functional properties of analogous human and songbird vocal learning brain regions. These clusters provide insight into the molecular basis for known properties of speech and song-associated brain regions, as well as suggest new molecular pathways that characterize these circuits. These findings provide information on the transcriptome changes that could serve as a causal mechanism in the evolution of vocal learning and other complex behaviors.

Disclosures: S. Annaldasula: None. **M. Wirthlin:** None. **G. Gedman:** None. **E.D. Jarvis:** None. **A.R. Pfenning:** None.

Poster

319. Birdsong: From Neurogenesis to Genetics and Epigenetics

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Topic: F.01. Neuroethology

Support: APVV-15-0077

VEGA 2/0177/14

Title: Behavioral plasticity is related to adult neurogenesis in songbirds

Authors: *L. NIEDEROVA-KUBIKOVA¹, K. LUKACOVA¹, J. POLOMOVA¹, L. BACIAK², S. KASPAROVA²

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Abstract: Injury within striatum leads to neurogenic (acquired) stuttering in humans and abnormally high syllable repetition in songbirds such as Bengalese finch (*Lonchura striata domestica*) and zebra finch (*Taeniopygia guttata*). While the stuttering-like song occurs soon after the injury in Bengalese finches, it occurs only after a month in zebra finches. These

behavioral changes are accompanied with lesion recovery which is mostly due to the incorporation of newly born neurons. It has been proposed that the function of adult neurogenesis is to facilitate new memory formation and promote behavioral plasticity. Here we investigated 1/ if the neurogenesis and/or new neuron addition to vocal nuclei are associated with song variability in zebra finch and Bengalese finch and 2/ if the different time-course of stuttering may be caused by the different recovery rate and the newly added neurons into the injured striatum. First we studied the basal levels of neurogenesis in the neurogenic subventricular zone (SVZ) and new neuron incorporation into the striatal vocal nucleus Area X. We found that Bengalese finches had higher level of neurogenesis in most of SVZ and they showed also higher number of new neurons in Area X. The songs of zebra finches were more stereotypical than the songs of Bengalese finches, as shown by linearity and stereotypy scores. The level of neurogenesis in SVZ negatively correlated with the song linearity and stereotypy. In the second part of this study we performed bilateral neurotoxic lesions of Area X in zebra finches and Bengalese finches and employed magnetic resonance imaging for the longitudinal examination of the injury and its recovery in the same birds. This analysis is in progress. The results show that the behavioral plasticity is associated with higher neurogenesis in songbirds.

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Poster

319. Birdsong: From Neurogenesis to Genetics and Epigenetics

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Topic: F.01. Neuroethology

Support: PSC-CUNY

Title: Unilateral vocal nerve section alters new neuron survival in the zebra finch song system

Authors: *J. V. ARONOWITZ¹, C. O'BRIEN¹, A. PEREZ², S. RIBEIRO³, K. WASNER⁴, A. LOPEZ¹, E. RODRIGUEZ⁵, B. KOO¹, C. PYTTE¹

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Abstract: Adult neurogenesis allows new neurons to be continually added to the adult brain. In the adult male zebra finch, the survival of adult-formed neurons in the song motor pathway nucleus HVC has been shown to be positively correlated with the quality of song production. This led to the idea that the degree of matching between expected and received auditory feedback during singing may contribute to the survival of new neurons engaged in song

production. Other regions of the song system that receive new neurons in adulthood are the basal ganglia nucleus Area X and the auditory region caudomedial nidopallium (NCM). These regions also respond to playback of the birds own song and therefore new neuron survival in these areas may similarly be sensitive to song feedback. To test this, we altered song structure by unilaterally denervating the syrinx and quantified new neurons in Area X and NCM in both hemispheres. Mitotically active cells were labeled with i.m. injections of bromodeoxyuridine (BrdU, 3x/day, for three days). Three weeks after the last BrdU injection, birds underwent left or right syringeal denervation by sectioning the tracheosyringeal nerve (nXIIts), which innervates the syrinx. Control birds received sham surgery. One week after surgery, we used immunohistochemistry to label BrdU and the neuron-specific protein Hu. There were no differences in numbers of new neurons between left and right hemisphere Area X in either control or nerve cut birds. Moreover, either a left or right nXIIts nerve cut resulted in a significant bilateral decrease in new neurons in Area X. Confirming earlier work, we found more new neurons in left than in right NCM in control birds. Interestingly, denervation primarily disrupted the left-side lateralization of new neurons. A left nXIIts nerve cut resulted in a significant decrease in new neurons in both hemispheres of NCM. A right nXIIts nerve cut did not alter overall numbers of new neurons but resulted in a reversal of lateralization such that there were more new neurons in the right NCM relative to the left. These results replicate and extend previously reported findings and suggest that new neuron survival throughout the song system may be influenced by song-related feedback, and differs depending upon brain region.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 320.01/II18

Topic: F.04. Stress and the Brain

Title: Sex differences in the effects of chronic stress on dendritic remodeling in orbitofrontal cortex

Authors: *A. GUTIERREZ¹, V. SZALAVARI¹, C. WELLMAN^{1,2} ¹Psychological and Brain Sci., Indiana Univ. Bloomington, Bloomington, IN; ²Program in Neurosci., Indiana Univ., Bloomington, IN

Abstract: Stressful life events are linked to the development of several psychological disorders such as depression and posttraumatic stress disorder (Kendler et al., 1999). This link may be related to sex, with twice as many women suffering from stress-related disorders than men (Solomon & Herman, 2009). Dysfunction of medial prefrontal cortex (mPFC) and the

orbitofrontal cortex (OFC) has been implicated in stress-sensitive disorders, and both mPFC and OFC are sensitive to the effects of stress. For instance, dendritic remodeling in mPFC after chronic stress is sex-specific. Whereas male rats exhibit dendritic retraction after chronic stress, female rats show dendritic growth (Garrett & Wellman, 2009). Liston and colleagues (2006) demonstrated that male rats show dendritic growth in OFC following chronic stress. However, no studies have examined the effect of stress on neuronal morphology in OFC in female rats. Therefore, we compared dendritic remodeling of pyramidal neurons in OFC in male and female rats after exposure to chronic stress. Rats underwent chronic restraint stress (3h/d for 10d) and were perfused on the final day of restraint. Brains were removed and stained using the Golgi-Cox method. Pyramidal neurons in ventral and lateral OFC were reconstructed in three dimensions using Neurolucida and their apical and basilar morphologies were quantified. Spine density on apical and basilar terminal dendrites was also quantified using Neurolucida. We found a basal sex difference in unstressed rats for both apical and basilar dendrites, with female rats having longer dendrites compared to males. This sex difference is opposite that found in mPFC, where unstressed males have longer apical dendrites (Garrett & Wellman, 2009). Moreover, unstressed females had reduced spine densities relative to unstressed males. In agreement with previous studies (Liston et al., 2006), chronic stress increased apical dendritic length in OFC of male rats. This effect was most robust distal to the soma. In contrast, little dendritic change was observed in female rats. We also found a decrease in overall spine density in stressed male rats compared with unstressed rats, which parallels the effects of chronic corticosterone exposure (Gourley et all, 2013). There was no difference between stressed and unstressed females. These results expand upon previous data, and further suggest that stress can have differing effects on neuronal morphology in the prefrontal cortex of male and female rats, and that these effects can differ by subregion. These sex differences in dendritic remodeling in OFC may contribute to differential effects of stress on OFC-mediated behaviors such as reversal learning.

Disclosures: A. Gutierrez: None. V. Szalavari: None. C. Wellman: None.

Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

Location: Halls A-C

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Topic: F.04. Stress and the Brain

Support: NIH Grant MH102065

HDRF Grant

Title: BAG-1 deficiency impairs stress-induced neuroplasticity and alters gene expression in the mouse prefrontal cortex

Authors: *J. KOGAN¹, M. W. SCHELKE², T. G. RUBIN³, R. DAVIDSON¹, N. P. BOWLES¹, B. S. MCEWEN¹, J. D. GRAY¹ ¹Rockefeller Univ., New York, NY; ²Weill Cornell Med. Col., New York, NY; ³Albert Einstein

Col. of Med., Bronx, NY

Abstract: Bcl-2 associated athanogene 1 (BAG-1) is a molecular chaperone that binds to the glucocorticoid receptor (GR) and inhibits nuclear signaling. Decreased levels of BAG-1 mRNA have been observed in the prefrontal cortex of patients with bipolar disorder and schizophrenia. Additionally, BAG-1 protein levels are up regulated in the hippocampus of rats given chronic lithium or valproate, two drugs commonly used to treat bipolar disorder. While the etiology of these disorders is not well understood, exposure to chronic stress can exacerbate their symptoms and precipitate their onset. Repeated GR activation in response to chronic stress causes dendritic shrinkage in the prefrontal cortex in both rodents and humans. Molecular chaperones such as BAG-1 are critical for silencing GR signaling at low CORT levels and allowing for proper activation at high CORT levels. Studying BAG-1 deficiency in vivo will provide important insight into the molecular mechanisms of altered CORT signaling associated with certain mental illnesses and identify new potential targets for pharmacologic intervention. Here we evaluated the effects of BAG-1 deficiency on stress-induced neuroplasticity and gene expression in the mouse medial prefrontal cortex (mPFC). Separate cohorts of adult BAG-1 heterozygous knockout (BKO), and wild type (WT) mice were subjected to a 21d chronic restraint stress (CRS) and sacrificed 24h following the final stress. Brains were either processed for Golgi impregnation, transcardially perfused with 4% paraformaldehyde for immunohistochemical (IHC) analysis or dissected and flash frozen on dry ice for qRT-PCR analysis. Blood was collected for measurement of circulating CORT levels. Unlike WT mice, BKO animals failed to show dendritic remodeling after CRS. Previous work has also implicated the polysialated neural cell adhesion molecule (PSA-NCAM) in stress-induced plasticity. We observed increased levels of PSA-NCAM in the mPFC of BKO animals by IHC and increased mRNA levels of NCAM and the sialating enzyme ST8SiaIV. We hypothesize that deficient BAG-1 levels would lead to excess activation of GR signaling at baseline, potentially mimicking the effects of chronic stress and elevating PSA-NCAM, which then impairs neural remodeling when BKO are subjected to chronic stress. Experiments to evaluate differences in GR activation before and after stress, as well as other markers of stress are underway. These studies demonstrate the importance of BAG-1 levels in stress-induced neuroplasticity in the mPFC and suggest that BAG-1 deficiency may mimic the effects of chronic stress.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

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NIMH BRAINS Award

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AAUW American Fellowship

Title: The unusual suspects: Oligodendrocytes predict resilience vs. vulnerability in response to stress

Authors: *K. LONG¹, L. CHAO², T. C. NEYLAN³, D. KAUFER¹ ¹UC Berkeley, Berkeley, CA; ²Radiology, ³Psychiatry, Univ. of California, San Francisco, San Francisco, CA

Abstract: Post-traumatic stress disorder (PTSD) is a debilitating anxiety disorder that affects nearly 20% of individuals who experience a traumatic event; however, the majority of individuals will recover from the experience. Understanding the neural underpinnings of individual variation in resilience and susceptibility to stress is of critical importance to public health; however, the basis for differential susceptibility to anxiety remains poorly understood. Recent research has shown that PTSD patients have greater myelin content in the hippocampus when compared to trauma-exposed, yet unaffected individuals. Furthermore, previous work from the Kaufer lab has found that stress increases the production of oligodendrocytes (myelinproducing cells) in the hippocampus of rats; however, whether white matter is a contributing factor to these disorders or merely a secondary consequence remains largely unknown. In the present work, we investigated the hypothesis that stress-induced changes to oligodendrocytes and myelin are both predictive and causative of high anxiety. To test this, we used an animal model of severe stress to induce a spectrum of anxiety-like behavior in rats. Brains collected from animals were analyzed for oligodendrocyte, myelin, and synaptic markers using immunohistochemistry and confocal microscopy. Interestingly, we found that increased oligodendrocyte and myelin density of the hippocampus following stress predicted higher rates of anxiety. In a second set of experiments, we experimentally increase or block hippocampal oligodendrogenesis to test whether increasing oligodendrogenesis is necessary and sufficient to generate the behavioral expression of anxiety. These studies contribute to the understanding of

differential susceptibility to stress and may provide new avenues for PTSD biomarkers and therapies.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

Location: Halls A-C

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Topic: F.04. Stress and the Brain

Support: BSD Office of Diversity & Inclusion at The University of Chicago

Title: HIF1a signaling modulates synaptic plasticity and adult neurogenesis following chronic intermittent hypoxia

Authors: *A. J. GARCIA, III¹, M. A. KHUU³, T. NALLAMOTHOU² ¹Emergency Med., ²The Univ. of Chicago, Chicago, IL; ³Emergency Med., Univ. of Chicago, Chicago, IL

Abstract: Several sleep disorders, such as sleep apnea, often correlate with a decline in cognitive performance among children and adults. Previous studies have shown that hippocampal plasticity and cognitive performance are impaired in animal models exposed to chronic intermittent hypoxia (CIH), a cardinal trait of sleep apnea. Moreover, our recent work has shown that CIH modulates adult neurogenesis in the hippocampus. While the effects of CIH appear to involve oxidative stress, the role of the pro-oxidant transcription factor, hypoxia inducible factor 1a (HIF1a) plays in this phenomenon remains to be resolved. Here we test the hypothesis that HIF1a signaling is necessary part of the signaling pathway for CIH to suppress synaptic plasticity and modulate neurogenesis in the hippocampus. Following ten days of CIH, hippocampal brain slices were prepared from wild-type and HIF1a heterozygous knockout (HIF^{+/-}) mice for electrophysiological or immunohistochemical studies. CIH suppressed long term potentiation in area CA1 and appeared to enhance adult neurogenesis in wild-type mice. However, long term potentiation from HIF1a^{+/-} mice was unaffected by CIH suggesting the involvement of HIF1a signaling for CIH to suppress synaptic plasticity. Ongoing experiments will identify the role CIH-induced HIF1a signaling plays to modulate hippocampal neurogenesis. These findings for role of HIF1a may be critical to understanding the molecular basis by which sleep apnea leads to cognitive decline.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

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Topic: F.04. Stress and the Brain

Support: National Research Foundation Graduate Research Fellowship Program

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NARSAD

Hartwell Foundation

Title: Network analysis of frontal cortical microcirrcuit dynamics after chronic stress hormone exposure and ketamine treatment

Authors: *R. N. MODA^{1,2}, M. MURDOCK^{1,2}, R. FETCHO^{1,2}, E. ALWAY^{1,2}, D. ROSENTHAL^{1,2}, K. LOPEZ^{1,2}, Y. MENG^{1,2}, T. HUYNH^{1,2}, C. LISTON^{1,2} ¹Neurosci., Weill Cornell Med. Col., New York, NY; ²Feil Family Brain & Mind Res. Inst., New York, NY

Abstract: Chronic stress alters neuronal morphology within the prefrontal cortex (PFC), inducing changes such as dendritic retraction and spine loss. Subanesthetic doses of ketamine, an NMDA antagonist, can act as a fast-acting antidepressant in treatment-resistant depressed patients, and these effects are associated with increased postsynaptic dendritic spine density in PFC pyramidal cells in fixed tissue studies. How changes in synapse number and dendritic morphology affect PFC microcircuit function, however, has yet to be established. In an effort to address this question, we investigated neural network dynamics in PFC microcircuits using two-photon calcium imaging and fiber photometry. Adult mice were intrancranially injected in the prefrontal cortex with a pan-neuronal GCaMP-expressing AAV virus (driven by the hSyn promoter), and either a microprism or an optic fiber was implanted to monitor the activity of GCaMP-expressing cells. PFC microcircuit activity was first quantified by two-photon calcium imaging through a microprism before and after a chronic, 10-day exposure to corticosterone, the principal murine stress hormone. The mice were then injected with an acute intraperitoneal dose of ketamine, and repeat imaging was performed 24-hours later from the same neural populations. PFC microcircuit activity was analyzed, and results suggest that chronic corticosterone exposure reduces correlated activity and disrupts multi-cell ensembles in network populations, which are rescued by ketamine treatment. Following the same chronic corticosterone protocol, we also used fiber photometry to record neural activity during tail

suspension. We found that an increase in mPFC activity precedes the shift from immobility to struggling in controls, which is reduced in mice subjected to chronic corticosterone and partially rescued in mice receiving ketamine treatment.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

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Topic: F.04. Stress and the Brain

Support: R01 MH109685-01

T32GM007739 (to the Weill Cornell/Rockefeller/Sloan Kettering Tri-Institutional MD/PhD Program)

Title: Prefrontal somatostatin interneurons in action valuation processing and motivational anhedonia

Authors: ***R. N. FETCHO**¹, T. N. HUYNH³, B. S. HALL¹, F. LEE¹, C. M. LISTON² ²Brain and Mind Res. Inst., ¹Weill Cornell Med. Col., New York, NY; ³Weill Cornell Med., New York, NY

Abstract: Major depressive disorder (MDD) is a highly prevalent and debilitating mood disorder that is diagnosed based on a mixture of symptoms, but anhedonia-decreased interest in rewarding activities—is a core feature. Anhedonia can be caused by an inability to experience pleasure or by a lack of motivation to work towards obtaining a reward. Every decision to exert effort to obtain a reward involves action valuation computations- essentially a cost-benefit analysis of whether the positive value of the expected outcome of an action outweighs the negative value of the expected effort associated with that action. The anterior cingulate cortex (ACC), a stress-sensitive region of prefrontal cortex (PFC), has been consistently implicated in action valuation computations across species. Whether and how dysfunctional action valuation computations drive anhedonic symptoms in stress-related psychiatric disease is unclear. While extensive, groundbreaking work has characterized the effects of stress on PFC glutamatergic circuitry, less is known about the role of local inhibitory circuitry in stress-induced depressivelike behaviors, despite substantial evidence for changes in the inhibitory system in MDD patients. Here we investigated the role of one cellular subtype, somatostatin (SST)- expressing interneurons, in driving both healthy reward-seeking and chronic stress-induced anhedonic behavior. We recorded the activity of the ACC SST interneuron population through a chronically
implanted optical fiber (fiber photometry) in awake, freely behaving mice performing an effortful reward-seeking task under normal and chronically stressed conditions. Our initial results indicate that SST interneurons facilitate action valuation computations by dynamically regulating synaptic inputs to the ACC and that this process is disrupted in chronic stress states. This study will advance our understanding of stress-induced anhedonic behavior by focusing on a sparse but essential cell population that is understudied in the context of stress and depression.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

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Topic: F.04. Stress and the Brain

Support: NIH R01 MH109685-01

Title: FAAH Genetic variation in mice leads to enhanced auditory fear extinction and increased frontolimbic circuit activity

Authors: *T. N. HUYNH, R. N. FETCHO, F. S. LEE, C. LISTON Weill Cornell Med., New York, NY

Abstract: Encoding and modulating memories of dangerous and threatening stimuli are critical adaptations, particularly when threats vary over time. PTSD is a debilitating disease involving intrusive memories of a traumatic event, which are due in part to the inability to modify responses to stimuli that are no longer threatening—a process known as extinction. Interestingly, there is a common human single nucleotide polymorphism (SNP) in the fatty acid amid hydrolase (FAAH) gene, the primary catabolic enzyme of the prototypical endocannabinoid, anandamide, which leads to the enhanced ability to extinguish fearful memories. Moreover, recently developed knock-in mice expressing this FAAH SNP display the same traits as observed in humans with this mutation. Previous studies demonstrate that humans containing the FAAH SNP and FAAH KI mice exhibit decreased anxiety as well as enhanced auditory fear extinction memory. Furthermore, humans and mice containing the FAAH SNP also exhibit increased fronto-amygdala projections which emerge during adolescence. It is well established that the medial prefrontal cortex (mPFC) and basolateral amygdala (BLA) are involved with the regulation fear extinction learning and memory, thus the FAAH KI mice provides a unique, clinically-relevant tool to investigate how the activity in this circuit is modulated to strengthen extinction memory. We used fiber photometry to acquire activity dependent calcium dynamics in freely moving rodents via a chronically implanted fiber. Using GCaMP6S, which we virally

injected into the basolateral amygdala, we found that *FAAH* KI mice exhibit enhanced tonelocked activity at the beginning of an auditory fear extinction session which attenuates by the end of the extinction session relative to WT mice. Interestingly, BLA activity is similar in *FAAH* KI mice and WT mice on day 2 of extinction, despite decreased freezing levels in *FAAH* KI mice relative to WT mice. We next investigated projection specific activity using a dual virus approach to record from BLA-projecting mPFC neurons. We found that on day 1 of extinction, activity in BLA-projecting mPFC cells is similar in *FAAH* KI mice and WT mice; on the other hand, there is an increase in tone-locked activity in BLA-projecting mPFC cells on day 2 of extinction in *FAAH* KI mice. These results indicate that BLA-projecting mPFC neurons may be critically involved with the encoding of auditory fear extinction memories that is dependent on the endocannabinoid system.

Disclosures: T.N. Huynh: None. R.N. Fetcho: None. F.S. Lee: None. C. Liston: None.

Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

Location: Halls A-C

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Program#/Poster#: 320.08/II25

Topic: F.04. Stress and the Brain

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R01 MH109685-01

Title: Frontostriatal circuit function in social interaction behavior and chronic social stress

Authors: ***B. S. HALL**¹, R. N. FETCHO¹, T. N. HUYNH³, A. M. RAJADHYAKSHA⁴, C. M. LISTON²

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Abstract: Impairments in social functioning are a core component of many stress-related neuropsychiatric conditions including depression, schizophrenia and anxiety disorders. The underlying mechanisms that lead to social dysfunction in these conditions are not well understood, but are thought to involve the nucleus accumbens (NAc), a stress-sensitive area of the ventral striatum that regulates drives and motivation. The NAc integrates signals from a reward-processing network that includes the infralimbic (IL) region of the prefrontal cortex. The mechanisms by which IL:NAc projections influence social behavior and how they are altered by chronic stress have not been well defined. This work aims to investigate how projections from the IL modulate activity in the NAc and influence social interaction behavior in order to define circuit mechanisms by which these processes are altered by chronic stress.

Additionally, the cell population in the NAc is heterogeneous, comprised predominantly of D1 and D2 subtype medium spiny neurons (MSNs). This study also looks to understand the contribution of these different cell types to the stress responses observed. Using a rodent model of chronic stress (chronic social defeat stress) and fiber photometry in order to record from and manipulate specific neural populations and circuitry, we show how chronic stress affects IL:NAc signaling and whole NAc, as well as D1 and D2, activity and in the context of social interaction behavior. This work may elucidate mechanisms by which stress can lead to changes in reward circuitry that impact social interaction and contribute to social dysfunction in psychiatric diseases.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

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Program#/Poster#: 320.09/II26

Topic: F.04. Stress and the Brain

Support: CIHR Grant 134059

Title: Hippocampal engrams: Roles in stress susceptibility

Authors: *T. ZHANG, A. S. WONG, T. P. WONG Douglas Mental Hlth. Univ. Inst., Verdun, QC, Canada

Abstract: Apart from mood changes, depression has been associated with a biased memory for negative stimuli. Neuroimaging studies suggest this cognitive bias is related to the enhanced functioning of the hippocampus. We hypothesize that the facilitated formation of hippocampal engram cells, known as cellular substrates for memory, is related to the cognitive bias for negative stimuli in an animal model of depression.

We employed a chronic social defeat model to examine the relationship between hippocampal engram cells and depression-related behaviours. The TetTag mouse model allows the tagging of activated neurons by a reporter gene LacZ. TetTag mice were stressed by social defeat, consisting of daily attacks by and co-housing with an aggressive mouse. Neurons activated in the first 2 days of social defeat were labeled by LacZ. After 8 total days of defeat stress, mice were separated into susceptible (exhibiting social avoidance) and resilient groups according to their social behaviour. Hippocampal engram cells were reactivated by an extra episode of social defeat to induce immediate early gene cFos expression. Neurons with both LacZ and cFos labeling represent engram cells.

We found more LacZ labeled hippocampal CA1 neurons in susceptible mice compared with

resilient and nonstressed control mice. Such group difference was still present when the dorsal and ventral hippocampus were analyzed separately. This finding suggests there are inherent differences in hippocampal activation between susceptible and resilient mice before the onset of depressive symptoms in susceptible mice.

Intriguingly, we also found significantly more engram cells in susceptible mice than other mouse groups in the CA1 region of both dorsal and ventral hippocampus. No difference in LacZ labeled and engram cells was found in the dentate gyrus. Our findings suggest susceptible mice may have an enhanced hippocampal memory for social stress. Given that a hyperactive hippocampus may be related to rumination in depression, our findings suggest that targeting hippocampal engram cells may be a novel therapy for depression.

Disclosures: T. Zhang: None. A.S. Wong: None. T.P. Wong: None.

Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

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Topic: F.04. Stress and the Brain

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Title: Medial prefrontal cortex versus orbitofrontal cortex: Teasing apart differences in plasticity after stress

Authors: *S. M. ADLER, S. E. BULIN, M. S. PATTON, M. GIROTTI, D. A. MORILAK Pharmacol., Univ. of Texas Hlth. At San Antonio, San Antonio, TX

Abstract: Cognitive inflexibility is a symptom dimension shared by several stress-related psychiatric disorders; it crucially contributes to their etiology and is exacerbated by stress. Unfortunately, deficits in cognitive flexibility are poorly treated by current medications. In order to create more efficacious treatments, we need to know more about the neurobiology of stress effects on cognitive flexibility. In rodents, the attentional set-shifting task (AST) can be used to evaluate two types of cognitive flexibility: reversal learning, which is mediated by the orbitofrontal cortex (OFC), and extra-dimensional set-shifting, which is mediated by the medial prefrontal cortex (mPFC).

We have identified two types of chronic stress that elicit behavioral deficits in each of these functions: chronic intermittent cold stress (CIC) selectively impairs reversal learning (p<.01), while chronic unpredictable stress (CUS) most robustly impairs extra-dimensional set-shifting

(p<.05). In recent work, we have found that each stress paradigm affects the functional outputs of the two brain regions very differently: after chronic stress, the OFC becomes hyper-excitable, while the mPFC becomes hypo-excitable. We have previously demonstrated this using mediodorsothalamus (MDT) afferent stimulation to evoke field potentials in these regions. Evoked field potentials in the OFC are considerably potentiated after 2 weeks of CIC stress compared to baseline (before stress); however, in the mPFC, evoked field potentials are decreased after 2 weeks of CUS.

Since the literature suggests that the mPFC has a decrease in apical spine density and dendritic complexity after chronic stress, we wanted to determine if, similar to our electrophysiological data, the OFC experiences the opposite effect. We have determined that, in male Sprague Dawley rats that undergo CIC stress, distal apical spine density in the medial OFC is significantly increased relative to non-stressed controls (p=0.0128). We plan on pursuing this further by using a larger cohort of animals in order to determine whether other regions of the OFC also have a similar effect. Additionally, we will be comparing this data to rats that undergo CUS in order to see if our stressor produces a decrease in spine density similar to what has been found in the literature. We will also perform Scholl analysis to determine if these groups also have differences in dendritic complexity. Finally, since functional changes in excitatory transmission correlate with altered AMPA receptor surface expression, we will also measure surface labeling of AMPA receptor subunits in the OFC and mPFC after chronic stress.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

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Topic: F.04. Stress and the Brain

Support: NSF CAREER Award 1553067

Title: HPA axis disruption alters glutamate signaling in the prefrontal cortex: Consequences for acute stress exposure and stress adaptation

Authors: *S. KINLEIN, F. SHAFFER, M. SAVENKOVA, I. N. KARATSOREOS Integrative Physiol. and Neurosci., Washington State Univ., Pullman, WA

Abstract: The brain-body response to stress is a set of biological countermeasures that promote adaptation and are crucial to survival. However, frequent, prolonged, or otherwise inappropriate responses to stress can lead to strain on physiologic function and eventually disease. The hypothalamic-pituitary-adrenal (HPA) axis is a key mediator of the stress response, and plays a

large role in the process of stress adaptation in the brain and hence in behavior. Here, we experimentally disrupt normal HPA function in male C57/B6 mice via noninvasive, oral corticosterone administration in order to determine how this system contributes to stress adaptation in the medial prefrontal cortex (mPFC), a brain area that is known to be affected by both acute and chronic stress. Our published data show this model blunts stress-induced corticosterone secretion, leads to abnormal behavioral responses to stress, and enhances stressinduced cfos mRNA expression in the mPFC. We hypothesized that this altered activity in the mPFC may be driven by changes in glutamate signaling. To test this, we measured the expression of genes coding for glutamate receptor subunits in the mPFC and also performed invivo biosensor recording of extracellular glutamate in the mPFC before, during, and after an acute stress exposure. We report altered glutamate signaling in the mPFC of HPA-disrupted mice that may account for the enhanced cfos expression we previously observed. As the mPFC undergoes structural and functional adaptation in response to repeated stress, which we propose occurs to prevent excitotoxic damage resulting from prolonged glutamate release, we also hypothesized that disruption of the HPA axis may affect the ability of neurons in the mPFC to properly adapt to repeated stress exposure. To test this, we exposed mice to repeated stress and quantified markers of oxidative stress in the mPFC. Together, our results suggest that disruption of normal HPA axis function can alter stress-induced glutamate signaling in the mPFC, and may also impair the ability of the mPFC to adapt to conditions of repeated stress exposure.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

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Topic: F.04. Stress and the Brain

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The William N. & Bernice E. Bumpus Foundation (RRDA Pilot: 2013.1)

Title: Hodgkin-Huxley models of excitatory-inhibitory balance in a cortico-striosomal circuit underlying aberrant cost-benefit decision-making caused by chronic stress

Authors: *L. G. GIBB, A. FRIEDMAN, D. HOMMA, B. BLOEM, K.-I. AMEMORI, D. HU, S. DELCASSO, A. S. HOOD, J. YANG, K. A. MIKOFALVY, T. F. TRUONG, S. E. TORO ARANA, D. W. BECK, N. NGUYEN, R. H. VORDER BRUEGGE, E. D. NELSON, K. A. GOOSENS, A. M. GRAYBIEL MIBR/BCS, MIT, Cambridge, MA

Abstract: For many decisions, the brain must combine both negative and positive motivators (costs and benefits). We recorded activity in a cortico-striosomal circuit in control and previously chronically stressed rats and mice during cost-benefit conflict task performance, and found aberrant activity in all elements of the circuit (Friedman et al. SfN 2017; Homma et al. SfN 2017). Based on our recordings, we hypothesized that a major effect of chronic stress on the cortico-striosomal circuit is a shift in excitatory-inhibitory (E-I) balance. To examine this hypothesis, we created Hodgkin-Huxley models of a normal circuit and a circuit whose connectivity (and therefore E-I balance) was altered by stress. We implemented a network consisting of excitatory, striosome-projecting prefrontal-prelimbic cortex neurons (PFC-PLs), striosomal spiny projection neurons (SPNs), and a fast-spiking interneuron (FSI; a well known source of feed-forward inhibition in the striatum). We adjusted the parameters of the model based on the experimentally observed responses of striosomal SPNs and FSIs to microstimulation of PFC-PL. After this tuning, we inserted into the model the activity of the PFC-PLs recorded in the control and stressed animals. The model of the control case successfully predicted a cascade of activation starting from PFC-PLs and continuing to FSIs, resulting in the inhibition of striosomal SPNs, as observed experimentally. The model also predicted the abnormal activity of striosomal SPNs and FSIs caused by stress. Additionally, our modeling approach enabled us to investigate the relative importance of abnormal PFC-PL activity versus the shift in E-I balance in accounting for the stress-induced changes in the activity of SPNs and FSIs. In order to do so, we inserted into the 'control' model the activity of the PFC-PLs recorded in stressed animals, and similarly we inserted into the 'stressed' model the activity of the PFC-PLs recorded in control animals. We demonstrate that a shift in the E-I balance is critical in explaining the effect of stress on the cortico-striosomal circuit using this model. This work has important potential implications for many neuropsychiatric disorders, in which a shift of E-I balance and/or a cortico-striosomal circuit may occur in conjunction with abnormal decision-making.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

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Topic: F.04. Stress and the Brain

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Title: Rescue and mimicking of chronic stress effects on cost-benefit decision-making by manipulation of a cortico-striosomal circuit

Authors: *D. HOMMA, A. FRIEDMAN, B. BLOEM, L. G. GIBB, K.-I. AMEMORI, D. HU, S. DELCASSO, A. S. HOOD, J. YANG, K. A. MIKOFALVY, T. F. TRUONG, S. E. TORO ARANA, D. W. BECK, N. NGUYEN, R. H. VORDER BRUEGGE, E. D. NELSON, K. A. GOOSENS, A. M. GRAYBIEL MIBR, MIT, Cambridge, MA

Abstract: Chronic stress often induces abnormal decision-making, but the underlying neuronal mechanisms for this effect are largely unknown. To understand the long-term effect of chronic stress on decision-making, we compared the behavior of normal and chronically stressed rats and mice in four different decision-making tasks performed on a T-maze. The cost-benefit conflict decision-making (CBC) options consist of high reward paired with high cost versus low reward paired with low cost. The benefit-benefit decision-making tasks consist of choices made between two rewards. The cost-cost decision-making task (CC) consists of equal rewards on each option paired with different cost levels. We over-trained rats and mice to stabilize choice variability and constructed psychometric functions. Rats and mice then underwent chronic stress procedures: immobilization or foot shock. Previously stressed animals were significantly more likely to choose high-reward, high-cost options than unstressed controls in the CBC task. However, they did not exhibit altered choice behavior in other tasks. We tested for a causal relationship between the aberrant choices in the CBC task and changes in the prefronto-striatal input preferentially targeting striosomes by injecting the prelimbic cortex (PFC-PL) with either AAV5-CaMKIIaeNpHR3.0-EYFP or AAV5-CaMKIIa-C1V1(E122T/E162T)-TS-EYFP. We found that optogenetic manipulation of activity in the striosome-targeting PFC-PL pathway can either

counteract or mimic the effects of chronic stress on choice. In our electrophysiological recordings (Friedman et al. SfN 2017; Gibb et al. SfN 2017), we found aberrant activity of striatal fast-spiking interneurons (FSIs) produced by chronic stress. Therefore, we performed optogenetic manipulation of parvalbumin interneurons (putative FSIs). Here, we show that optogenetic stimulation partially rescued the behavioral effects of stress on decision-making, increasing the frequency of low-cost, low-reward choices in the CBC task. We found long-term effects of the optogenetic manipulation, which lasted for up to one month without additional stimulation. To further test the involvement of striatal inhibitory interneurons, we injected IEM-1460, an inhibitor of GluR2-lacking AMPARs that preferentially blocks excitation of striatal FSIs. IEM-1460 infusion into the striatum shifted the animals' choice behavior in the CBC task, which was strongly correlated with the changes in firing rates of striosomal projection neurons and FSIs. Our study demonstrates that manipulation of elements of the cortico-striosomal circuit can rescue or mimic chronic stress effects on decision-making.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

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Topic: F.04. Stress and the Brain

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and the William N. & Bernice E. Bumpus Foundation (RRDA Pilot: 2013.1)

Title: A shift in the excitation-inhibition balance of a cortico-striosomal circuit underlies aberrant cost-benefit decision-making caused by chronic stress

Authors: *A. FRIEDMAN, D. HOMMA, B. BLOEM, L. G. GIBB, K.-I. AMEMORI, D. HU, S. DELCASSO, A. S. HOOD, J. YANG, K. A. MIKOFALVY, T. F. TRUONG, S. E. TORO

ARANA, D. W. BECK, N. NGUYEN, R. H. VORDER BRUEGGE, E. D. NELSON, K. A. GOOSENS, A. M. GRAYBIEL McGovern Inst. for Brain Res., MIT, Cambridge, MA

Abstract: The ability to combine costs and benefits is critical for resolution of motivational conflict during decision-making. Abnormal decision-making is a symptom in many neurological and neuropsychiatric disorders. We examined the long-term effects of chronic stress on rats and mice across four T-maze decision-making tasks: a cost-benefit conflict (CBC) task, in which a high-reward option is paired with high cost; two benefit-benefit tasks with similar or dissimilar rewards; and a cost-cost task with equal rewards but different costs. We find that chronic stress changed the ability to perform the CBC task (Homma et al. SfN 2017). In order to identify the mechanism underlying this abnormal CBC task performance, we focused on a cortico-striosomal circuit implicated in evaluation of cost and reward (Friedman et al. 2015). We previously demonstrated that in non-stressed rats, the striosome-projecting neurons in the rodent prelimbic region of the prefrontal cortex (PFC-PLs) are selectively engaged in CBC decision-making, and that PFC-PL activation inhibits striosomal spiny projection neurons (SPNs) via striatal fastspiking interneurons (FSIs). In the current study, we identified putative PFC-PLs neurons antidromically and identified putative striosomal SPNs orthodromically. Using waveform characteristics, we identified FSIs, which are a well known source of feed-forward inhibition in striatum. We demonstrate that chronic stress radically alters the functioning of each element of the cortico-striosomal circuit during performance of the CBC task. We simultaneously recorded from pairs of PFC-PLs with striosomal SPNs, PFC-PLs with FSIs, and FSIs with striosomal SPNs. We examined the effect of chronic stress on functional connections in these pairs. We found altered neural excitation-inhibition balance during abnormal behavioral integration of cost and benefit in the CBC task. We delivered electrical microstimulation in the PFC-PL of control and previously stressed rats and confirmed that chronic stress reduces activation of FSIs by the PFC-PL, reducing feed-forward inhibition of the SPNs and shifting the balance of excitationinhibition in the striosomes toward excitation (Gibb et al. SfN 2017). Our findings demonstrate that chronic exposure to stress has a profound long-term effect on a cortico-striosomal circuit. We suggest that the cortico-striosomal circuit is a potential therapeutic target for neuropsychiatric disorders with abnormal decision-making.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 320.15/JJ5

Topic: F.04. Stress and the Brain

Title: Corticohippocampal NMDAR-GluN2B subunit deletion protects against stress-induced dendritic remodelling

Authors: *S. E. KANDIGIAN¹, A. NG¹, C. R. PINARD², A. HOLMES², H. C. BERGSTROM¹ ¹Dept. of Psychological Sci., Vassar Col., Poughkeepsie, NY; ²Lab. of Behavioral and Genomic Neurosci., NIAAA/NIH, Rockville, MD

Abstract: Glutamate transmission at NMDA receptors (NMDARs) play a crucial role in stressinduced structural plasticity in prefrontal cortex. NMDARs containing the GluN2B subunit are linked with various forms of developmental and experience-induced synaptic plasticity. One well-supported form of neuroplasticity is dendritic remodeling in the prefrontal cortex in response to stress, but the contribution of GluN2B-containing NMDARs in this process is unclear. Here we sought to establish a role of GluN2B in stress-related dendrite remodeling in the prelimbic cortex (PL) using mice lacking GluN2B in cortical and hippocampal CA1 subfield pyramidal neurons. Adult male GluN2B-floxed (Cre-positive; mutant) and GluN2B-floxed (Crenegative; control) mice (Brigman et al., 2012) underwent a chronic restraint stress regimen; mice were placed in ventilated 50 mL Falcon tubes for 2 hr/day for 10 consecutive days. Nonrestrained mice remained in the home-cage. On the final day of stress, adrenal glands were removed, weighed, and whole brains processed for Golgi staining to visualize dendritic structure. Layer II/III PL dendrites were completely reconstructed in 3D and morphometrically analyzed. Results revealed remodeling of the apical, but not basilar, dendritic tree after stress in controls, supporting previous work (Wellman, 2004). Stress-induced differences in PL apical dendritic structure were absent in mutants, suggesting NR2B expression in cortical and CA1 hippocampal pyramidal neurons is required for stress-induced apical dendrite remodeling in this brain region. There was also some indication for a retraction of the basilar tree after stress in mutants, as compared with controls. These findings extend prior evidence that NMDARs are critical mediators of stress-induced dendrite remodeling in the prefrontal cortex (Martin and Wellman, 2011), and advance our understanding of GluN2B in stress-induced synaptic plasticity.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 320.16/JJ6

Topic: F.04. Stress and the Brain

Title: Rapid brain changes in cortical midline structures after acute psychosocial stress

Authors: *M. UHLIG^{1,2}, J. REINELT¹, M. E. LAUCKNER¹, D. KUMRAL^{1,3}, L. SCHAARE^{1,2}, A. REITER^{4,1}, M. ERBEY^{1,3}, J. ROEBBIG¹, T. HENDLER⁵, Y.-J. BAE⁶, J. KRATZSCH⁶, A. BABAYAN¹, A. VILLRINGER^{1,3}, M. GAEBLER^{7,1,3} ¹MPI for Human Cognitive and Brain Sci., Leipzig, Germany; ²Intl. Max Planck Res. Sch. NeuroCom, Leipzig, Germany; ³Berlin Sch. of Mind and Brain, Humboldt-Universität zu Berlin, Berlin, Germany; ⁴Lifespan Developmental Neuroscience, Technische Univ. Dresden, Dresden, Germany; ⁵Fac. of Medicine, Sagol Sch. of Neurosci. Tel Aviv Univ., Tel Aviv, Israel; ⁶Inst. of Lab. Medicine, Clin. Chem. and Mol. Diagnostics, Leipzig, Germany; ⁷Leipzig Res. Ctr. for Civilization Diseases, Univ. of Leipzig, Leipzig, Germany

Abstract: In the presence of a stressor, the brain orchestrates an adaptive biobehavioral response (e.g., by increasing stress hormone levels, McEwen 1998). These processes, in turn, can influence brain structure and function, as shown by human brain morphological changes after chronic or severe stressors (Sapolsky 1996). Rodent models suggest that neuroplastic changes can also occur rapidly following acute stress (Gould et al. 1998). While rapid neuroplasticity in humans has been demonstrated e.g. after motor learning (Taubert et al. 2016), it has not been shown after acute stress. We used 3T MRI to investigate human brain plasticity after an acute psychosocial stressor. 67 healthy men underwent either the Trier Social Stress Test (TSST, Kirschbaum et al. 1993) or a placebo version (Het et al. 2009) while blood, saliva, and psychometrics were sampled. T1-weighted MP2RAGE images (Marques et al. 2010) and pulsed arterial spin labelling (pASL) scans were acquired 45 min before and 65 min after the intervention. Changes in grey matter density (GMD) and cerebral blood flow (CBF) were identified with voxel-based morphometry (VBM, Ashburner & Friston 2000) and a voxel-based analysis of pASL data. The TSST elicited a pronounced physiological stress response relative to the placebo (e.g., ACTH, group-by-time: F(1.58,69.69)=20.58, $p_{GG}<0.0001$). The VBM-analysis showed a significant group-by-time interaction in two clusters at the cortical midline: a cluster in the anterior cingulate cortex (ACC; [-2,40,21], p_{FWE}=0.037) showed a decrease in GMD in the control and no change in the stressor group, while a cluster in the midcingulate cortex ([4,-12,48], p(FWE)=0.021) showed an effect in the opposite direction. GMD changes in the ACC were significantly associated with endocrine stress markers in the stressor group only (e.g., ACTH: $\rho=0.47$, p=0.02). Neither cluster showed a significant change in CBF (both p>0.1 uncorrected). We found rapid brain plasticity after acute psychosocial stress in areas that have

been associated with processing stressful (Dedovic et al. 2009) and self-relevant negative information (Grimm et al. 2009). While fast morphological changes in the adult brain have been related to cell-level phenomena (e.g., glial plasticity, Sagi et al. 2012), VBM findings have also been associated with other physiological parameters such as blood oxygenation (Tardif et al. 2017). It is conceivable that such parameters are affected by a stressful intervention and thus contribute to our effects. We here provide first evidence for rapid brain plasticity following acute psychosocial stress.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 320.17/JJ7

Topic: F.04. Stress and the Brain

Support: Arizona State University's College of Liberal Arts and Sciences

NSF Graduate Research Fellowship Program grant DGE-1311230

Arizona State University's School of Life Sciences Undergraduate Research Program

Title: Subtle hippocampal CA1 dendritic restructuring following chronic stress: Influence of a post-stress rest period and hippocampal CA3 BDNF downregulation

Authors: *J. B. ORTIZ¹, E. J. DAAS², A. FLEGENHEIMER², B. Q. LE², C. D. CONRAD² ¹Dept. of Psychology, ²Psychology, Arizona State Univ., Tempe, AZ

Abstract: Chronic stress often leads to hippocampal dendritic retraction in the apical region of CA3 pyramidal neurons without quantifiable changes in the dendritic complexity of CA1 pyramidal neurons. When chronic stress ends and a post-stress rest period without restraint is given, the reduction in CA3 dendritic complexity improves and becomes more complex. However, how a post-stress rest period affects CA1 neurons is unclear. Here, we investigated the relationship between CA3 and CA1 pyramidal neurons to determine whether dendritic restructuring in CA3 neurons corresponds with region-specific changes in the dendritic complexity of CA1 neurons. Young adult male Sprague-Dawley rats were chronically stressed by wire mesh restraint (6h/d/21d) and brains were removed soon after restraint ended (Str-Imm) or after a 21d post-stress rest period (Str-Rest). In addition, prior to restraint, we used RNA interference to downregulate BDNF in the CA3 region of the hippocampus, providing robust conditions to investigate the CA3-CA1 relationship. Consequently, rats were infused into the

CA3 area with either an AAV vector with a coding sequence against BDNF (shRNA) or a sequence with no known mRNA complements (Scr). Apical and basal dendritic complexity of CA3 and CA1 neurons was quantified by counting total dendritic bifurcations and total dendritic intersections using the Sholl analysis (20 µm distances from soma). The data reveal CA3 apical dendritic retraction in Str-Imm-Scr and Str-Rest-shRNA. For the CA1 apical area, gross dendritic arborization differences were not detected, as often found in the field, but the Sholl quantification revealed regionally-enhanced dendritic complexity that varied by distance from the soma at the distal apical dendrites (Str-Imm-Scr) and proximal basal dendrites (Str-Rest-shRNA). For the latter, significant increases in basal branch points were also detected with total branch point quantification method. Moreover, a correlation using all groups revealed a significant inverse relationship between CA3 apical dendritic complexity and CA1 basal dendritic complexity. The results demonstrate that chronic stress-induced CA3 apical dendritic retraction may relate to region-specific changes in CA1 dendritic complexity.

Disclosures: J.B. Ortiz: None. E.J. Daas: None. A. Flegenheimer: None. B.Q. Le: None. C.D. Conrad: None.

Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 320.18/JJ8

Topic: F.04. Stress and the Brain

Title: Attenuated nucleus accumbens dopamine neurotransmission in a rodent model of antidepressant resistance

Authors: *S. J. TYE¹, A. WALKER¹, R. P. KALE², B. MORATH² ¹Psychiatry & Psychology, ²Mayo Clin., Rochester, MN

Abstract: Chronic adrenocorticotropic hormone-(1-24) (ACTH) treatment (100 µg/day; 14 days) induces antidepressant resistance in rodents. In this model, antidepressant response can be elicited via activation of nucleus accumbens (NAc) dopamine signaling. The present study aimed to determine the effects of ACTH pre-treatment on NAc dopamine neurotransmission using fast scan cyclic voltammetry and behavioral response to imipramine (10mg/kg), fluoxetine (10mg/kg), armodafinil (10mg/kg) or bupropion (10mg/kg) in the forced swim test. All treatments reduced immobility time in saline-treated control animals, however, only armodafinil and bupropion reduced immobility behaviors in ACTH-treated animals. NAc dopamine neurotransmission was attenuated in ACTH-treated animals relative to controls, and potentiated with either armodafinil or bupropion treatment. NAc dopamine receptor expression was upregulated in ACTH-treated animals relative to controls and Akt/GSK3 signaling was facilitated by armodafinil or bupropion to a greater degree in these animals. These data suggest

that ACTH treatment impairs NAc dopamine neurotransmission and induces a potentially compensatory up-regulation of dopamine receptors. Together, these data support a role for targeted antidepressant treatment with armodafinil or bupropion in individuals with deficits in dopamine neurotransmission, potentially resulting from HPA-axis dysfunction.

Disclosures: S.J. Tye: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Teva P/L. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Xencor P/L. **A. Walker:** None. **R.P. Kale:** None. **B. Morath:** None.

Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

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Program#/Poster#: 320.19/JJ9

Topic: F.04. Stress and the Brain

Support: NIMH Grant T32MH103213

Title: Prior chronic stress exposure alters medial prefrontal cortex response to a novel stressor in a sex-dependent manner

Authors: *K. M. MOENCH, C. L. WELLMAN

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Abstract: Recurring exposure to stressful life events increases risk for certain psychological disorders, including depression and posttraumatic stress disorder. Further, women are twice as likely to be diagnosed with many of these disorder during their lifetime. Currently, the neurobiological mechanisms underlying these risk factors are poorly understood. One possibility is that lasting stress-induced changes in brain regions associated with stress-linked disorders, including prefrontal cortex, might result in sex-specific vulnerability to subsequent stress exposure. Brain-derived neurotrophic factor (BDNF) and neuropeptide Y (NPY) are potential stress-resiliency factors, and importantly, these two factors are capable of differentially modulating neural activity. Thus, we examined activity (via fos expression), BDNF, and NPY expression in medial prefrontal cortex (mPFC) subregions of chronically stressed male and female rats in response to a novel, acute stressor. Adult rats underwent chronic restraint stress (CRS; 3h/day for 10 days) or were left unstressed (No CRS). Either 24 hours (CRS-24h) or 7 days later (CRS-7d), rats were exposed to a 30-minute elevated platform stressor. Rats were euthanized ~60 min later and brains were removed and stained for fos, BDNF, and NPY. Stereological estimates of fos-positive cells and relative luminosities of BDNF-

expressing neurons were obtained. As subregions of mPFC can bidirectionally modulate the periventricular nucleus (PVN) of the hypothalamus, fos expression was also measured in this region to examine potential chronic stress-induced changes in neuroendocrine function. Compared to No CRS male rats, CRS-24h and CRS-7d male rats had suppressed fos expression in prelimbic cortex in response to an acute novel stressor, along with increases in BDNF and NPY expression. A similar pattern of changes was observed in infralimbic cortex, though only in CRS-24h male rats, suggesting subregion-specific differences in the lasting effects of chronic stress. Importantly, no acute stress-induced changes in fos, BDNF, or NPY expression in mPFC were observed in CRS females at either timepoint. In response to acute novel stress, CRS-24h, but not CRS-7d, male rats had decreased PVN fos expression. In contrast, while CRS-24h females appeared to have a typical PVN response to acute stress, CRS-7d females had a significant increase in fos expression. These data suggest that a lack of neural changes in stress-sensitive brain regions that modulate HPA axis responses immediately following chronic stress may increase female vulnerability to subsequent stress exposure, perhaps due to potentiated responses in the PVN.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

Location: Halls A-C

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Program#/Poster#: 320.20/JJ10

Topic: F.04. Stress and the Brain

Support: DARPA Grant W911NF1010093

T32 Training Grant NS077413

Title: Sphingosine-1-phosphate receptor 3 in the medial prefrontal cortex: A novel target to promote resilience to stress

Authors: *B. CORBETT, N. SOTUYO, S. LUZ, J. PEARSON-LEARY, J. STAIB, S. BHATNAGAR Abramson Res. Ctr., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Repeated exposure to stress promotes depressive- and anxiety-like behaviors in animals and the development of psychiatric disorders in humans, including depression and posttraumatic stress disorder. However, not all stressed individuals develop stress-related psychiatric disorders. That is, some individuals are resilient to the effects of stress whereas others are vulnerable. In a paradigm of chronic social defeat in rats, we have identified subpopulations that are resilient or vulnerable to the neuroendocrine and behavioral effects of defeat. Rats that

exhibit passive coping strategies as evidenced by short latencies to be defeated (SL) in the resident-intruder paradigm are vulnerable to the effects of stress whereas rats that exhibit active coping strategies as evidenced by longer defeat latencies (LL) are more resilient. SL rats display increased depressive-like and anxiety-like behaviors compared to LL rats. However, the mechanisms underlying stress resilience and vulnerability remain unclear. We used a targeted PCR array approach to identify novel neural substrates underlying resilience and vulnerability. We found that in LL rats, the expression of sphingosine-1-phosphate receptor 3 (S1PR3) was increased in medial prefrontal cortex (mPFC) neurons. S1PR3 is a G-protein coupled receptor that is associated with reductions in inflammation in the periphery. The mPFC regulates the stress response and affective-like behaviors, morphological changes occur in the mPFC after stress and its activity is dysfunctional in stress-related affective disorders. We used a viral vector to overexpress S1PR3 in the mPFC of rats and found that S1PR3 overexpression decreased depressive- and anxiety-like behavior, reduced c-Fos expression, and reduced expression of the inflammatory cytokines interleukin 1 beta (IL1 β) and tumor necrosis factor alpha (TNF α). Virally mediated S1PR3 knockdown in the mPFC produced the opposite effects. We investigated whether the ability of S1PR3 to decrease neuronal activity or to reduce inflammation in the mPFC may be mechanisms that promote resilience. We found that chemogenetic inhibition of the mPFC simulated some, but not all, behaviors associated with resilience. In S1PR3 knockdown rats, we found that preventing increases in TNF α by viral knock down reversed the increased depressive- and anxiety-like behavior produced by S1PR3 knockdown. Based on these results, we propose that S1PR3 in the mPFC promotes resilience by reducing neuronal activity and reducing TNF α in the mPFC. We conclude that the S1PR3 is a novel receptor that promotes behavioral and neuroendocrine responses characteristic of resilient individuals.

Disclosures: B. Corbett: None. **N. Sotuyo:** None. **S. Luz:** None. **J. Pearson-Leary:** None. **J. Staib:** None. **S. Bhatnagar:** None.

Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

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Program#/Poster#: 320.21/JJ11

Topic: F.04. Stress and the Brain

Support: Korea Institute of Oriental Medicine.(201700230001)

Title: Vagus nerve stimulation upregulates the production of 5HT1A and 5HT1B receptors in hippocampal neurons of a chronic restraint stress model of depression

Authors: H. SHIN¹, J. PARK², *U. NAMGUNG²

¹Daejeon Univ., Daehak-ro 62, Korea, Republic of; ²Daejeon Univ., 96-3 Yongun-dong, Korea, Republic of

Abstract: Recent studies show that vagus nerve stimulation (VNS) can be used to improve neurological disorders including depression. Here we investigated whether VNS is involved in regulating the responsiveness of hippocampal neurons in relation to depression-like behavior in rats. We induced depression-like state by chronic restraint stress (CRS) in rats and mice, and applied them acute and chronic modes of VNS. Chronic VNS was performed on a daily basis for 2 weeks using a cervically implanted microelectrode. c-Fos protein was induced in the solitary nucleus and serotonergic raphe nucleus 2 h after VNS. We found that 5HT1A and 1B receptor levels were decreased in the hippocampus of CRS animals and elevated to a similar level as in control 24 h after the acute VNS. Immunofluorescence analysis confirmed the hippocampal localization of receptors in the dentate granule cells and CA1 and 3 pyramidal neurons. In CRS animals having chronic VNS, hippocampal 5HT1A and 1B receptors were increased 2 weeks after VNS, and phospho-Erk1/2 protein levels were similarly regulated in hippocampal neurons as well. Behavioral forced swimming test revealed that the chronic VNS in CRS animals improved the immobility score compared to sham-stimulated control. Our results suggest that VNS may improve depression-like behavior via the activation of serotonergic neurons in the hippocampus.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

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Topic: F.04. Stress and the Brain

Support: NIMH R01 (MH111604)

WhiteHall Foundation (APP131146)

Title: Development of a hippocampal circuit-specific knockout of FOSB gene

Authors: *A. J. WIRTZ¹, A. L. EAGLE², A. ROBISON³ ¹Physiol., ²Dept. of Physiol., ³Neurosci., Michigan State Univ., East Lansing, MI

Abstract: Hippocampal (HPC) circuits, such as the neurons that extend from ventral HPC (vHPC) to nucleus accumbens (NAc), are important for memory and play a critical role in depression and addiction, however the molecular mechanisms underlying this are not understood. We have recently discovered a role for Δ FosB, a chronic activity-dependent transcription factor encoded by the *FosB* gene that drives gene expression underlying cellular function of HPC neurons. In order to better understand the role of Δ FosB in vHPC-NAc projection neurons, we sought to develop a mouse model with a knockout (KO) of the *FosB*

gene. We mated a *floxed FosB* strain (*FosBfl/fl*) with a Cre driver line (Nts2-Cre) with expression in HPC in order to knockout (KO) Δ FosB in HPC. NtsR2-Cre-driven *FosB* KO mice had a lack of Δ FosB expression in sub granular neurons in the dentate gyrus of the hippocampus. These mice also displayed decreased memory and were susceptible to stress, similar to what we had previously found when we silenced Δ FosB transcriptional activity in HPC. We next injected a retrograde HSV-Cre into NAc to drive GFP expression and KO *FosB* in vHPC projections to this region and found that Δ FosB is induced in these projections by cocaine, which is blocked in *FosB* KO mice. Studies are currently ongoing to produce a circuit-specific KO. However, these preliminary findings indicate that Δ FosB is important for normal hippocampal function and its expression (and inhibition) in the HPC-NAc circuit may be specifically crucial to stress susceptibility and drug seeking.

Disclosures: A.J. Wirtz: None. A.L. Eagle: None. A. Robison: None.

Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

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Topic: F.04. Stress and the Brain

Support: NIMH RO1 (MH111604)

WhiteHall Foundation (APP131146)

Beckman Foundation

MSU SUPER Program

Title: Chronic cocaine and stress alter spine morphology of hippocampal pyramidal neurons

Authors: *A. R. ST. GERMAIN¹, A. L. EAGLE², A. ROBISON³ ¹Physiol., Michigan State Univ., Brighton, MI; ²Dept. of Physiol., ³Neurosci., Michigan State Univ., East Lansing, MI

Abstract: Drug addiction is characterized by the continued seeking and taking of drugs despite adverse consequences, and this relapse to drug seeking can be precipitated by stress. Moreover, stress-related diseases, such as mood and anxiety-related disorders, are highly comorbid with drug addiction. The hippocampus, a brain region essential for learning and memory, has been implicated in addiction, as the user typically makes associations between the drug and the environment that can prompt craving and relapse, and in stress responses and mood disorders. Thus, in order to better treat these diseases, we require a better understanding of the synaptic underpinnings of both hippocampus-driven drug associations and hippocampal responses to

stress. Excitatory signaling in the hippocampus occurs at dendritic spines, small postsynaptic specializations whose number, size, and shape correspond to synaptic number, strength, and maturity, respectively, and we sought to measure the effects of chronic drug or stress exposure on the morphology of these spines. To visualize spines, we performed stereotaxic surgery to inject herpes simplex virus (HSV) expressing green fluorescent protein (GFP) into the dorsal and ventral hippocampus of adult male C57/Bl6J mice. We treated mice chronically with cocaine (20 mg/kg i.p. for 10 days) in a novel environment or exposed mice to 10 days of chronic social defeat stress (CSDS). We then conducted epiflourescent confocal microscopy of fixed brain tissue to quantify the morphologic changes in dendritic spines. Our initial observations suggest that, in dorsal hippocampus CA1 pyramidal neurons, chronic cocaine increased mushroom (mature) spines and decreased thin (immature) spines with no change in total spine density. We are also using HSVs to explore the molecular mechanisms of drug- and stress-dependent hippocampal spine changes, with particular emphasis on drug- and stress-induced transcription factors and their regulators. Ultimately, this research could lead to a better understanding of the common cellular, synaptic, and molecular pathways dysfunctional in addiction and mood disorders, and potentially provide novel targets for therapeutic intervention in these comorbid diseases.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

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Support: NIMH R01 (MH111604)

Whitehall Foundation

NARSAD Young Investigator Grant

NIEHS T32 Fellowship (ES725525)

Title: Circuit-specific genomic and functional dissection of male and female resilience to social stress

Authors: *E. S. WILLIAMS¹, A. L. EAGLE², C. E. MANNING³, R. L. NEVE⁴, A. ROBISON³

¹Pharmacol. & Toxicology, ²Physiol., ³Neurosci., Michigan State Univ., East Lansing, MI; ⁴MIT, Cambridge, MA

Abstract: Depressive syndromes are a major cause of morbidity, affecting nearly 300 million globally, and often arise in response to life stress. Aside from its obvious social and financial burden, a striking characteristic of depression is that it affects women nearly twice as often as men. The impact of depression and the disparity in numbers of affected men and women have been recognized for some time, but the molecular underpinnings of the disease remain unknown. This knowledge gap is critical, as treatments remain ineffective in a large number of patients and no sex-specific therapies are known. Neurons in the hippocampus, particularly those that project to the nucleus accumbens, are mediators of stress responses, but little is known of the regulation of this circuit at the level of cell function or gene expression. Here, I use a novel witness chronic social defeat stress (CSDS) mouse model that is amenable to both sexes to investigate the role of the transcription factor Δ FosB in the regulation of ventral hippocampal-nucleus accumbens (vHPC-NAc) projection cells in susceptibility or resilience to CSDS. This circuit has gained recent attention in mood disorder research as it has been shown that reduced activity in these neurons promotes resilience to CSDS. Additionally, our group has shown that the transcription factor Δ FosB is required for hippocampal learning, and its expression is induced in the vHPC by stress or antidepressant treatment. Taken together with our knowledge of its role in resilience in other brain regions, this makes Δ FosB an exciting prospective target for the regulation of vHPC-NAc neuronal function in response to stress. Here, I follow work from our group showing that general inhibition of Δ FosB function throughout the vHPC (but not dHPC) promotes susceptibility to subchronic stress, and show that overexpression of Δ FosB in vHPC reduces cell excitability. Moreover, we explore both basal and stress-induced sex differences in the excitability of vHPC-NAc projection cells, and uncover differences that may underlie sexual dimorphism in susceptibility to mood disorders. With these findings, I hypothesize that stressinduced Δ FosB in vHPC-NAc neurons mediates changes in the function of these neurons and regulates gene expression to promote resilience to social stress in a sex-dependent manner.

Disclosures: E.S. Williams: None. **A.L. Eagle:** None. **C.E. Manning:** None. **R.L. Neve:** None. **A. Robison:** None.

Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

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Topic: F.04. Stress and the Brain

Support: NIMH R01 (MH111604)

Whitehall Foundation (APP131146)

Title: Circuit-specific ventral hippocampus Δ Fosb expression mediates resilience in the social defeat model of depression

Authors: *C. MANNING¹, A. L. EAGLE², D. T. CARABALLO⁷, E. S. WILLIAMS³, P. A. GAJEWSKI⁴, R. L. NEVE⁸, M. S. MAZEI-ROBISON⁵, A. ROBISON⁶ ²Dept. of Physiol., ¹Michigan State Univ., East Lansing, MI; ³Pharmacol. & Toxicology, Michigan State Univ., Lansing, MI; ⁴Genet., ⁵Physiol., ⁶Neurosci., Michigan State Univ., East Lansing, MI; ⁷Univ. of Peurto Rico Arecibo, Arecibo, Puerto Rico; ⁸MIT, Cambridge, MA

Abstract: Stressful and traumatic experiences can contribute to mood disorders in some individuals while others are resilient. Though we know that the hippocampus plays a crucial role in stress responses, it is unknown how changes in hippocampal function, particularly at the level of gene expression, may drive resilience to stress. We have recently described a critical role for hippocampal expression of the transcription factor Δ FosB, encoded by the *FosB* gene, in learning, but its role in stress is unknown. Using the chronic social defeat stress (CSDS) model of depression, we have investigated the role of hippocampal Δ FosB in stress resilience in male mice. Here we show that Δ FosB is induced by CSDS and antidepressants. Inhibition of Δ FosB transcriptional activity in ventral, but not dorsal, hippocampus of adult male mice enhances susceptibility to CSDS. Moreover, using a novel dual-virus CRISPR system, we show that silencing of the *FosB* gene in adulthood in specific hippocampal cells projecting to nucleus accumbens also increases susceptibility to the social withdrawal phenotype of CSDS, while silencing of the *FosB* gene in ventral hippocampal cells projecting to amygdala is anxiolytic. These behavioral phenotypes are reversed by overexpression of Δ FosB in the same cells. Taken together, these data suggest that Δ FosB expression in response to stress regulates hippocampal cells projecting to nucleus accumbens to promote resilience to the social withdrawal phenotype of depression, while Δ FosB expression in hippocampal cells projecting to amygdala may drive stress-induced anxiety. We are currently investigating potential sex differences in Δ FosB's role in these hippocampal projections in response to social stress using a modified witness model of CSDS. These findings offer new insight into the transcriptional mechanisms underlying the etiology of mood- and anxiety-related disorders.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

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Support: NIEHS T32 Fellowship (ES725525)

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NIMH R01 (MH111604)

Whitehall Foundation

Title: Circuit-specific FOSB gene silencing in ventral hippocampal projections underlies differential behavioral phenotypes associated with psychiatric disease

Authors: ***A. L. EAGLE**¹, C. E. MANNING², E. S. WILLIAMS³, P. A. GAJEWSKI⁴, F. M. BOYCE⁶, R. L. NEVE⁷, I. S. MAZE⁸, A. ROBISON⁵

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Abstract: Ventral hippocampus (vHPC) projection circuits play a critical role in psychiatric disease, including depression, addiction, posttraumatic stress disorder, and anxiety disorders. Glutamatergic vHPC afferents to limbic regions such as the amygdala (AMY) and nucleus accumbens (NAc) are uniquely important in maladaptive emotional behaviors associated with psychiatric disease. Gene expression regulates HPC function, however the role of altered gene expression in vHPC circuits and its contribution to maladaptive emotional behaviors is unknown. Δ FosB, a truncated splice product of the FOSB gene, is a stable activity-dependent transcription factor that we have previously demonstrated to be critical for normal HPC function, making it a potential mediator of gene expression-dependent changes in vHPC projections. The goal of this project was to determine the role of Δ FosB in vHPC circuits and how it may contribute to maladaptive behaviors related to psychiatric disease. We used circuit-specific viral-mediated CRISPR silencing of the FOSB gene in vHPC projections to AMY or NAc and assessed a battery of emotional behaviors. We first found that non-specific inhibition of Δ FosB in vHPC impaired place preference conditioning to cocaine, and altered anxiety & fear learning. Moreover, FOSB silencing specifically in vHPC-NAc projections impaired place preference conditioning but did not affect fear & anxiety. Conversely, FOSB silencing specifically in vHPC-AMY decreased anxiety and impaired avoidance learning, but did not affect place preference conditioning. Viral-mediated rescue of Δ FosB expression in the FOSB silenced circuits reversed these effects. TRAP-Seq (Translating Ribosomal Affinity Purification followed by RNA sequencing) in FOSB silenced circuits indicates that these effects are driven by specific patterns of gene expression which may underlie physiological changes in vHPC projection neuron activity. Collectively, these findings demonstrate that Δ FosB regulates circuit-specific vHPC neuron function via changes in gene expression, and that this may underlie maladaptive emotional behaviors associated with psychiatric disease. Furthermore, they suggest that Δ FosB and its gene targets in vHPC projections neurons may serve as promising therapeutic inroads for multiple psychiatric diseases.

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Poster

320. Stress-Modulated Pathways: Networks, Circuits, and Morphology

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Topic: F.04. Stress and the Brain

Support: NSERC Discovery Grant

Early Researcher Award

Canada Research Chair in Developmental Cortical Physiology

Title: Distinct physiological and molecular characteristics of cortical S100a10 neurons in response to stress and antidepressant treatment

Authors: D. SARGIN¹, R. C. UTHAIAH², D. T. CHU¹, K. E. PERIT¹, E. F. SCHMIDT³, N. HEINTZ³, P. GREENGARD², *E. K. LAMBE¹

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Abstract: Pyramidal neurons in cortex expressing S100a10 (p11) are considered essential for the antidepressant action of selective serotonin reuptake inhibitors (SSRIs), yet the physiology and serotonergic modulation of these neurons are not well understood. Here, we examine the characteristic properties and serotonergic modulation of GFP-positive S100a10 cortical neurons, then probe their response to the stress of prolonged social isolation and to chronic treatment with fluoxetine. In group-housed mice, S100a10 neurons display a biphasic serotonin response with brief inhibition followed by excitation. In socially-isolated mice, however, S100a10 neurons lose this serotonergic "signature" and the excitatory component is supplanted by strong inhibition. In these stressed mice, chronic treatment with fluoxetine in drinking water restores serotonergic excitation of S100a10 neurons. However, we observed that the fluoxetine-treated mice display behavioral heterogeneity with suggestive parallels to human reactions to SSRIs. Additional experiments revealed that a subset of SSRI-treated mice displayed anxiety-like behaviors together with an apparent overshoot of normal excitatory responses to serotonin in S100a10 neurons. To investigate state-dependent molecular changes in S100a10 cortical neurons, we pursued cell type-specific mRNA purification by translating ribosome affinity purification. Data from 4 groups (group-housed, socially-isolated, fluoxetine-treated, and fluoxetine-anxious mice) reveal substantial changes in the gene expression of S100a10 neurons when social isolation disrupts and chronic fluoxetine restores or exaggerates serotonergic excitability. Intriguingly, anxiety upon fluoxetine appears to reflect SSRI-hyper-responsiveness in S100a10 neurons, rather than a failure to respond to treatment.

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Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

Location: Halls A-C

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Program#/Poster#: 321.01/DP10/JJ18 (Dynamic Poster)

Topic: F.04. Stress and the Brain

Support: NIH Grant HL122454 (Myers)

Title: Infralimbic prefrontal cortical structural and functional projections to the limbic forebrain: A combined viral genetic and optogenetic analysis

Authors: *B. MYERS¹, M. WOOD², O. ADIL², S. FOURMAN², J. P. HERMAN² ¹Biomed. Sci., Colorado State Univ., Fort Collins, CO; ²Univ. of Cincinnati, Cincinnati, OH

Abstract: The prefrontal cortex is critical for contextual appraisal, executive function, and goaldirected behavior. Additionally, the infralimbic (IL) subregion of the prefrontal cortex has been implicated in stress responding, mood, and fear memory. However, the specific circuit mechanisms that mediate these effects are largely unknown. To date, IL output to the limbic forebrain has been examined largely qualitatively or within a restricted number of downstream sites. In order to quantify IL presynaptic input to structures throughout the forebrain, we utilized a lentiviral construct expressing synaptophysin conjugated to mCherry. Thus, allowing quantification of IL efferents that are specifically synaptic, as opposed to fibers of passage. Additionally, this approach permits IL innervation to be determined on a sub-structural level within the multiple heterogeneous nuclei of the limbic system. To examine the functional output of the IL, as well as structure-function relationships, we optogenetically activated IL output neurons and histochemically quantified neuronal activation throughout the limbic forebrain using fos-related antigen (Fra). Our quantification of synaptophysin-mCherry indicated that the IL provides robust synaptic input to a number of regions within the thalamus, hypothalamus, amygdala, and bed nucleus of the stria terminalis (BST), with limited input to the hippocampus and nucleus accumbens. Specific nuclei with the greatest innervation density included the medial dorsal and paraventricular nuclei of the thalamus, the posterior and lateral nuclei of the hypothalamus, the medial and basomedial regions of the amygdala, and anteromedial and anterolateral aspects of the BST. Furthermore, there was a high concordance between structural connectivity and functional activation. Optogenetic activation of IL output neurons induced the greatest activation in the posterior hypothalamic nucleus, medial amygdala, basolateral amygdala, and midline thalamic nuclei. Interestingly, some regions receiving substantial synaptic input did not have significant increases in Fra immunoreactivity, including the lateral hypothalamus and BST. Collectively, these studies represent a step toward a comprehensive and

quantitative analysis of output circuits of the IL. This large scale efferent quantification or 'projectome' also opens the door for data-driven analyses of the downstream synaptic mechanisms that mediate the integrative aspects of organismal stress responding.

Disclosures: B. Myers: None. **M. Wood:** None. **O. Adil:** None. **S. Fourman:** None. **J.P. Herman:** None.

Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

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Topic: F.04. Stress and the Brain

Support: Research reported in this publication was supported by the National Institute of Mental Health of the National Institutes of Health under award number R44MH098595.

Title: Long-term lactate and oxygen measurements in group housed animals

Authors: D. V. AILLON, S. GABBERT, E. KERNER, S. GROTH, E. NAYLOR, D. GWARTNEY, B. BARRETT, *D. A. JOHNSON, D. A. JOHNSON Pinnacle Technology, Inc, Lawrence, KS

Abstract: Wireless recording of in vivo biosensor activity provides second-by-second resolution of brain analytes within a specific region of a freely-moving animal. Observation of these parameters from multiple animals in a co-housed environment can provide valuable insight into the physiology underlying group housed behavior. Pinnacle developed a cage system capable of long-term housing, video tracking, RFID monitoring and wireless recording of physiological parameters from two animals over multiple days. Lactate and oxygen changes were measured continuously for seven days from two rats co-housed in the enclosure and compared to animals that were individually housed. Physiologically relevant changes in oxygen consumption corresponding with play behavior were measured. Lactate concentration was observed to change with sleep/wake state in a manner consistent to previously reported studies. Movement artifact was negligible even during periods of active animal activity. Sleep/Wake analysis noted differences between singly housed animals and group housed animals. The cage system is designed to accommodate food, water and bedding as well as programmable IR lighting for tracking animal behavior during lights off periods. Rats were implanted with guide cannulas (BASi) bilaterally into the frontal cortex region. After recovery from surgery (1 wk) a lactate biosensor (80 um diameter) and an oxygen sensor (180 um disc) were inserted into the cannulas and the sensors were connected to a wireless potentiostat in a head-mounted enclosure. Rats were co-housed for seven days with sensor, video and RFID data collected. All procedures were previously approved by the University of Kansas ACUC. Post-processing software was used to

continually differentiate both rats using a combination of telemetry, RFID tagging and video tracking algorithms. Resolution of video occlusion events was accomplished by verification of the RFID tag signature for an unknown animal on one of four RFID antennas placed in each quadrant of the cage.

Disclosures: D.V. Aillon: A. Employment/Salary (full or part-time):; Pinnacle Technology Inc. **S. Gabbert:** A. Employment/Salary (full or part-time):; Pinnacle Technology Inc. **E. Kerner:** A. Employment/Salary (full or part-time):; Pinnacle Technology Inc. **S. Groth:** A. Employment/Salary (full or part-time):; Pinnacle Technology Inc. **E. Naylor:** A. Employment/Salary (full or part-time):; Pinnacle Technology Inc. **D. Gwartney:** A. Employment/Salary (full or part-time):; Pinnacle Technology Inc. **B. Barrett:** A. Employment/Salary (full or part-time):; Pinnacle Technology Inc. **D.A. Johnson:** A. Employment/Salary (full or part-time):; Pinnacle Technology Inc.. **E.** Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pinnacle Technology Inc. **D.A. Johnson:** A. Employment/Salary (full or parttime):; Pinnacle Technology Inc. **B.** Employment/Salary (full or parttime):; Pinnacle Technology Inc. **D.A. Johnson:** A. Employment/Salary (full or parttime):; Pinnacle Technology Inc. **D.A. Johnson:** A. Employment/Salary (full or parttime):; Pinnacle Technology Inc. **E.** Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pinnacle Technology Inc..

Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

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Topic: F.04. Stress and the Brain

Support: This work is supported to professor Sun Shin Yi by Soonchunhyang research fund.

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Title: Neurogenic and stress-ameliorating effect in hippocampus and hypothalamus of OVX mice model with chronic TM extraction administration

Authors: *K. KIM, H. BAEK, M. PARK, B. KO, K. KIM, S. YI Soonchunhyang Univ., Asan, Korea, Republic of

Abstract: *Tenebrio molitor* (TM) is an edible insect-derived material. In our previous study, as a result of chronic oral administration of TM extract to the OVX-mice model, density and thickness of bone tissues were increased and it appears that neurogenesis in hippocampal dentate gyrus tended to be improved. Therefore we hypothesized this ameliorating effect of menopause-related problems in TM-treated mice model might be related to interaction between hormonal

changes and stress regulation, particularly hypothalamic-pituitary-adrenal (HPA) axis in the body, together with neurogenesis mechanism. In this study, we administered Vehicle or TM extract to non-OVX-mice or OVX-mice model for 4 weeks. We collected brain tissues and blood, and performed blood chemistry, immunohistochemistry (IHC; AVP, GR, CRF and DCX) and Western blotting (WB; GR, CRF and DCX) at the hippocampus and hypothalamus. According to our metabolic data, body weight was significantly increased in OVX/TM group than OVX/Veh group and that was not different between NON groups. Serum estradiol-17β level and glucocorticoid level did not show difference between TM- and Veh-administered groups. However, it was demonstrated by IHC and WB results that doublecortin (DCX) expression was increased in TM- groups than Veh-administered groups. On the other hand, glucocorticoid receptor (GR) expression was significantly decreased in TM- groups than veh-administered groups and very interestingly, the GR expression site tended to be particularly concentrated in the subgranular zone (sgz). This is very meaningful because it is the place where the neuroblasts are located. In addition, the expression of vasopressin (AVP) and corticotropin releasing factor (CRF) was significantly reduced in the PVN area. Taken together, it is thought that chronic administration of TM extract may have the potential to alleviate systemic stress in postmenopausal animal models, and may have an impact on the regulation of HPA axis and neurogenesis correlation.

Disclosures: K. Kim: None. H. Baek: None. M. Park: None. B. Ko: None. K. Kim: None. S. Yi: None.

Poster

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Program#/Poster#: 321.04/JJ21

Topic: F.04. Stress and the Brain

Support: CSIR-BSC0115/miND

Title: Chronic Predator Stress in zebrafish as a simpler, cost effective in-vivo depression model: Characteristic evaluation to be used as a tool in potential drug screening

Authors: B. R. REDDY^{1,3}, A. GOLLA^{1,4}, T. DAS^{1,3}, D. BHATTACHARYA¹, A. KUMAR^{4,3}, *S. CHAKRAVARTY^{2,3}

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Abstract: During the past decade, various behavioral and molecular manifestations of the neuropsychiatric disorders in zebrafish were well characterized to be equivalent to humans.

Unlike rodents, a standard depression model in zebrafish is still a prerequisite. Seeking a resemblance to the subordination, aggression, humiliation and bulling of the social defeat model in rodents, we designed the Chronic Predator Stress (CPS) model in zebrafish (Danio rerio). This model follows resident-intruder paradigm where the intruder (Danio rerio) is exposed to (direct) belligerent encounters of an aggressive resident fish (Metriaclima callainos) for a very short period and later separated by a partition (indirect). After the 5-day CPS paradigm, depression-like phenotype was evidently observed through relevant behavior tests (Social Interaction, Anhedonia, Novel Tank Test. Further molecular studies using telencephalon region (corresponding to hippocampus of mammals) of the subject fish indicated significant reduction in the expression of the neurotrophic factors and dysregulation of the downstream intracellular signaling cascade associated with CREB activation. CPS exposure indicated a significant contrariety in the expression of various neuronal and glial populations in the telencephalon compared to the control fish.

After the accomplishment of the construct, face and discriminative validity, we evaluated this model for predictive validity using two antidepressants of different origin i.e, Fluoxetine (slow) and Ketamine (rapid) and their actions on phenotype and physiologiocal recovery. Our results indicate that the CPS model can be utilized as an alternative in in vivo animal model for depression which eventually can be useful as a tool to screen of novel potential antidepressants.

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Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

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Program#/Poster#: 321.05/JJ22

Topic: F.04. Stress and the Brain

Support: NIH Grant 1R01MH099192-01

Title: A new framework for identifying molecular drivers of resilient neural circuit activity

Authors: ***R. HULTMAN**¹, M. JONES³, L. MA², K. ULRICH¹, D. CARLSON¹, L. CARIN¹, K. DZIRASA⁴

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Abstract: Neural circuit disruptions have been increasingly appreciated as contributing directly to behavioral dysfunction. Identifying molecular contributors to specific neural circuit activity may provide a more promising route to efficacious drugs that target specific circuit-level causes of behavioral dysfunction. Here we describe an innovative, transdisciplinary pipeline that takes

advantage of multisite in vivo neurophysiology, RNA-Seq, machine learning, and targeted viral manipulation strategies, for identifying and validating molecular drivers of specific neural circuit activity. We dissect neural circuitry underlying stress-susceptibility in a well-characterized mouse model of susceptibility to chronic stress: The chronic social defeat stress (CSDS) paradigm.

Using in vivo neurophysiology in freely behaving mice, we recorded an endophenotype of stresssusceptibility: pre-frontal cortex 2-7Hz oscillatory activity. We then measured the reactivity of this neural signature in a forced interaction test (FIT). Prefrontal cortex tissue was microdissected and gene expression was detected according to neural circuit activity using RNA-Seq. We found that gene pathways associated with the mitochondrial complex ii were upregulated in animals with the putative resilient neural signature prior to stress exposure. We then used AAV manipulations to demonstrate that differential regulation of this pathway drove resilience to chronic social defeat stress. Finally, we demonstrate that we can enhance the predictor of resilience using multi-site in vivo recordings that incorporate other brain regions that contribute to a PFC-driven neural circuit signature, including nucleus accumbens, basolateral and central amygdala, ventral hippocampus and ventral tegmental area.

Here we demonstrate that combining in vivo neurophysiology and Next Generation Sequencing can be a powerful approach for the identification of molecular drivers of specific neural circuit function with regard to stress-related pathology. Applying such approaches more widely will be essential for the development of circuit-based psychopharmacotherapeutics.

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Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

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Topic: F.04. Stress and the Brain

Support: T32DK059803-13 Training Program in Neuroendocrinology of Homeostasis

Title: Local microinfusion of pituitary adenylate cyclase-activating polypeptide into the infralimbic cortex alters ACTH sensitivity and behavioral coping strategies

Authors: *S. E. MARTELLE¹, B. A. PACKARD¹, E. M. COTELLA², J. HERMAN² ¹Psychiatry, Univ. of Cincinnati, Cincinnati, OH; ²Psychiatry, Univ. of Cincinnati, CINCINNATI, OH

Abstract: Among its many roles, pituitary adenylate cyclase-activating polypeptide (PACAP) acts as an excitatory neuromodulator influencing neurotransmitter release and reuptake.

Recently, PACAP has emerged as a regulator of stress responding. For instance, PACAP knockout mice exhibit abnormal anxiety-like behaviors and polymorphisms of PACAP and its receptor are associated with post-traumatic stress disorder in humans. In the current study, we investigated the effects of PACAP in the infralimbic cortex (IL), known for its role in maladaptive stress responses. We hypothesized that IL injections of PACAP would increase excitatory input to the intercalated cells of the amygdala and thus inhibit the central amygdala, reducing anxiety-like behavior. Cannulated adult male Sprague-Dawley rats were microinjected with PACAP (1 ug/side; n=12) or vehicle (sterile saline, 0.5 ul/side; n=10) into the IL 30 minutes prior to a forced swim test (FST). Blood was sampled via tail clip at 15, 30, 60, and 120 minutes post initiation of the FST for analysis of HPA responsiveness. Animals pretreated with PACAP had decreased incidence of swimming (p=0.02) and dived more often (p=0.006) than controls, suggesting alternative active coping approaches. Furthermore, PACAP-treated animals had decreased adrenal responsivity (p=0.03), a measure of adrenocortical function that could be an indication of reduced sensitivity to ACTH at the level of the adrenal cortex. When undergoing a passive avoidance test one week later, animals that had previously been treated with PACAP displayed a decreased cross-over latency during habituation, further suggestive of alternate coping strategies. Analysis of Fos indicated that intra-IL PACAP does not differentially activate primary targets in the basolateral or central nuclei of the amygdala, necessitating future studies investigating how PACAP is affecting HPA axis responsiveness and coping strategies.

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Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

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Program#/Poster#: 321.07/JJ24

Topic: F.04. Stress and the Brain

Support: NIH Grant R01 DA019921

Title: Differential effects of ventral hippocampal corticosterone and its receptors on accumbal dopamine output in drug-naïve and amphetamine withdrawn rats

Authors: *B. BRAY^{1,2}, M. A. WEBER^{1,2}, G. L. FORSTER^{1,2} ¹Basic Biomed. Sci., ²Ctr. for Brain and Behavior Res., Univ. of South Dakota, Vermillion, SD

Abstract: Alterations in stress and dopamine levels can motivate goal-oriented behavior and prompt drug use in withdrawal. Amphetamine withdrawal is associated with dysphoria and hypersensitivity to stress in humans and rats that can prompt relapse. This effect may be mediated by enhanced stress-induced corticosterone (CORT) in the ventral hippocampus

(vHipp), as is observed in rats. vHipp CORT can induce glutamate release and the vHipp sends glutamatergic projections to the nucleus accumbens shell (NAcS) to enhance dopamine output and reward salience. To directly test whether vHipp CORT can enhance NAcS dopamine output, a stress-relevant concentration of CORT (0.24 ng total) was infused into the vHipp of anesthetized drug-naïve adult male rats, and rats in amphetamine withdrawal. Resulting NAcS dopamine output was measured by in vivo chronoamperometry. In drug-naive rats, vHipp CORT enhances NAcS dopamine output, peaking 55 min post infusion. This suggests a novel mechanism by which stress exposure could enhance reward value to promote goal-oriented behavior. In amphetamine withdrawal, vHipp CORT reduces NAcS dopamine output, peaking 20 min post-infusion, returning to baseline at 55 min post-infusion, then decreasing again, peaking at 80 min post-infusion. This suggests stress-induced vHipp CORT could contribute to dysphoric states that prompt relapse in withdrawal. These findings support an opponent-process theory of addiction, in which blunted dopamine reward responses and enhanced CORT stress responses negatively reinforce drug-taking. Preliminary data suggests these findings are specific to CORT in the ventral dentate gyrus/subiculum regions of the vHipp, as CORT infused into the dorsal vHipp or anteriorly into the CA1/CA3 regions do not alter NAcS dopamine output. CORT can act on glucocorticoid (GR) or mineralocorticoid (MR) receptors. vHipp GRs are thought to be excitatory; whereas vHipp MRs may be excitatory or inhibitory. Here we show that blocking vHipp MRs or vHipp GRs abolishes the CORT-induced NAcS dopamine increase observed in control conditions and augments CORT-induced decreases of NAcS dopamine output in amphetamine withdrawal, although GR antagonism produces these effects at a greater magnitude than that observed for the MR antagonist. These findings suggest that vHipp GRs and MRs contribute to vHipp CORT induction of NAcS dopamine output both in control conditions and in amphetamine withdrawal. Overall, our findings suggest vHipp CORT could play an important role in driving positive stress coping mechanisms in healthy conditions, with dysregulation of this system potentially contributing to relapse in withdrawal.

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Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

Location: Halls A-C

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Program#/Poster#: 321.08/JJ25

Topic: F.04. Stress and the Brain

Support: MH049698

Title: Cell type specific and region specific knockdown of glucocorticoid receptors: sex specific effects on the HPA axis and behavior

Authors: *J. SCHEIMANN, R. MORANO, P. MAHBOD, J. P. HERMAN Univ. of Cincinnati, Cincinnati, OH

Abstract: Hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis has been implicated in psychiatric disorders such as major depressive disorder (MDD), Post-Traumatic Stress Disorder (PTSD), and possibly anxiety. Normally, glucocorticoid release consequent to HPA activation promotes adaptive responses that may equip the animal for future threats, via enhanced memory and altered emotionality. However, under conditions of excessive activation, these changes may be maladaptive leading to different excitatory and inhibitory drives within important limbic regions and their projections.

Lesion studies suggest that the medial prefrontal cortex (mPFC) and ventral subiculum (vSUB) are critical for cessation of the HPA axis via polysynaptic signaling to the hypothalamus. We have shown that knocking down GR in the PFC leads to changes in HPA activation and depressive like behavior (as shown as increased immobility in the forced swim test). In this study, we sought to determine if the HPA and behavioral effects of GR downregulation are primarily due to loss of GR on excitatory projection neurons of the PFC and vSUB. To achieve region-specific down-regulation, we injected an adeno-associated virus (AAV) expressing Cre Recombinase under the CAMKIIa promotor into either the PFC or VSUB of mice with a floxed GR exon 3 (f/f) or wildtype (wt) littermate controls. Three weeks after injection of virus, male (n=9-11), and female (n=11-12) mice, aged 30-60 weeks were given a battery of behavioral tests that are thought to assess anxiety-like behavior (open field or elevated plus maze), depressive-like behavior(forced swim test), and behavioral inhibition (passive avoidance). There was no effect of PFC-specific GR deletion on open field or passive avoidance behavior in either males or female mice. Moreover, there were no differences in HPA axis activation after an acute stressor in f/f mice versus wildtype controls. In contrast, vSUB GR deletion in male mice decreased open arm entries in the elevated plus maze and decreased cross-over latency in the passive avoidance task (reduced behavioral inhibition). There were no differences in female mice. In addition, vSUB GR deletion increased stress-induced corticosterone release, again, only in males. These data suggest an important role for the GR in modulating vSUB, but not PFCmediated, stress responsiveness and emotional reactivity in a sex-dependent manner.

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Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

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Topic: F.04. Stress and the Brain

Support: College of Human Sciences Seed Grant

Title: The impact of acute stress on the expression of brain adenosine receptors

Authors: *B. A. BAUSTIAN, E. BAUER, A. BELL, P. J. CLARK Iowa State Univ., Ames, IA

Abstract: Our lab and others have shown that exposing rodents to a single episode of acute stress can produce long-lasting reductions in wheel running that persist months after the stressor is no longer present. We hypothesize that chronically reduced running may be related to maladaptive changes of central-mediated fatigue pathways. Adenosine signaling in the brain is a key contributor to central-mediated fatigue. Under heightened metabolic demand, adenosine is produced from the catabolization of the cellular energy source, adenosine triphosphate (ATP), and can act as a neuromodulator by promoting fatigue-related behavior primarily through activity at adenosine A1 and A2a receptors. Adenosine receptor expression may upregulate following metabolic demands of sufficient intensity, such as acute stress, which may produce a more rapid onset of central fatigue and contribute to persistently reduced wheel running activity. Therefore, we explored the effects of a single episode of acute uncontrollable stress on the expression of adenosine receptor protein in the striatum, as well as other brain areas where adenosine modulates fatigue behavior including the hippocampus, prefrontal cortex, and hypothalamus. We exposed adult C57BL/6J mice to a series of uncontrollable tail shocks (acute stress) or left them undisturbed in home cages (control). The mice were sampled at either 24 hours or 4 weeks by transcardial perfusion with phosphate buffer and paraformaldehyde. Immunohistochemistry was performed on thin brain sections to detected A1 and A2A receptors. Brain sections will be imaged and densitometry will be performed to semi-quantitatively measure the density of adenosine receptors in brain regions. Data collection is underway. We hypothesize A₁ and A_{2a} receptor expression will upregulate across all brain regions following acute stress by at least the 4-week time point. A widespread increase in brain adenosine receptors may be relevant to the modulation of increased fatigue-related behavior and reductions in physical activity following stressful experiences of sufficient intensity.

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Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

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Fulbright Graduate Study Grant

Title: Food restriction alone exercise alone or the combined during adolescence rescue elevated anxiety and deficits in memory and social function resulting from variant BDNF Val66Met single nucleotide polymorphism through GABAergic mechanisms in the dorsal hippocampus

Authors: *Y.-W. CHEN¹, H. ACTOR-ENGEL¹, F. LEE², C. J. AOKI¹ ¹Ctr. for Neural Sci., New York Univ., New York, NY; ²Dept of Psychiatry, Weill Cornell Med. Col., New York, NY

Abstract: Val to Met substitution at codon 66 (Val66Met) in Brain-Derived Neurotrophic Factor (BDNF) leads to reduced activity-dependent secretion of BDNF. In humans, this SNP has been associated with anxiety disorder, structural abnormalities of the hippocampal formation and cognitive impairments. Our previous data link anxiety-like differences in BDNF-Val66Met knock-in (BDNF^{Met/Met}) mice with reduced GABAergic innervation of the dorsal hippocampus (doi: 10.1093/cercor/bhw210). Others have also shown that both food restriction (FR) and exercise (EX) elevate BDNF levels in the hippocampus.

We investigated whether FR and/or EX during adolescence restore behavioral deficits in BDNF^{Met/Met} mice. Four groups of male and female pubertal BDNF^{Met/Met} mice and their wild-type littermates, all singly housed, were reared as follows during adolescence: EX (housed with a wheel from P36-44), FR (from P41-44), and FR-plus-EX (housed with a running wheel from P36-44 and FR from P41-44, an animal model of activity-based anorexia) and CON (no FR and no EX). After recovery from these treatments for >7 days, we assessed the animals' anxiety-like behavior, spatial and object recognition memory and social preference and recognition. Animals were sacrificed at P60 to measure GABAergic innervations and $\alpha4\beta\delta$ -GABA_A receptor levels on their pyramidal neurons in the dorsal hippocampal CA1 by electron microscopic immunocytochemistry.

During early adulthood, male BDNF^{Met/Met} mice display increased anxiety-like behavior and impairment of spatial and object recognition memory and social recognition. EX, FR, and FR-plus-EX to BDNF^{Met/Met} mice during adolescence had no effect on anxiety-like levels, and EM confirmed that GABAergic innervation remains low. However, EX, FR, and FR-plus-EX showed differential restorative effects on spatial, object recognition memory, and social recognition deficits, and EM suggests that these are mediated by changes in $\alpha 4\beta\delta$ -GABA_AR levels. Female BDNF^{Met/Met} mice display normal spatial memory, but elevated anxiety-like behavior and impairments in object recognition memory and social recognition. FR and FR-plus-EX normalized their anxiety-like levels and improved their object recognition memory, with changes in $\alpha 4\beta\delta$ -GABA_AR levels. Social recognition deficits found in BDNF^{Met/Met} female mice may be via altered GABAergic innervation in distal dendrites.

Thus, FR and/or EX during adolescence normalizes anxiety, cognitive and social abnormalities exhibited by BDNF^{Met/Met} mice. Our study highlights sex differences in the effects of BDNF

Val66Met SNP on anxiety and cognitive functions, and their potential causal and restoring mechanisms.

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Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

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Topic: F.04. Stress and the Brain

Support: MH053851

Title: Optogenetic induction of LTD in the Medial Prefrontal Cortex results in set-shifting deficits

Authors: *S. E. BULIN¹, K. M. HOHL¹, D. A. MORILAK²

¹Pharmacol., UT Hlth. Sci. Ctr. at San Antonio, San Antonio, TX; ²Pharmacol. and Ctr. for Biomed. Neurosci., Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: Stress-related mood and anxiety disorders, like depression and posttraumatic stress disorder (PTSD), are highly prevalent yet poorly treated. Relapse and residual symptoms remain problematic, and a poor understanding of the neurobiology underlying these illnesses has limited the development of new treatments. Imaging studies have shown reduced activity in the mPFC in both depression and PTSD, associated with deficits in executive functions, including impaired cognitive flexibility, that not only represent symptoms of these illnesses, but also contribute causally to their development and maintenance. Such changes presumably involve aberrant forms of neural plasticity in the mPFC. Similarly, chronic stress compromises cognitive flexibility, and is likely to disrupt the plasticity that normally underlies this executive process. Cognitive flexibility mediated in the mPFC can be measured using the attentional set-shifting test, and chronic unpredictable stress (CUS) induces a deficit of cognitive flexibility on this test. Further, we have previously shown that CUS attenuates the response of the mPFC to stimulation of the excitatory afferent from the mediodorsal thalamus (MDT). Thus, we are using optogenetics to investigate if directly inducing plastic changes in the mPFC is sufficient to alter cognitive flexibility. Glutamatergic neurons in the MDT were selectively infected either with the ChETA variant of channelrhodopsin by injecting an AAV5-CAMKII-ChETA viral vector, or with control AAV5-CAMKII-GFP, and allowed 6 weeks for expression and trafficking of the channel to terminals innervating the mPFC. Rats were anesthetized with chloral hydrate and baseline field potentials evoked by MDT stimulation were recorded in the mPFC for 15 min. Optical LTD was induced by stimulating mPFC terminals. The mean amplitude of evoked responses, recorded for 3 hr after stimulation, was reduced to 75% of baseline, indicating a
depressed response (n=4; p <0.0001). This depressed response was not observed in control GFP animals. (n=4; p>0.05) Then using this procedure, we induced LTD in awake rats to test the effect of LTD on set-shifting. ChETA injected rats (n=9) that received the LTD protocol 1-hr prior to set-shifting took significantly more trials to reach criteria than controls (n=8; p <0.0001). These results suggest that inducing LTD in the MDT-mPFC pathway is sufficient to produce cognitive deficits similar to those observed after CUS. By contrast, future experiments will explore if inducing LTP in the mPFC will restore healthy behaviors compromised by CUS.

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Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

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Topic: F.04. Stress and the Brain

Support: NIH Grant MH093981

Title: Social stress produces a long-term activation of locus coeruleus neurons in adolescent vs. adult female rats

Authors: *A. L. CURTIS, H. GUAJARDO, G. ZITNIK, R. VALENTINO Dept Anesthesiol., Children's Hosp. Philadelphia, Philadelphia, PA

Abstract: Women suffer double the incidence of psychiatric disorders related to stress compared to men. While the symptoms of such psychiatric disorders like depression are similar in males and females the range of vulnerabilities and sensitivity to likely causal factors remains unknown. This has encouraged investigations of factors thought to participate in the onset of such disorders like stress. A component of the stress response is activation of the locus coeruleus (LC)norepinephrine system. Stress-induced LC activation is mediated by corticotropin-releasing factor (CRF) and is associated with enhanced arousal and changes in cognitive flexibility. This study examined the effects of adolescent and adult social stress using the resident-intruder model on LC activity in unanesthetized female rats. Single unit LC activity was recorded before and during resident-intruder stress on the first and fifth days of 5 consecutive daily exposures. The resident was a lactating female and LC activity was recorded from the intruder. Spontaneous LC neuronal activity was compared between adult and adolescent rats before and during the stressor. We found that LC activity was similarly increased in adult ($160 \pm 19\%$ C, 27 cells, 3 rats) and adolescent (156 + 10%C, 60 cells, 4 rats) females on day-1 of social defeat (SD). However, by day-5 of SD, LC neuronal activation in adolescents further increased to $201 \pm 10\%$ C (43 cells, 4 rats), but stress-induced LC activation in adults declined to 123 + 10%C (24 cells, 2 rats) on day5 of SD. This suggests that LC activity in adolescents may be sensitized by repeated stress and this differs from the reduced LC response to stress shown in adult females. This finding is interesting because it is suggested that adolescence is a period of stress vulnerability when brain circuits associated with emotion and decision-making are in ongoing maturation. In conclusion, we found that LC neurons of adolescents were more sensitive to social stress than those of adult female rats. These findings of enhanced reactivity to stressful experiences during adolescence may serve to underscore the critical vulnerability of this developmental stage and how it may lead to an elevated incidence of stress-related psychiatric disorders in women.

Disclosures: A.L. Curtis: None. H. Guajardo: None. G. Zitnik: None. R. Valentino: None.

Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

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Topic: F.04. Stress and the Brain

Support: MH 093981

DA 09082

Title: Social stress activates amygdalar corticotropin releasing factor and brainstem enkephalinergic afferents to the rat locus coeruleus in adolescent male rats depending on coping strategy

Authors: *M. URQUHART¹, B. A. REYES², X.-Y. ZHANG³, R. VALENTINO³, E. J. VAN BOCKSTAELE⁴

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Abstract: Adolescence is a crucial time of increased neuronal plasticity, rendering this period particularly vulnerable to stress. A common stressor encountered during this period is social stress. An ethologically relevant animal model of social stress is the resident-intruder model. Using this model, we have shown that social stress engages distinct amygdalar corticotropin-releasing factor (CRF)- and nucleus paragigantocellularis (PGi) opioid-containing afferents to the locus coeruleus (LC) in adult male and female rats, that segregate according to coping strategy as determined by the latency of the rats to assume a defeat posture. Here, we identified the neural circuitry that activates the LC following social stress in adolescent male Sprague-Dawley rats. Prior to social defeat exposure, rats were injected with the retrograde tracer,

Fluorogold (FG) into the LC. Three days following FG injection, rats were subjected to repeated (5 days) social defeat or control manipulation, and perfused 90 minutes after the last session. Sections through the lower brain stem and forebrain were collected and processed for immunocytochemical detection of c-fos, a marker of neuronal activity, FG and CRF or enkephalin (ENK). Consistent with our previous tracing studies, retrogradely labeled neurons from the LC were distributed throughout the rostro-caudal segments of the central nucleus of the amygdala (CeA) and PGi. Cell counts revealed that c-fos expression in the CeA was significantly increased in the short latency to defeat (SL) rats (P < 0.01) when compared to control and LL latency (LL) rats. Triple labeling of c-fos, FG and CRF revealed that 78% of c-fos and FGimmunoreactive neurons in the CeA of SL rats also expressed CRF, which was significantly higher (P < 0.05) when compared to control and LL rats. In the PGi, approximately 34% of c-Fos and FG-immunoreactive neurons of LL rats also expressed ENK, which was significantly higher (P < 0.05) when compared to control and SL rats. These results are consistent with previous studies in male adult rats and underscore that that social stress engages distinctly different regulation of the LC depending on coping strategy that may translate to distinct behavioral and physiological consequences.

Disclosures: M. Urquhart: None. B.A. Reyes: None. X. Zhang: None. R. Valentino: None. E.J. Van Bockstaele: None.

Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

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Topic: F.04. Stress and the Brain

Support: Canada Research Chair in Developmental Cortical Physiology

NSERC Discovery Grant

Early Researcher Award

NSERC Undergraduate Summer Research Award

Title: Sex-specific behavior and dorsal raphe 5-HT neuronal excitability in response to acute and chronic stress

Authors: *D. T. CHU, D. K. OLIVER, D. SARGIN, E. K. LAMBE Dept. of Physiol., Univ. of Toronto, Toronto, ON, Canada

Abstract: A severe and widespread mood disorder, major depression affects about 15 % of the population. Significantly, women are twice as likely to develop a depressive illness as men.

Despite the high prevalence of this disease, its neurophysiological correlates are not completely understood. The most widely used antidepressant treatments target the serotonin (5-HT) system, especially the 5-HT neurons in the dorsal raphe nucleus, which play an important role in stress response and emotional regulation. We have recently shown that chronic social isolation stress in male mice decreases 5-HT neuronal excitability in the dorsal raphe nucleus and produces a depressive-like phenotype (Sargin et al., eLife, 2016). Here, we examined potential sex differences in the regulation of 5-HT neuronal excitability at baseline and in response to stress. In a combined set of behavioral and electrophysiological studies, we investigated anxiety- and depressive-like behaviors and dorsal raphe 5-HT neuronal excitability in male and female mice. Compared to male mice, female mice showed significantly increased anxiety in the open field test at baseline. Under acute forced swim stress, female mice showed a significantly greater depressive-like response. To investigate 5-HT neuronal excitability, we performed whole cell patch clamp electrophysiology in dorsal raphe slices obtained from adult male and female mice. At baseline, in response to strong depolarization 5-HT neurons of female mice showed attenuated action potential firing compared with 5-HT neurons of male mice indicating reduced excitability. In response to acute stress, 5-HT neurons in male mice were less excitable, while those in females remained unaffected. Ongoing experiments are further probing the cellular mechanisms underlying the sex differences in behavior and 5-HT neuronal physiology under baseline conditions and in response to stress.

Disclosures: D.T. Chu: None. D.K. Oliver: None. D. Sargin: None. E.K. Lambe: None.

Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

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Program#/Poster#: 321.15/KK2

Topic: F.04. Stress and the Brain

Support: DA 020129

Title: Cellular sites for interactions between the endocannabinoid metabolic enzyme monoacylglycerol lipase and norepinephrine in the rat frontal cortex

Authors: *E. J. VAN BOCKSTAELE¹, K. MACKIE², B. A. REYES³ ¹Dept. of Pharmacol. & Physiol., Drexel Univ. Col. Of Med., Philadelphia, PA; ²Dept. of Psychological and Brain Sci., Indiana Univ., Bloomington, IN; ³Pharmacol. & Physiol., Drexel Univ., Philadelphia, PA

Abstract: Endogenous cannabinoids or endocannabinoids (eCB), derived from phospholipids are one of the most ubiquitously distributed modulators in the brain. Involved in various physiological and pathological conditions, eCB release from post-synaptic neurons mediate their

actions through the activation of cannabinoid receptors that are often localized on pre-synaptic axon terminals. We have shown that cannabinoid type 1 receptors (CB1r) are positioned to presynaptically modulate norepinephrine (NE) release in the rat frontal cortex (FC) and that these are directly apposed to neurons containing the eCB synthesizing enzyme diacylglycerol lipase (DGL). DGL functions to synthesize 2-arachidonoylglycerol (2AG) in the post-synaptic neuron. While DGL synthesizes the eCB 2AG, the metabolic enzyme monoacylglycerol lipase (MGL) serves to degrades the eCB. These enzymes are also thought to be differentially distributed, with DGL being localized post-synaptically in somato-dendritic processes and MGL more frequently localized pre-synaptically, in axon terminals. In the present study, we investigated cellular sites for interactions between noradrenergic afferents and cortical neurons expressing MGL using immunofluorescence and immunoelectron microscopy. Tissue sections were collected through the FC and processed for immunocytochemical detection of MGL, CB1r and the norepinephrine transporter (NET) or the NE synthesizing enzyme, dopamine-beta-hydroxylase (DBH). Immunofluorescence microscopy revealed co-existence between MGL, CB1r and DβH or NET in varicose processes in the FC. Ultrastructural analysis, using immunogold-silver labeling for MGL and immunoperoxidase labeling for NET or D\u00dfH, confirmed that NET or D\u00ffH-labeled axon terminals also exhibited MGL-immunoreactivity in the FC. Taken together, these data indicate anatomical substrates for proposed interactions between the eCB metabolic enzyme, MGL, and the noradrenergic system in the FC.

Disclosures: E.J. Van Bockstaele: None. K. Mackie: None. B.A. Reyes: None.

Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

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Topic: F.04. Stress and the Brain

Support: Agreement "CNR-Sardegna Ricerche-Universita' di Cagliari"

Title: Gender-specific changes in hippocampal synaptic plasticity and cognitive performance in C57BL/6J mice exposed to maternal separation

Authors: *G. TALANI¹, G. SARIGU², F. VEDELE², M. PETRELLA², D. COLOMBO², E. SANNA²

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Abstract: Repeated maternal separation (MS) in newborn rodents is a powerful experimental model for investigating the consequences of neonatal stress on ethanol (EtOH) sensitivity as well as the neurochemical mechanisms involved in the long-term impairments at brain level in

adulthood. Because previous work suggested that the effect of MS on voluntary EtOH drinking appears to be gender-specific, the aim of the present study was to evaluate the long-term effects of MS on EtOH voluntary consumption and long-term hippocampal plasticity and compare the outcome between males and females C57BL/6J adult mice. Male or female pups were separated daily from the dam for 360 min (from PND 2 to 17). On PND 60, mice were tested for their voluntary EtOH consumption by using the two-bottle free choice paradigm. Extracellular and patch-clamp recordings were performed in order to evaluate possible changes in hippocampal synaptic plasticity as well as GABAergic and GLUergic transmission. As previously shown, MS male but not females mice showed a marked increase of EtOH intake and preference. Long-term depression (LTD) but not potentiation (LTP), induced in the CA1 hippocampal subfield, was enhanced in MS male but not females. In parallel, MS male mice showed an impaired performance on the Barnes maze with a decrease of long-term spatial memory when compared with control animals. Conversely, females exposed to MS did not differ from their control counterparts when tested in the Barnes maze. Patch-clamp experiments revealed, in the CA1 pyramidal cells, a significant enhancement of GABAergic sIPSC frequency and a decrease in the amplitude and decay time constant of GLUergic sEPSCs. Interestingly, a single injection of βethynyl estradiol in MS male mice at PND2, completely prevented the MS-associated long-term changes of both voluntary EtOH consumption and hippocampal synaptic plasticity, suggesting that this treatment produces a protective effect against MS stress. Overall, our findings strengthen the idea that MS produces long-lasting modifications on plasticity at both excitatory and inhibitory synapses, effects that may be relevant for the altered cognitive performance and sensitivity to EtOH. These results, herein add to previous evidence supporting the idea of genderspecific sensitivity to stress associated to MS, and suggest that hormones may affect the outcome of early life although the neurobiological mechanisms behind these changes need further investigation. Founded by the Agreement "CNR-Sardegna Ricerche-Universita' di Cagliari"

Disclosures: G. Talani: None. G. Sarigu: None. F. Vedele: None. M. Petrella: None. D. Colombo: None. E. Sanna: None.

Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

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Topic: F.04. Stress and the Brain

Support: DARPA/ARO W911NF1010093

Title: Paraventricular thalamic regulation of habituation to repeated stress: Molecular and network mechanisms

Authors: B. CORBETT¹, S. LUZ², *S. BHATNAGAR³

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Abstract: In the field of stress neurobiology, habituation is defined as a decreasing and learned response to a familiar stressor over time. Disrupted habituation is a signature of post-traumatic stress disorder (PTSD), causing devastating effects for those afflicted. Understanding the molecular and neural substrates underlying habituation may allow for improved therapies for PTSD patients. In rats, we model habituation using the repeated restraint stress paradigm. Exposure to this moderately intense stressor increases the expression of immediate early genes in certain brain regions, induces the production of stress-related hormones, and elicits struggle behavior. All of these responses are highest on day 1 of restraint and attenuate by the 5th to 7th exposure. We have previously identified the posterior paraventricular thalamic nucleus (pPVT) as a crucial brain region that promotes habituation. However, the underlying molecular mechanisms and specific neural connections among the pPVT and other brain regions that mediate habituation are unknown. Using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), we investigated the role of the pPVT in regulating the stress response. We demonstrated that chemogenetic inhibition of the pPVT disrupted neuroendocrine habituation to 5 days of 30min daily restraint exposure, consistent with our previous data using neurotoxic lesions. Additionally, we found that chemogenetic inhibition of a subset of pPVT neurons that project to the medial prefrontal cortex (mPFC), which negatively regulates the stress response, was sufficient to attenuate habituation. We hypothesize that pPVT neurons, in particular those that project to the mPFC, play a critical role in the abiity to habituate to stress. Finally, we investigated whether activity-regulated cytoskeleton-associated protein (Arc) induction within the pPVT was a molecular mechanism of habituation. Arc is an immediate early gene that reduces excitatory synapse number. We found that Arc expression was increased in the pPVT on day 1, but not day 5 of restraint in naïve rats and that Arc knockdown in the pPVT attenuated habituation. We hypothesize that Arc-mediated restrictions in excitatory synapse number during the first exposures to restraint are a critical mechanism underlying habituation of the stress response. Our findings offer new insight into the role of the pPVT in regulating the stress response and are amongst the first to provide potential molecular and network mechanisms underlying stress habituation.

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Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

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Topic: F.04. Stress and the Brain

Support: DA020129

Title: Effect of corticotropin-releasing factor on noradrenergic locus coeruleus neurons in cannabinoid type 1 receptor knock out mice

Authors: *R. WYROFSKY¹, L. G. KIRBY², E. J. VAN BOCKSTAELE¹ ¹Pharmacol. & Physiol., Drexel Univ. Col. of Med., Philadelphia, PA; ²Ctr. for Substance Abuse Res. and Dept Anat. and Cell Biol, Lewis Katz Sch. of Med. At Temple Univ., Philadelphia, PA

Abstract: The noradrenergic system has been shown to play a key role in the modulation and regulation of stress responses, arousal, mood, and emotional states. Dysregulation of noradrenergic transmission, which has been shown to play a key role in the modulation and regulation of stress responses and emotional states, can lead to the development of several stressrelated psychiatric disorders. As the primary mediator of stress-related inputs to the noradrenergic locus coeruleus (LC), corticotropin-releasing factor (CRF) increases LC neuronal discharge rates and induces norepinephrine (NE) release in target regions. The endocannabinoid (eCB) system has been shown to modulate stress responses in multiple brain regions and is thought to act as an "anti-stress" neuromediator. We have shown that cannabinoid receptor type 1 (CB1r) and CRF co-localize within individual axon terminals in the core and peri-LC areas, and that these are characterized by morphologically distinct synapses as revealed by electron microscopy. Using anterograde tract-tracing from the amydala, we also demonstrated that the eCB system is positioned to directly regulate CRF release from this limbic afferent. Mice lacking the CB1r are known to exhibit anxiogenic-like phenotypes and have altered stress responses. In the present study, we sought to investigate the effect of CRF on LC-NE activity in male and female wild type (WT) and CB1r knock-out (KO) mice. Whole-cell patch-clamp recordings of LC-NE neurons were conducted in 250 µm thick horizontal brain slices obtained from 4-5 week old C57/B6 CB1r KO mice and their WT littermates. An optimal dose of 300nM CRF was first determined in male WT mice. Preliminary results, in WT subjects, show that bath application of 300nM CRF significantly increases LC-NE excitability compared to baseline in both sexes, as measured by the number of spikes elicited by a range of increasing current pulses. Additionally, 300nM CRF caused an increase in input resistance in both males and females. Interestingly, both male and female CB1r KO mice had heightened basal LC-NE excitability that was comparable to the level of excitability caused by 300nM CRF in WT littermates. Bath application of 300nM CRF did not cause any further increase in LC excitability. Taken with our previous anatomical data showing co-existence of CB1r with CRF in single afferents innervating the LC, some of which originate from the amygdala, these data indicate that the eCB system regulates LC-NE neuronal activity, which has significant implications for mediating stress responses.

Disclosures: R. Wyrofsky: None. L.G. Kirby: None. E.J. Van Bockstaele: None.

Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

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Program#/Poster#: 321.19/KK6

Topic: F.05. Neuroimmunology

Support: NARSAD Young Investigator Award from the Brain & Behavior Research Foundation to GEH

Title: Sex differences in cytokine expression following sub-chronic variable stress

Authors: *J. R. RAINVILLE, G. E. HODES

Sch. of Neurosci., Virginia Tech., Blacksburg, VA

Abstract: Depression affects more than 300 million people worldwide, and the risk of experiencing a depressive episode is twice as high for women as it is for men. Pro-inflammatory cytokines and the activation of inflammatory pathways have been associated with depression. Many autoimmune and inflammatory diseases also have a higher incidence in women, presenting the possibility that sex-specific peripheral immune system responses to stress may influence sex differences in susceptibility to depression. The purpose of this study is to examine how peripheral cytokine expression may reflect differences in stress susceptibility between sexes. We used a 6-day subchronic variable stress (SCVS) model, in which stressed female but not male mice express depression-associated behaviors (Hodes et al., 2015) to observe the effects of sex and stress on peripheral cytokine expression. Animals were exposed to a combination of foot shock (0.45 mA/ 2 sec/ 100 in 1 hour), tail suspension and restraint stress, one a day, two times, over the course of 6 days. Blood was drawn from animals within 20 minutes of stress exposure (restraint stress) on the last day of SCVS. Multiplex ELISA was used as an unbiased screen to quantify plasma expression levels of 27 different cytokines. The overall pattern of expression indicated that male and female peripheral immune systems respond to stress differently following 6 day SCVS. In general, cytokines that were significantly affected by stress in males were increased compared to male controls. In females, stress generally decreased cytokine levels compared to same sex controls. The following cytokines were significantly (p<0.05), and oppositely regulated in stressed male and female mice after 6 day SCVS: CXCL1, VEGF, CCL5, IL-1α, Eotaxin, and IL-13. The exceptions to this pattern were the cytokines CXCL2 and M-CSF which were both significantly increased males and females following stress. The expression of VEGF, IL-13, CCL5, IL-1α in all treatment groups were positively correlated with each other, suggesting a potential interconnection of these inflammatory pathways in response to stress in both sexes. These results were further correlated with behavior results from a novelty suppressed feeding test that was conducted after the blood draw. CCL5 levels were positively correlated with latency to eat in males (p<0.05), but not in females. LIX levels were positively correlated

with latency to eat in all groups. Eotaxin levels were negatively correlated with latency to eat in stressed animals of both sexes. Together these data suggest that sex specific cytokine patterns of expression may be able to indicate stress susceptibility in both sexes.

Disclosures: J.R. Rainville: None. G.E. Hodes: None.

Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

Location: Halls A-C

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Topic: F.05. Neuroimmunology

Support: NARSAD Young investigator award from the Brain and Behavior Research Foundation

Title: Transcriptional sex differences in the nucleus accumbens and hippocampus following subchronic and chronic variable stress

Authors: M. TSYGLAKOVA¹, J. RAINVILLE², A. JOHNSON², B. SMITH², *G. E. HODES² ¹Translational Biology, Med. and Hlth., Virginia Tech. Carilion Sch. of Med. and Res. Institute, Roanoke, VA; ²Neurosci., Virginia Tech., Blacksburg, VA

Abstract: Depression and anxiety are common and debilitating mood disorders, and they are more prevalent in females than males. Previously, we reported that sub-chronic (6 days) variable stress (SCVS) induced behavioral stress susceptibility in females but not males. These behavioral effects were accompanied by sex specific changes in transcription of the Nucleus Accumbens (NAc) determined by RNA sequencing (Hodes et al. 2015). Here, we took a candidate gene approach based on pathway analysis of genes that sex specifically regulated the effects of stress on plasticity, neurotransmitter levels and immune function in the nucleus accumbens (NAc) following 6 days of stress. We examined expression of these genes in the NAc and hippocampus of male and female mice following chronic (28 days) variable stress (CVS). These two brain regions were examined as they both show sex specific regulation of plasticity that has been associated with the effects of stress on behavior (Bangasser et al, 2007, Brancato et al., 2017, Christoffel et al, 2011, 2012, McEwen et al. 2001, 2016, McKittrick et al, 2000, Shors et al. 2001, 2004). Males and females exposed to CVS both demonstrate stress susceptibility across a behavioral test battery. We hypothesized that different transcriptional patterns would emerge at 6 and 28 days within the same brain areas. Specifically we characterized whether expression in males and females at 28 days followed the same or opposite sex patterns found at 6 days. We found that for many of these candidate genes males expressed transcriptional patterns more similar to females (than males) at 6 days. For example, After 6 days of variable stress we found that transcription for the adonosine receptor 1 (Adora1) which can regulate both dopamine and

glutamate was significantly down regulated in the NAc of females whereas by 28 days it was down regulated both sexes in the NAc (trend: p=0.08) and hippocampus (p=0.01). The gene encoding the serotonin transporter (SLc6a4) was initially upregulated in the NAc of stressed females following SCVS whereas following CVS it was upregulated in males compared to females (p=0.02). Vascular endothelial growth factor A (Vegfa) was significantly down regulated in the NAc of stressed females following SCVS, at 28 days it was no longer significantly regulated by stress in the NAc of either sex but was down regulated in the hippocampus of both sexes (p=0.003). These data suggest that changes in gene expression occur first in the NAc of females after SCVS and may be followed by similar changes in the NAc of males and the hippocampus of both sexes following 28 days of variable stress.

Disclosures: M. Tsyglakova: None. **J. Rainville:** None. **A. Johnson:** None. **B. Smith:** None. **G.E. Hodes:** None.

Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

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Topic: F.05. Neuroimmunology

Support: NIMH R01-MH109165

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NIDCR T32-DE014320

Title: Paired fighting causes social withdrawal and leukocyte recruitment to the cerebral ventricle in an IL-1R1 dependent manner

Authors: *D. J. DISABATO¹, D. P. NEMETH², X. LIU², G. GORANTLA³, J. P. GODBOUT¹, N. QUAN²

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Abstract: Chronic stress is associated with an increase in prevalence of mental health complications such as anxiety and depression. We have previously reported that repeated social defeat (RSD) causes microglial activation, monocyte infiltration into the brain, and increased IL-1 β signaling in association with prolonged anxiety-like behavior. We hypothesized that interleukin-1 β (IL-1 β) signaling in the brain was the key player in this stress-mood disorder interaction. In the present study, we used a modified social stress paradigm paired fighting (PF) to induce social stress. Following exposure to six days of PF, C57BL/6 mice displayed general withdrawal in a social interactivity test with juvenile C57BL/6 mice. The experimental mice also experienced a decreased latency to immobility in the tail suspension test. Mice exposed to paired

fighting had increases in neutrophils and Ly6C^{hi} reactive monocytes in circulation. Parallel to this, PF mice had an increased number of peripherally-derived leukocytes in the cerebral ventricle. To examine the significance of IL-1 signaling in these processes, we used our genetic model of global IL-1 receptor 1 knockout, the IL-1 restore model (IL-1R1^{r/r}). After PF, IL-1R1^{r/r} mice did not display the same social withdrawal or reduced latency to first immobility in tail suspension. In addition, IL-1R1 deletion did not prevent the increase in circulating Ly6C^{hi} monocytes after PF, but it did reduce the number of peripheral leukocytes in the cerebral ventricle. Next, we injected IL-1 β intracerebroventricularly (icv) in mice exposed to PF. Following PF, icv-IL-1 β injection did not affect microglial morphological alterations. Nonetheless, icv-IL-1 β injection in PF increased peripheral macrophages within the cerebral ventricle and exacerbated affective behavior. Taken together, these results show that IL-1R1 signaling plays a crucial role in stress-induced affective behavior and central IL-1 β administration augments the neurobehavioral deficits associated with paired fighting stress.

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Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

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Topic: F.05. Neuroimmunology

Support: NIH Grant NS026880

NIH Grant DA019521

NIDA T32 DA007135

Title: Exploring the role of GPR83, a newly deorphanized G-protein coupled receptor, in stress and immune function

Authors: *L. M. LUEPTOW¹, L. MIORIN², A. FAKIRA³, M. SCHOTSAERT², A. GARCIA-SASTRE², L. DEVI³

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Abstract: Considerable research has demonstrated the complex interplay between the brain, stress, and immune function. Additionally, most immune organs have direct innervation from the CNS, along with the presence of many neurotransmitters and neuropeptides, and often, these signaling molecules can have a profound effect on immune function. GPR83, a G-protein coupled receptor recently identified as the receptor for the neuropeptide PEN, is expressed in a

number of rodent brain regions that are critically involved in the regulation of stress, mood and behavior. In addition, previous research suggests GPR83 is also highly expressed in the spleen, thymus and specific T-cell populations. GPR83 has shown to be differentially regulated following exposure to the glucocorticoid dexamethasone, with an up-regulation of GPR83 in rodent thymocytes, but a down-regulation in various brain regions. This suggests dynamic regulation of GPR83 in neuroendocrine activation, potentially in stress and anxiety. Previous research has also shown GPR83 knockout mice to be resilient to stress-induced anxiety following an acute stressor. To further investigate the role of GPR83 in stress, wild type and GPR83 knockout mice were exposed to chronic stress. Both WT and KO mice showed significantly decreased latency to immobility in the forced swim test as compared to non-stressed mice, suggesting knockout of GPR83 does not protect against stress-induced depressive-like behavior. To better characterize GPR83 in immune function, we investigated GPR83 expression in various immune cell types. Using flow cytometry, we found GPR83 to be highly expressed on numerous immune cell types, including CD4+ and CD8+ T-cells, FoxP3+ T-cells, and NK cells. Low dose infection of PR8 (H1N1) to female GPR83 KO mice resulted in elevated viral RNA levels, and reduced levels of various cytokines in the lungs as compared to wild type control mice at day 5 after infection. In addition, reduced infiltration of CD4+ and CD8+ T-cells, as well as FoxP3+ T-cells to the lungs of GPR83 KO mice was observed by flow cytometry analysis. To explore the potential neuronal pathways that activate GPR83 in peripheral immune organs, we injected attenuated pseudorabies virus tagged with GFP into the spleen or thymus of mice, and examined viral expression following 72 hrs of retrograde transport. GFP expression was observed in parts of the raphe nucleus, periaqueductal grey and central amygdala, regions involved in somatic regulation and the modulation of pain and stress. Based on these observations, we believe further characterization will reveal the GPR83-PEN receptor system to be an important mediator of stress and immune function.

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Poster

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Topic: F.05. Neuroimmunology

Support: NIH Grant R01 MH-109165

Title: Distribution of type 1 interleukin-1 receptor in the central nervous and neuroendocrine systems in mouse

Authors: *X. LIU¹, A. SONG¹, D. NEMETH¹, L. ZHU¹, N. QUAN² ¹The Ohio State Univ., Columbus, OH; ²Dept Biosci., Ohio State Univ., Columbus, OH

Abstract: Interleukin 1 is a pleiotropic cytokine that mediates diverse functions in the central nervous system and neuroendocrine system through its receptor, type I interleukin-1 receptor (IL-1R1). In the past, efforts have been made to visualize IL-1R1 mRNA and protein. However, due to the fact that IL-1R1 is often expressed at very low levels, it remains difficult to distinguish IL-1R1 expressing cells from labeling artifacts generated by the visualization procedures including *in situ* hybridization and immunohistochemistry. In the present study, we remapped the IL-1R1 distribution in an IL-1R1 reporter mouse line in which IL-1R1 mRNA and protein were tracked by the endogenous tdTomato fluorescence and HA tag respectively. Endothelial IL-1R1 mRNA and protein was found throughout the brain and spinal cord, with highly elevated expression in the olfactory bulb and paraventricular nucleus of hypothalamus. Neuronal IL-1R1 mRNA was identified in selective brain regions including dentate gyrus, dorsal raphe nucleus, arcuate nucleus and cerebellum. In addition, IL-1R1 mRNA and protein were found in the cells of the anterior lobe of pituitary, but not in the posterior lobe of pituitary and adrenal gland. The distribution of IL-1R1 in the CNS and neuroendocrine systems shown by the current study suggests additional targets of IL-1 stimulation.

Disclosures: X. Liu: None. A. Song: None. D. Nemeth: None. L. Zhu: None. N. Quan: None.

Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 321.24/KK11

Topic: F.05. Neuroimmunology

Support: NIH grant R01 MH109165

Title: Chronic IL-1 suppresses hippocampal neurogenesis via dentate neuronal IL-1R1

Authors: *D. NEMETH¹, X. LIU², G. GORANTLA², D. J. DISABATO³, N. QUAN⁴ ¹Inst. For Behavioral Med. Res., Columbus, OH; ³Neurosci., ²The Ohio State Univ., Columbus, OH; ⁴Dept Biosci., Ohio State Univ., Columbus, OH

Abstract: Neurogenesis in the central nervous system plays a critical role in neuronal plasticity and memory. Previous reports showed chronic Interleukin-1 β (IL-1 β) decreases neurogenesis; however the mechanism of this phenomenon is not understood. In this study, we examined the expression patterns of Interleukin-1 Receptor 1 (IL-1R1) and the impact of chronically expressed IL-1 β on hippocampal neurogenesis. Using a genetic IL-1R1 reporter mouse (IL-1R1^{GR/GR}), IL-1R1 mRNA expression is visualized via tdTomato fluorescence. IL-1R1 expression was found in

brain endothelial cells and neurons of the dentate gyrus. Restricting IL-1R1 expression to glutamatergic neurons (VglutCre-IL-1R1^{r/r}), allowed IL-1R1 to be expressed exclusively in these dentate gyrus neurons. Interestingly, IL-1R1 expressing neurons do not express doublecortin, indicating neuronal IL-1R1 is not expressed on the proliferating neural progenitor cells. Chronic IL-1 β expression in the DG, via an adenoviral vector, induced local activation of microglia and neuronal death in the WT and VGLUTCre-IL-1R1^{r/r} animals but not IL-1R1 deficient (IL-1R1^{r/r}) animals. When proliferating neurons are tracked by EDU labeling, chronic IL-1 β caused decreased EDU+ cells within the subgranular zone (SGZ) in WT and VGlutCre-IL-1R1^{r/r} but not IL-1R1^{r/r} animals. These results show IL-1R1 is located on the mature neuronal population of the dentate gyrus and IL-1 acts upon these receptors to indirectly suppress neurogenesis.

Disclosures: D. Nemeth: None. X. Liu: None. G. Gorantla: None. D.J. Disabato: None. N. Quan: None.

Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 321.25/KK12

Topic: F.05. Neuroimmunology

Support: NIH Grant MH097243

Title: Microglial recruitment of monocytes to the brain underlies reoccurring anxiety in stress-sensitized mice

Authors: *J. P. GODBOUT¹, M. D. WEBER², J. F. SHERIDAN³

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Abstract: Repeated social defeat (RSD) is a murine stress model that promotes long-term "stress-sensitization", in which exposure to sub-threshold stress causes re-establishment of anxiety. We previously reported that RSD-induced anxiety depends on trafficking of monocytes to fear and threat appraisal brain regions. We hypothesize that RSD drives the production of inflammatory monocytes that are recruited to the brain by microglia, and these monocytes augment pro-inflammatory signaling that reinforces activation of threat circuitry. To address this, we used the CSF-1R inhibitor, PLX5622, to deplete microglia. Subsequent removal of the inhibitor allows microglia to rapidly repopulate. Here, we show that elimination of microglia after RSD prevented the recruitment of monocytes to the brain and recall of anxiety in stress-sensitized mice. Furthermore, microglia were depleted prior to RSD and allowed to repopulate. The phenotype of microglia was assessed by measuring behavioral and cytokine reactivity to peripheral LPS administered 24 days after RSD. We report that mice exposed to RSD displayed

a prolonged reduction in social interaction to peripheral LPS and exaggerated pro-inflammatory cytokine expression from microglia. Importantly, repopulating microglia in mice exposed to RSD normalized social interaction and reduced microglial pro-inflammatory cytokine expression to levels similar to non-stressed mice. These data demonstrate that microglia depletion/repopulation can be used to prevent microglial priming. Collectively, microglial priming may underlie monocyte recruitment to the brain and re-establishment of anxiety in stress-sensitized mice.

Disclosures: J.P. Godbout: None. M.D. Weber: None. J.F. Sheridan: None.

Poster

321. Stress-Modulated Pathways: Physiological and Behavioral Outcomes

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 321.26/KK13

Topic: F.05. Neuroimmunology

Support: F31 MH 109234

R01- MH-093473

R01-MH093472

Title: Extramedullary monopoiesis underlies stress-sensitization and recurring anxiety

Authors: *D. B. MCKIM¹, J. P. GODBOUT², J. F. SHERIDAN³ ¹The Ohio State Univ., Columbus, OH; ²Neurosci., Ohio State Univ. Dept. of Neurosci., Columbus, OH; ³Inst. for Behavioral Med. Res., Ohio State Univ., Columbus, OH

Abstract: In humans, chronic stress is associated with an increased prevalence of mental health complications including anxiety and depression. Repeated social defeat (RSD) in mice recapitulates key deficits associated with psychosocial stress in humans. We showed that exposure to sub threshold stress 24 days after RSD caused the recurrence of anxiety that was dependent on monocyte trafficking from the spleen to the brain. We hypothesized that extramedullary production of monocytes in the spleen contributed to enhanced monocyte trafficking and recurring anxiety following psychosocial stress. RSD caused profound mobilization of hematopoietic stem progenitor cells (HSPCs) into circulation that accumulated and substantially proliferated in the spleen. For instance, RSD significantly increased the presence of all sub-types of hematopoietic colony forming units (CFU) in blood and spleen. Moreover, there was a 30-fold increase in the number of Lin-/Sca1+/cKit+ (LSK) progenitor cells in the spleen after stress that were actively proliferating (S/G2/M cell cycle phase). Next, competitive bone marrow adoptive transfer was used to assess the presence of splenic HSPCs. RSD significantly increased the presence HSPCs in the spleen (28-fold) that engrafted into host

bone marrow. EdU pulse chase experiment revealed that RSD potently induced proliferation of HSPCs within the red pulp of the spleen. Moreover, a BrdU pulse chase experiment showed that RSD significantly increased the presence of proliferating splenic CD11b+/BrdU+ monocytes. This increase in monocyte proliferation in the spleen persisted for at least 1 month as detected by BrdU pulse chase. These data indicate that RSD caused extramedullary monopoiesis in the spleen that persisted for at least 1 month. To determine the necessity of this splenic reservoir for recurring anxiety, mice were splenectomized. Splenectomy either before or after RSD both prevented the recurrence of anxiety following acute stress 1 month after RSD. We conclude that splenic monopoiesis promotes recurring anxiety following psychosocial stress in mice. The fundamental observation that psychosocial stress caused chronic extramedullary monopoiesis in the spleen has numerous implications for negative health outcomes associated with chronic stress. We specifically showed here that extramedullary monopoiesis underlies monocyte trafficking and recurring anxiety following psychosocial stress. These data reveal a novel immune mechanism connecting stress to prolonged behavioral complications.

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Poster

322. Stress and Cognition: Clinical Studies

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 322.01/KK14

Topic: F.04. Stress and the Brain

Support: JSPS KAKENHI 15H02502

JSPS KAKENHI 16K16585

Title: Disorganization between default mode and attention networks is the feature of acute mental fatigue

Authors: *A. T. SASAKI^{1,3,4}, K. MIZUNO^{1,3,4,5}, K. WATANABE^{1,6}, K. TAJIMA³, T. HAYASHI², Y. WATANABE^{1,3,4}

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Abstract: Fatigue may be a bio-alarm for sustainable working performance. Past neuroimaging studies have shown differences in gray matter morphometry and in task-related brain activities between fatigued and unfatigued subjects or states. Recent studies using resting state functional

magnetic resonance imaging (RS-fMRI) have suggested that spontaneous co-activating brain regions, termed as resting state networks (RSNs), may potentially differ from fatigue states. However, it is still unclear how RSNs are associated with subjective fatigue during cognitive performance. In this study, we conducted a RS-fMRI study to clarify temporal changes in RSNs before and after mental fatigue. We recruited 32 healthy volunteers who did not complain any fatigue in daily life. RS-fMRI scans were performed before and after fatigue-inducing session in which participants performed working memory task for 45 minutes. During a period of the 5-min RS-fMRI scans, subjects were asked to view a fixation cross, and not to sleep. As a working memory task, we adopted a 2-back task where subjects were asked to view successively presented numeric characters (form 1 to 4), and to answer whether a newly presented character was identical or not compared with that appeared two presentation before. For the data analysis, we used the predefined 20 components of RSNs (Smith et al., 2002), and run dual regression to compare the RSNs connectivity between before and after fatigue. The comparison of RSNs revealed that the visual RSN significantly decreased after fatigue session. The analysis of correlation between RSNs revealed that the correlation between default mode and attention networks significantly changed after fatigue session: these two networks were in significantly negative correlation before, but not after the fatigue. It is known that the default mode network is deactivated when attention network is active during cognitive tasks directed towards external stimuli, indicating that these two networks function in antagonistic manner. Taken together, the present study indicates that acute mental fatigue may induce disorganization between default mode and attention networks, potentially underlining a mechanism for impairment of sustainability in cognitive performance.

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Poster

322. Stress and Cognition: Clinical Studies

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Program#/Poster#: 322.02/KK15

Topic: F.04. Stress and the Brain

Support: German Research Foundation Grant SCHW1357/10-1

Title: A haplotype of the mineralocorticoid receptor gene promotes the stress-induced shift from 'cognitive' to 'habit' learning

Authors: *L. WIRZ¹, M. REUTER², J. WACKER³, A. FELTEN², L. SCHWABE¹ ¹Cognitive Psychology, Hamburg, Germany; ²Differential and Biol. Psychology, Bonn, Germany; ³Differential Psychology, Hamburg, Germany

Abstract: Stress induces a shift from hippocampus-dependent, 'cognitive' toward dorsal striatum-dependent, 'habitual' memory. Not all individuals are susceptible to this shift under stress, yet the source of these individual differences is largely unknown. Based on pharmacological studies pointing to a critical role of the mineralocorticoid receptor (MR) in the stress-induced shift toward striatal learning, we hypothesized that variants in the MR gene contribute to individual differences in the effects of stress on the engagement of multiple memory systems. In two independent experiments, healthy participants were genotyped, exposed to a stressor (Trier Social Stress Test) or control manipulation and performed a probabilistic classification learning task that can be solved using hippocampus-dependent single-cue or dorsal striatum-dependent multi-cue strategies, while EEG (experiment I) or fMRI (experiment II) measurements were taken. Stress did not affect learning performance per se, but led to a shift from hippocampal to striatal learning strategies and this shift was more pronounced in carriers of a six SNPs-comprising haplotype containing at least one copy of the alleles of two MR variants associated with increased MR functionality ([rs2070951] MR-2G/C C, [rs5522] MR-I180V I). The stress-induced shift toward dorsal striatal processing was paralleled by an increase in the feedback-related negativity, an event-related potential thought to reflect, at least partly, striatal processing, as well as by enhanced amygdala and caudate nucleus activation. Carriers of the MR haplotype showed reduced amygdala and hippocampus activation under stress. Additionally, stress resulted in reduced functional connectivity between the amygdala and the hippocampus and this reduction was particularly pronounced in MR haplotype carriers. Already at rest, the MR haplotype was linked to increased amygdala-caudate nucleus connectivity. Our results indicate that genetic variants associated with enhanced MR expression facilitate a stress-induced shift from hippocampal toward dorsal striatal learning, most likely via impaired hippocampal processing and reduced amygdala-hippocampus crosstalk, which allows the dorsal striatum to guide behavior under stress.

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Poster

322. Stress and Cognition: Clinical Studies

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 322.03/KK16

Topic: F.04. Stress and the Brain

Support: This research and ND are supported by funds from the Kinesiology Department of KU Leuven.

Title: Stress modulates motor memory consolidation

Authors: *N. DOLFEN¹, B. R. KING¹, L. SCHWABE², S. P. SWINNEN¹, G. ALBOUY¹ ¹Movement Control and Neuroplasticity Res. Group, Dept. of Kinesiology, KU Leuven, Leuven, Belgium; ²Univ. of Hamburg, Hamburg, Germany

Abstract: It is suggested that the specific combination of hippocampal activity during initial motor sequence learning (MSL) and post-training sleep is necessary to optimize motor memory consolidation. The current study investigates whether exposure to stress, a factor known to disrupt hippocampal activity during learning, compromises the subsequent consolidation of motor sequence memory. Thirty-seven healthy young adults were exposed to a stressor (i.e. socially evaluated cold pressor test; SECPT) (stress group, n = 20) or control procedure (no stress group, n = 17) *before* they were trained on an eight-element bimanual MSL task. Participants were retested on the motor task 24 hours (including a night of sleep) after initial training to assess memory consolidation. Autonomic and endocrine responses to stress were assessed by measuring heart rate and blood pressure as well as salivary cortisol (i.e., principal stress mediator hormone), respectively. Results revealed that participants in the stress group, as compared to the no stress group, showed significant increases in blood pressure and heart rate during stress exposure. 80% of the participants in the stress group were classified as "cortisol responders" (n = 16) as they showed an increase in cortisol of at least 2 nmol/l after the SECPT intervention (Schwabe et al., 2008, PNEC). Cortisol concentration in responders was significantly elevated as compared to their own baseline as well as the no stress group prior to and immediately following initial training. Importantly, while both groups showed comparable motor performance (in terms of both movement speed and accuracy) during the initial training session, the magnitude of performance improvement was significantly lower in the stress group as compared to the no stress group during the 24h-retest. Our results provide the first evidence that stress exposure prior to motor learning selectively disrupts subsequent memory consolidation processes. It is speculated that stress exposure challenged the recruitment of the hippocampus during initial acquisition of the motor memory and therefore compromised subsequent sleep-related motor memory consolidation.

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Poster

322. Stress and Cognition: Clinical Studies

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 322.04/KK17

Topic: F.04. Stress and the Brain

Title: The effects of psychological and physiological stress on spatial reasoning skills

Authors: *B. ROMAGNA, B. P. GEE

Psychology, Western Connecticut State Univ., Danbury, CT

Abstract: Research has identified stress as a paradoxical pressure, acutely impeding cognitive processing (Sliwinski, Smyth, Hofer & Stawski 2006) while simultaneously improving memory and mental health in chronic, predictable doses (Buchanan & Lovallo 2001; Parihar, Hattiangady, Kuruba, Shuai & Shetty 2011). The question of how stress impacts our mind and body remains largely unanswered. This study evaluated spatial reasoning ability as a function of stressor type. Human subjects performed a mental rotation task (Shepard & Metzler 1971) during psychological (loud, misleading feedback) and physiological (cold pressor test) stress. For each trial, participants viewed a pair of geometrical shapes varying in spatial orientation, and decided whether the stimuli were identical or mirrored objects. Task performance was based on reaction time and proportion correct. After a block of trials were completed for each stress and control condition, stress levels were measured, such as self-report scales, cardiovascular function, and salivary concentrations of cortisol and alpha-amylase. In the cold pressor test condition, both speed and accuracy were better than the control condition (warm water hand submersion). The psychological stress condition did not affect performance as much as the physiological stressor. Biomarkers of stress were also most affected by the cold pressor test. The results showed that physical stress can adversely affect cognitive function, while psychological stress may need to be high before significant cognitive impairment is observed. We theorized that physiological and psychological stressors contradistinctively activate the stress response.

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Poster

322. Stress and Cognition: Clinical Studies

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 322.05/KK18

Topic: F.04. Stress and the Brain

Support: NIH Grant R15MH104836

Title: Interactive influence of sex, stressor timing, and the BclI glucocorticoid receptor polymorphism on stress-induced alterations of long-term memory

Authors: *T. J. DUFFY¹, H. E. NAGLE¹, A. M. DAILEY¹, M. K. FIELY¹, B. E. MOSLEY¹, A. R. SCHARF¹, C. M. BROWN¹, M. B. EARLEY¹, K. L. HESS¹, J. K. HANDEL¹, M. R. RIGGENBACH¹, M. T. ROSELER¹, L. E. WIREMAN¹, J. J. HIPSKIND¹, B. R. RORABAUGH², P. R. ZOLADZ¹ ¹Psychology, Sociology, & Criminal Justice, ²Pharmaceut. & Biomed. Sci., Ohio Northern Univ., Ada, OH Abstract: The BclI polymorphism of the glucocorticoid receptor (GR) gene (NR3C1) is a common polymorphism that has been associated with increased GR sensitivity, blunted cortisol responses to stress, and increased susceptibility for stress-related psychological disorders. Given the extensive work implicating glucocorticoid signaling in the effects of stress on learning and memory, we predicted that the BclI polymorphism might influence such effects. Two hundred and thirty-five healthy individuals were exposed to the socially evaluated cold pressor test or a control condition immediately or 30 min prior to learning a list of words that varied in emotional valence and arousal level. Participants' memory for the words was tested immediately (recall) and 24 h after learning (recall and recognition), and saliva samples were collected to genotype participants for the *Bcl*I polymorphism. The results showed that stress administered immediately before learning exerted sex- and genotype-dependent effects on long-term memory. Specifically, stress enhanced long-term memory selectively in male non-carriers of the BclI risk allele (G). Stress had no effect on females or male risk allele carriers. Stress administered 30 min before learning selectively impaired long-term recognition memory in risk allele carriers. These findings suggest that carriers of the BclI polymorphism may retain a sensitized stress response system. They also suggest that the association between the *Bcl*I polymorphism and increased susceptibility for stress-related disorders might relate to its influence on emotional memory systems.

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Poster

322. Stress and Cognition: Clinical Studies

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Program#/Poster#: 322.06/KK19

Topic: F.04. Stress and the Brain

Support: NIH Grant R15MH104836

Title: FKBP5 polymorphisms influence pre-learning stress-induced alterations of learning and memory

Authors: *P. R. ZOLADZ¹, A. M. DAILEY¹, H. E. NAGLE¹, M. K. FIELY¹, B. E. MOSLEY¹, C. M. BROWN¹, T. J. DUFFY¹, A. R. SCHARF¹, M. B. EARLEY¹, K. L. HESS¹, J. K. HANDEL¹, M. R. RIGGENBACH¹, M. T. ROSELER¹, L. E. WIREMAN¹, J. J. HIPSKIND¹, B. R. RORABAUGH² ¹Psychology, Sociology, & Criminal Justice, ²Pharmaceut. & Biomed. Sci., Ohio Northern Univ., Ada, OH

Abstract: FK506 binding protein 51(FKBP5) is a co-chaperone of heat shock protein 90 and significantly influences glucocorticoid receptor sensitivity. Single nucleotide polymorphisms (SNPs) in the *FKBP5* gene are associated with altered hypothalamus-pituitary-adrenal (HPA) axis function, changes in the structure and function of several cognitive brain areas, and increased susceptibility to post-traumatic stress disorder, major depression, bipolar disorder, and suicidal events. The mechanisms underlying these associations are largely unknown, but it has been speculated that the influence of these SNPs on emotional memory systems may play a role. In the present study, 112 participants were exposed to the socially evaluated cold pressor test (stress) or control (no stress) conditions immediately prior to learning a list of 42 words. Participant memory was assessed immediately after learning (free recall) and 24 h later (free recall and recognition). Participants provided a saliva sample that enabled the genotyping of three FKBP5 polymorphisms: rs1360780, rs3800373, and rs9296158. Results showed that stress impaired immediate recall in risk allele carriers. More importantly, stress enhanced long-term recall and recognition memory in non-carriers of the risk alleles, effects that were completely absent in risk allele carriers. Follow-up analyses revealed that memory performance was correlated with salivary cortisol levels in non-carriers, but not in carriers. These findings suggest that *FKBP5* risk allele carriers may possess a sensitized stress response system, perhaps specifically for stress-induced changes in corticosteroid levels, which might aid our understanding of how SNPs in the FKBP5 gene confer increased risk for stress-related psychological disorders and their related phenotypes.

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Poster

322. Stress and Cognition: Clinical Studies

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 322.07/KK20

Topic: F.04. Stress and the Brain

Support: New York City Department of Design and Construction

Title: Stress responsiveness alters human performance on tests of executive function under varying air quality conditions in a built environment

Authors: *A. A. WALF¹, L. HALDERMAN², A. MURALI³, D. RIVERA⁴, Z. LIN³, A. DYSON^{5,6}, J. DRAPER^{5,6}

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Dept, ⁴Mathematics Dept, ⁵Sch. of Architecture, Rensselaer Polytechnic Inst., Troy, NY; ⁶Ctr. for Architectural Sci. and Ecology (CASE), New York, NY

Abstract: When considering how individuals' respond in a built environment some of the characteristics of the space that can be considered are related to air quality, such as carbon dioxide levels (CO2). Here the effects of exhaled CO2 levels in a room scale environment for human subjects' self-reports of their perception of the space, stress responding, and executive function were assessed. The hypotheses tested were that: 1) higher exhaled CO2 levels in room will increase self-reported stress ratings and reduce executive function of human room occupants; 2) the Amplified Modular Phytoremediation System (AMPS), designed by the Center for Architectural Science and Ecology (C.A.S.E.), will reduce carbon dioxide levels in the room coincident with reducing self-reported stress ratings and enhancing executive function of human room occupants; and 3) there are individual differences (relating to stress responsiveness/disposition) for these measures in different room air quality conditions. Human subjects were tested in three different air quality conditions (baseline low CO2, high CO2, and remediated low CO2 by AMPS) on standard self-report measures of stress/anxiety (State and Trait Inventory of Somatic and Cognitive Anxiety) and executive function (Trail Making, Digit-Symbol Substitution Task, Compound Remote Associates Test, short- and long-term verbal memory, Mental Rotation, n-back). The findings suggest differences in how individuals respond on standard measures of stress/mood and executive function under high CO2 conditions that can be remediated by AMPS. These patterns of poor responding under high carbon dioxide concentrations were particularly pronounced in individuals with high stress responsiveness. For example, subjects with low stress responsiveness had better performance in the Compound Remote Associates test than those with high stress responsiveness under baseline and remediated low CO2 conditions, compared to high CO2 conditions. Together, the data from this experiment provide proof-of-concept that AMPS has the capacity to reduce carbon dioxide levels in a room produced by individuals' occupancy in that space and that there are measurable effects on individuals' reported symptoms, stress, and cognitive function that depend upon stress responsive disposition of subjects.

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Poster

322. Stress and Cognition: Clinical Studies

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 322.08/KK21

Topic: F.04. Stress and the Brain

Support: The study was sponsored by Biologische Heilmittel Heel GmbH, Baden-Baden, Germany

Title: Influence of Neurexan® on brain activity in responses to deviant stimuli during an auditory oddball task

Authors: *M. A. KRYLOVA¹, G. SUROVA², S. ALIZADEH¹, H. JAMALABADI¹, M. SCHULTZ³, M. WALTER^{2,1,4}

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Abstract: Background: Neurexan®, a medicinal product sold over the counter (OTC), contains four ingredients; Passiflora incarnata (passionflower), Avena sativa (oats), Coffea arabica (coffee) and Zincum isovalerianicum (zinc valerianate). Neurexan® has been investigated in patients with symptoms related to acute stress, nervousness/restlessness, and insomnia. The previous research suggested an attenuated neuroendocrine stress response in healthy volunteers induced by Neurexan® (Doering et al. 2016). This study further explores the effects of Neurexan® on cognitive performance and attention that can be assessed by the oddball paradigm. It is generally recognized that stress is associated with cognitive impairments. Expecting that Neurexan[®] reduces the stress level, we hypothesized that the subjects in the placebo group would be more susceptible to distraction compared to treatment group. Methods: In a randomized, placebo-controlled, double-blind, two-period crossover trial, brain responses to the unattended auditory oddball task of 39 healthy, moderately stressed males were measured with 64-channel electroencephalogram (EEG) after intake of a single dose of Neurexan® or placebo. The paradigm consisted of 80% standard tones and two types of deviant tones (10% frequency deviant; 10% duration deviant), presented in a pseudo-randomized order. The standard tone was composed of eight equally loud sinusoidal tones (fundamental frequency 330 Hz and seven harmonic partials) and had the duration of 100 ms. The deviants were either 40 ms shorter (duration deviant) or 1.25 semitones higher (frequency deviant). *Results:* Significant effect of Neurexan® on the latency of mismatch negativity (MMN) (decreased latency under treatment) was observed with repeated-measures ANOVA. The main effect of the *treatment* (F(1,37)=4.297, p=0.045, $\eta^2 = 0.104$) and significant *treatment* x *devianttype* interaction (F(1,37)=8.828, p=0.005, η^2 =0.193) were found. Further Wilcoxon-test for paired samples showed that this reductione of latency was present for the frequency deviant

stimuli (z(37)=-2.85, p=0.004).

Conclusion: Significant reduction of MMN latency in the Neurexan treatment group suggests that Neurexan® induces subtle primary processing changes in term of reaction time. *References:* Doering B.K., Wegner A., Hadamitzky M., et. al. (2016) "Effects of Neurexan® in an experimental acute stress setting—An explorative double-blind study in healthy volunteers" *Life sciences 146*, 139–147.

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Poster

322. Stress and Cognition: Clinical Studies

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 322.09/KK22

Topic: F.04. Stress and the Brain

Support: NIA Grant R21AG-048463

Title: Differential effects of stress exposure on working memory performance across the hormonal contraceptive cycle

Authors: ***A. Y. HERRERA**¹, R. VELASCO¹, S. FAUDE², J. WHITE³, P. C. OPITZ⁴, R. HUANG¹, K. TU³, M. MATHER¹

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Abstract: Hormonal contraception (HC) can modify the stress response and memory processes. However, these studies often ignore HC type, delivery method, drugs delivered, and dosing schedule, all factors that differ across the many HC formulations and which might affect outcomes. Timing within one HC type might also exert effects. For example, unlike implants, oral HC and vaginal rings deliver hormones for a set period followed by a period of no hormones. Disregarding these factors make it difficult to interpret when and how HC may be exerting effects.

To examine if HC phase (active vs. inactive) differentially influences stress effects on free cortisol responses and working memory performance, we tested 16 women using monophasic 28-day HC containing 7 inactive days. Women were seen twice, once during days 8 to 21 (active phase) and once during days 24 to 28 (inactive phase). Women completed an n-back task, followed by a stressor (cold pressor), and later completed the n-back task again. Three saliva samples were collected; a baseline sample, immediately pre-stress, and 19-minutes post-stress. A 2 (HC phase) x 3 (time: baseline vs. pre-stress vs. post-stress) repeated-measures ANOVA on salivary cortisol levels (n=9) revealed a 2-way interaction with increases across all three time points during the active phase, and a decrease from baseline to pre-stress and increase from pre-stress to post-stress during the inactive phase. Additional 2 (HC phase) x 2 (time: baseline vs. post-stress) repeated-measures ANOVA revealed increases from baseline to post-stress during the active phase. ANOVA revealed increases from baseline to post-stress during the active phase. ANOVA revealed increases from baseline to post-stress during the active phase only.

A 2 (HC phase) x 2 (load: 0-back vs. 2-back) x 2 (time: pre-stress vs. post-stress) repeatedmeasures ANOVA on WM performance before and after stress (n=16) revealed stress-induced increases in correct responses during the 0-back but decrements during the 2-back. We also found a marginal 3-way interaction with the above pattern observed during both HC phases, but with smaller overall changes during the inactive phase. A similar 2 (HC phase) x 2 (load) x 2 (time) repeated-measures ANOVA on reaction time for correct responses revealed a significant 3-way interaction, with stress-induced increases during the 0-back and decreases during the 2-back during the active phase, but increases during both loads during the inactive phase. Our findings suggest that women experience differential effects of stress on salivary cortisol responses and cognition depending on HC cycle phase. Together, these results help inform how a woman's HC might affect her day to day life, and also suggest that HC type and position in the HC cycle must be considered in future studies investigating the potential effects of HC.

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Poster

322. Stress and Cognition: Clinical Studies

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 322.10/KK23

Topic: F.04. Stress and the Brain

Support: NIH Grant R15MH104836

Title: ADRA2B deletion variant influences time-dependent effects of pre-learning stress on long-term memory

Authors: *B. E. MOSLEY¹, A. M. DAILEY¹, H. E. NAGLE¹, M. K. FIELY¹, C. M. BROWN¹, T. J. DUFFY¹, A. R. SCHARF¹, M. B. EARLEY¹, K. L. HESS¹, J. K. HANDEL¹, M. R. RIGGENBACH¹, M. T. ROSELER¹, L. E. WIREMAN¹, J. J. HIPSKIND¹, B. R. RORABAUGH², P. R. ZOLADZ¹ ¹Psychology, Sociology, & Criminal Justice, ²Pharmaceut. & Biomed. Sci., Ohio Northern Univ., Ada, OH

Abstract: Extensive work over the past few decades has shown that certain genetic variations interact with life events to confer increased susceptibility for the development of psychological disorders. The deletion variant of the *ADRA2B* gene, which has been associated with enhanced emotional memory and heightened amygdala responses to emotional stimuli, might confer increased susceptibility for the development of post-traumatic stress disorder (PTSD) or related phenotypes by increasing the likelihood of traumatic memory formation. Thus, we examined whether this genetic variant would predict stress effects on learning and memory in a non-clinical sample. Two hundred and thirty-five individuals were exposed to the socially evaluated cold pressor test or a control condition immediately or 30 min prior to learning a list of words that varied in emotional valence and arousal level. Participants' memory for the words was tested

immediately (recall) and 24 h after learning (recall and recognition), and saliva samples were collected to genotype participants for the *ADRA2B* deletion variant. Results showed that stress administered immediately before learning selectively enhanced long-term recall in deletion carriers. Stress administered 30 min before learning impaired recognition memory in male deletion carriers, while enhancing recognition memory in female deletion carriers. These findings provide additional evidence to support the idea that *ADRA2B* deletion variant carriers retain a sensitized stress response system, which results in amplified effects of stress on learning and memory. The accumulating evidence regarding this genetic variant implicates it as a susceptibility factor for traumatic memory formation and PTSD-related phenotypes.

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Poster

322. Stress and Cognition: Clinical Studies

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 322.11/KK24

Topic: F.04. Stress and the Brain

Title: An Experimental Manipulation of hypothalamic pituitary axix in humans

Authors: *B. BARRERA-MERA

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Abstract: By searching the benefits of one remedy for metal fatigue and depression on elderly. We found an innocuous and inexpensive solution. A manner which improves mental and corporeal human discomfort is delivered by the use of a selective cooling of both gonads -the must peripheric component of hypothalamic-pituitary axis-. On the essays, we found the must spectacular benefits especially to solve the memory loss, depression, fatigue, sadness.

Disclosures: B. Barrera-Mera: None.

Poster

322. Stress and Cognition: Clinical Studies

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 322.12/KK25

Topic: F.04. Stress and the Brain

Support: T32 DA 028874

Title: Unhealed wounds: Childhood maltreatment is associated with heightened brain response to 33 msec subliminal aversive cues

Authors: *P. S. REGIER¹, A. M. TEITELMAN², K. JAGANNATHAN¹, A. R. CHILDRESS¹ ¹Dept. of Psychiatry, ²Sch. of Nursing, Univ. of Pennsylvania, Philadelphia, PA

Abstract: INTRODUCTION: Childhood maltreatment is associated with neurobiological changes, such as decreased grey matter and increased brain response to fearful, traumatic, and other emotional stimuli in fronto-limbic/striatal areas. Prior studies have suggested that maltreatment is more prevalent among women and low-income families. In this study, we recruited young women from a family planning clinic (Title X) that prioritizes low-income families, and we compared them to a group of young women recruited from the surrounding area (mostly college students). We tested whether a relationship between childhood maltreatment and the brain response to aversive cues is robust enough to be detected even when the aversive cues are presented subliminally - entirely outside conscious awareness. METHODS: Young women (age=21.9) were recruited from a Title X family planning clinic (clinic sample, n=34) and from the surrounding university (community sample, n=20). Childhood maltreatment was assessed with the childhood trauma questionnaire (CTQ), and brain activity was measured with BOLD fMRI in response to 33 msec evocative [food, romantic, and aversive (vs. neutral)] cues. Total scores from the CTQ were used as a covariate of interest for each of the evocative (vs. neutral) cue contrasts for all participants (n=54), the clinic sample (n=34), and the community sample (n=20). Thresholds for all whole-brain fMRI correlation analyses were set at p < 0.005 and k > 0.005200. RESULTS: The clinic population had significantly higher CTQ scores than the community sample (p < 0.05). There was a positive correlation of higher CTQ scores with heightened brain response to aversive (but not sexual or food) cues in one large cluster (k>2000) that extended from the anterior orbitofrontal cortex to the nucleus accumbens to the midbrain. Importantly, the clinic (but not the community) sample showed a positive correlation of increased brain response with CTQ scores that mirrored the results from the overall analysis. CONCLUSIONS: Increased prior adversity was associated with an increased brain response to aversive cues in frontolimbic/striatal regions, observed primarily in the clinic sample. These results suggest that the link between prior adversity and a heightened brain sensitivity to later stressful stimuli is strong enough to be detected even when the "stressor" is minimal (33 msec) and entirely outside awareness. The relationship was stronger in the clinic sample, highlighting a potential vulnerability to stressors in this disadvantaged group, while simultaneously offering a potential brain target for screening pharmacotherapeutic or behavioral interventions.

Disclosures: P.S. Regier: None. **A.M. Teitelman:** None. **K. Jagannathan:** None. **A.R. Childress:** None.

Poster

322. Stress and Cognition: Clinical Studies

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 322.13/KK26

Topic: F.04. Stress and the Brain

Support: BGN - Berufsgenossenschaft Nahrungsmittel und Gastgewerbe

Title: Magnetoencephalographic and cardiac parameters are changed in employees with permanent hearing loss - A quantitative analysis in elderly workers

Authors: R. HUONKER, J. MÜLLER, J. LUKAJEWSKI, P. JAUER, O. W. WITTE, *F. RICHTER

Univ. Hosp. Jena, Jena, Germany

Abstract: In Germany estimated 4 to 5 million employees work in jobs with hearing impairing noise exposure. Noise induced hearing loss is the second most common work-related disease. In hearing impaired people auditory recognition is worsened and listening effort is enhanced resulting in higher mental load. We assume that this induces not only psychical but also physical stress that could be analyzed qualitatively and quantitatively either by questionnaires or by recording of magnetoencephalographic (MEG) activity and of vegetative and cardiac parameters. Our two groups of probands worked either in the beverage industry or in symphonic orchestras. One control group (16 normal hearing workers, mean age 59 years) and two groups (13 participants, mean age 56 years) with moderate hearing impairments (average 45 dB SPL) in the frequency range of 3-4 kHz were stimulated with tone pips at 65 dB SPL with different pitches overlaid by random noise. In a "one back task" the subjects had to decide whether the next to last pip had a higher or lower frequency than the last pip, and correct responses were rewarded. Two different stimulus conditions were presented, either at a constant pitch difference or with pitch difference adjusted to the actual error rate. We recorded the MEG and auditory evoked magnetic fields for analyzing the distribution of the sources, the electrocardiogram, arterial oxygen saturation and breathing frequency. From the electrocardiogram we calculated the square root of the mean squared differences of successive heart beat intervals (RMSSD) and the HF-power as parameters of heart rate variability. The discrimination threshold in the control group was significantly lower (122 Hz) compared to the hearing impaired group (300 Hz). In both groups alpha activity in the MEG increased by 0.1 to 0.15 Hz towards higher frequencies during the hearing task. However, in the hearing impaired group the alpha activity was higher already at rest (9.5 Hz) than in the control group (9.25 Hz). Heart rate significantly increased during the hearing task in both groups. Heart rate variability and HF-power at rest were significantly lower in the hearing impaired group, and RMSSD and HF-power further decreased during the hearing task, thus indicating an attenuated influence of the parasympathetic nervous system. These data

provide objective evidence that cardiovascular parameters are affected already at rest by the increased mental load and have to be considered in occupational medical care in the prevention of worsening of work-related hearing damages. The long term alterations to the cardiovascular system caused by noise induced hearing damage can be objectified and reflect stress.

Disclosures: R. Huonker: None. **J. Müller:** None. **J. Lukajewski:** None. **P. Jauer:** None. **O.W. Witte:** None. **F. Richter:** None.

Poster

323. Stress and Cognition: Animal Studies

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 323.01/KK27

Topic: F.04. Stress and the Brain

Title: Anxiety/depressive-like behaviors without the change of the HPA axis by mild stress can be improved treadmill exercise in rats

Authors: *K. ISHIDA¹, K. KOIKE², A. MARUYAMA¹, Y. UENISHI¹, T. GYOUDA¹, Y. SUGIYAMA¹

¹Nagoya Univ. Grad. Sch. of Med., Nagoya, Japan; ²Saarland Univ., Homburg, Germany

Abstract: The onset or development of depression could be caused by chronic stress. While exercise has stress-buffering effects, the therapeutic effect of exercise against anxiety/depressivelike behaviors is not fully understood. The objectives of this study were, at first, confirm that chronic forced swim stress induces anxiety/depressive-like behaviors after stress exposure, and then investigate whether treadmill exercise alleviates the anxiety/depressive-like behaviors induced by forced swim stress through the alteration of hypothalamic-pituary-adrenal (HPA) axis response. It has been found that forced swim stress exposure as rats were placed in a glass cylinder (35 cm high \times 24 cm diameter) filled to a depth of 25 cm with water (25 \pm 1 degrees). A 6-min test was repeated each day, induced abnormal behaviors including forced swim 21 days after stress exposure using forced swim test, open field test and elevated open-platform test. These abnormalities were inhibited by repeated administration of the antidepressant, imipramine, suggesting that these behaviors are anxiety/depressive behaviors. Moreover, treadmill exercise ameliorated depressive behaviors induced by chronic forced swim stress. Interestingly, there was no significant difference of the HPA axis response including serum corticosterone level among groups. These data suggest that the treatment effect of exercise may be not through the alteration of the HPA axis. These results also suggest that the HPA axis changes may be not as responsive as behavioral measures. Further research is needed to explore the mechanism of chronic forced swim stress. In summary, forced swim stress induces anxiety/depression-like behaviors without the change of the HPA axis. Moreover, treadmill exercise ameliorates anxiety/depression-like behaviors induced by chronic forced swim stress not affecting on the HPA axis response. These

data suggest that treadmill exercise may rescue anxiety/depression-like behaviors without the HPA axis in mild stress rat model.

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Poster

323. Stress and Cognition: Animal Studies

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 323.02/KK28

Topic: F.04. Stress and the Brain

Title: Susceptibility to post-traumatic stress disorder is comorbid with reduced mitochondrial capacity in mice

Authors: *G. PRESTON¹, T. EMMERZAAL², F. KIRDAR¹, E. MORAVA-KOZICZ¹, T. L. KOZICZ²

¹Tulane Univ. Sch. of Med., New Orleans, LA; ²Anat., Radboud Univ. Nijmegen Med. Ctr., Nijmegen, Netherlands

Abstract: Psychopathology is frequently associated with mitochondrial dysfunction in patients. Post-traumatic stress disorder (PTSD) is a debilitating psychiatric disorder that affects millions of people worldwide. Induced by exposure to a traumatic event, the presentation of the disease in terms of duration and symptomatology is highly heterogenous, and the severity of the inducing trauma does not predict the severity of the disorder. This suggests the possibility of an underlying genetic metabolic factor. We therefore investigated whether susceptibility to PTSD is associated with mitochondrial dysfunction. We exposed 48 wildtype male mice of the FVB.129P2 ("sighted" FVB) background to a PTSD-induction paradigm previously shown to reliably induce PTSD-like symptomatology with a similar presentation to that of PTSD in humans. The inducing trauma was inescapable electric foot shock, administered in two decontextualized sessions over two consecutive days. PTSD-susceptible animals were diagnosed through a series of behavioral tests to identify some physical symptoms of PTSD: hyperarousal, hypervigilance, and sleep cycle disruption. Those 12 animals most and least clearly displaying PTSD-like symptomatology were identified, brain tissue was collected, and mitochondria were isolated. The activities of the electron transport chain (ETC) complexes I, II, III and IV, as well as citrate synthase, were measured by spectrophotometric assay using a Konelab autoanalyzer to determine mitochondrial activity and density, respectively. Comparing ETC activity and mitochondrial density to PTSD-like symptomatology reveals a statistically-significant inverse relationship between PTSD susceptibility and mitochondrial capacity (p=0.016). Our results indicate that susceptibility to PTSD in mice is comorbid with reduced mitochondrial capacity. To demonstrate that mitochondrial dysfunction is sufficient to induce PTSD susceptibility, we are

currently inducing PTSD in a strain of transgenic mice with genetically-induced suboptimal mitochondrial function, and assaying for a concordant increase in PTSD susceptibility. We are also investigating how reduced mitochondrial capacity affects activation of large scale brain networks and endocrine functions involved in stress response.

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Poster

323. Stress and Cognition: Animal Studies

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Topic: F.04. Stress and the Brain

Support: The College of Liberal Arts and Sciences

The National Science Foundation Graduate Research Fellowship Program (DGE-1311230, Ortiz)

Title: Antagonizing the GABA_A receptor during behavioral testing improves spatial memory in chronically stressed rats

Authors: *K. NISHIMURA, J. B. ORTIZ, C. D. CONRAD

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Abstract: Chronic stress leads to a dysregulated hippocampal inhibitory tone that may contribute to poor hippocampal-dependent spatial learning and memory. The present study examined whether the spatial memory deficits that occur following chronic stress could be overcome by antagonizing the γ -aminobutryic acid (GABA_A) receptors, a prominent inhibitory receptor for GABA in the hippocampus. Young adult male Sprague-Dawley rats were chronically stressed (STR, wire mesh restraint, 6h/d/21d) or assigned to a non-stressed control group (CON). When chronic restraint ended, rats were tested on a 2-trial object placement (OP) task at a challenging delay (3hr) that would result in chance performance without intervention. Rats were also tested over the next few days on an object recognition task to measure non-spatial memory and the elevated plus maze (EPM) to assess anxiety profile. In CON rats, injections of bicuculline (BIC, 0, 0.25, 0.5 mg/kg, i.p.) 30 min prior to testing, facilitated object placement at both the 0.25 and 0.5 mg/kg doses. In contrast, STR rats required the highest dose (0.5 mg/kg) of BIC to improve their performance on the object placement task, as the lower dose (0.25 mg/kg) and vehicle were ineffective. Motivation to explore the objects and anxiety profile were unlikely to explain these results, based upon the performances on object recognition and EPM. These findings reveal different dose response functions for BIC in control and chronically stressed rats, with

chronically stressed rats requiring a higher dose of BIC to achieve spatial memory ability as observed in the controls. While the literature demonstrates that chronic stress disrupts hippocampal inhibitory tone, the current study reveals that a single injection of a GABA_A receptor antagonist can restore hippocampal-dependent spatial memory in chronically stressed subjects.

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Poster

323. Stress and Cognition: Animal Studies

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Topic: F.04. Stress and the Brain

Support: CIHR

Title: Sex differences in the role of adult neurogenesis in the development of learning and memory dysfunction following chronic stress

Authors: *T. P. O'LEARY¹, B. LEE², D. ESPINUEVA¹, J. S. SNYDER¹ ²Psychology, ¹Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Adult neurogenesis in the hippocampus is involved in visuo-spatial learning and memory, and also regulation of the hypothamic-pituitary-adrenal axis and stress-related behaviour. Although, neurogenesis has been shown to buffer the effects of chronic stress on expression of depressive-like behaviours, it is not yet known if adult neurogenesis acts in a similar way to buffer negative effects of chronic stress on learning and memory function. Sex differences exist in the effects of chronic stress on learning and memory, and thus the role of adult neurogenesis during chronic stress likely differs between male and females. To address this possibility, we used a transgenic rat that expresses the herpes simplex virus thymidine kinase, under the glial fibrillary acidic protein promoter (GFAP-TK). Administration of the anti-viral drug valganciclovir leads to death of mitotic neural precursor cells in the GFAP-TK rat. Beginning at 6 weeks of age, GFAP-TK rats were given valganciclovir orally for 6 weeks (twice per week), which leads to a near-complete ablation of adult-neurogenesis in the dentate gyrus. At 13 weeks of age, rats then completed 21 days of chronic restraint stress, with 6 hours of daily restraint stress. Following restraint stress, visuo-spatial learning and memory was assessed with a modified Morris water maze procedure, in which stress during testing was increased using cold 16 °C water. Rats completed acquisition training for 3 days (4 trials per day) with a probe trial (60 sec) on the following day to assess memory for the escape platform location. Typical effects of chronic stress were observed, with reduced body-weight gain in restrained rats, and also increased adrenal weight in restrained male, but not female rats. Restraint stress did not impair

learning and memory ability in either GFAP-TK or wild-type male rats. In female rats, however, restraint stress improved learning performance in wild-type rats. In female GFAP-TK rats, learning performance of restrained and control rats was similar, but in the probe trial restrained GFAP-TK rats performed worse than controls. These results indicate that the chronic restraint stress paradigm used was not sufficient to impair performance in wild-type male and female rats. In GFAP-TK rats, however, restraint stress impaired memory performance in female rats, suggesting that neurogenesis may buffer the deleterious effects of chronic stress on memory ability specifically in females. These findings may indicate a sexually dimorphic role for adult neurogenesis in the etiology of cognitive dysfunction within depression and stress-related disorders.

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Poster

323. Stress and Cognition: Animal Studies

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Topic: F.04. Stress and the Brain

Support: 601152N.0000.000.A1308

Title: Method for affective profiling for anxiety-like behavior in rodent model

Authors: J. STATZ¹, R. MCCARRON², J. GOODRICH¹, S. L. CIARLONE¹, M. L. MEHALICK², S. T. AHLERS³, P. B. WALKER², *A. E. TSCHIFFELY⁴ ¹Naval Med. Res. Center, HJF, Silver Spring, MD; ²Neurotrauma, ⁴Dept. of NeuroTrauma, ³Naval Med. Res. Ctr., Silver Spring, MD

Abstract: Traumatic brain injury (TBI) is a frequent injury in military conflicts, often caused by exposure to overpressure (shock) waves from devices such as improvised explosive devices. Studies indicate high rates of co-morbid mTBI and posttraumatic stress disorder (PTSD). Specifically, Ahlers and colleagues (2012) demonstrated blast-exposed rats display PTSD-like behavior, indicating possible relationships between blast and PTSD development. Moreover, both stathmin and corticosterone levels are increased post-blast in the rodent brain and serum, respectively. These markers typically indicate increased anxiety and are altered as a result of both fear and stress responses. However, not every blast-exposed subject will display anxiety-like behavior and may display phenotypes similar to controls. Therefore, including animals from experimental groups that do not appear anxious may obscure results that would demonstrate differences due to blast. To account for this, normal and abnormal behaviors can be determined from the control group, and the experimental group can be split into those displaying normal behaviors ("unaffected") and anxiety-like behaviors ("affected"). These subgroups can then be

compared separately to controls for a better understanding of changes that blast exposure may cause in behavior and molecular variables. Data sets consist of molecular variables (e.g., blood or protein biomarkers) and behavioral data that identify the "affected" group. Using an affective profiling approach introduced by Richter-Levin and his colleagues (2015), control animal behavioral data was analyzed to determine boundary values for normal behavioral results. These boundary values were then applied to the experimental group's behavioral data, and animals whose behavior fell outside this range were separated into an "affected" experimental group; remaining animals were labeled "blast-unaffected". Using these procedures, data for molecular variables were then compared. When this method was used on test data containing behavioral data from an elevated zero maze and stathmin and corticosterone levels, clear differences in the average values between "affected" and "unaffected" groups were present. This method shows promise as another method to study effects of blast, while accounting for animals whose data may obscure results due to similarities to control animals.

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Poster

323. Stress and Cognition: Animal Studies

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Topic: F.04. Stress and the Brain

Support: NIH Grant NS090283

Brain & Behav Foundation

Title: Stress-induced deficits in latent inhibition in GDNF-deficient mice

Authors: *C. K. BROWN, C. V. BUHUSI, M. BUHUSI Dept. Psychology, Utah State Univ., Logan, UT

Abstract: Stress may induce adaptive or maladaptive organismal responses depending on the individual's genetic makeup. Glial-derived neurotrophic factor (GDNF), a growth factor supporting the survival and physiology of dopaminergic neurons, is upregulated in response to stress and is essential for adaptive brain responses to chronic stress. Heterozygous GDNF mice, which have reduced GDNF expression, and wild-type littermates were tested for learning and attention impairments under standard experimental conditions and after a chronic stress regimen. Freezing behavior in response to pre-exposed and non-pre-exposed stimuli paired with foot shocks was analyzed to assess latent inhibition, as a measure of selective attention. Analyses indicated a main effect of pre-exposure (F(1,47)=59.89, p<0.01), stress (F(1,47)=4.64, p<0.05),
and a pre-exposure x stress interaction (F(1,47)=6.35, p<0.05). Post-hoc analyses showed that all unstressed mice as well as the wild-type stressed mice showed latent inhibition (p<0.05); in contrast, GDNF-deficient mice did not show latent inhibition after chronic stress (p>0.05). Immunohistochemical staining procedures were used to evaluate neuronal activation in brain regions relevant to latent inhibition, and revealed significant differences between GDNF-deficient mice and controls in the prefrontal cortex and nucleus accumbens. Our results support the role of chronic stress in triggering maladaptive brain responses in vulnerable individuals, with important implications for the onset of psychiatric disorders such as schizophrenia or PTSD.

Disclosures: C.K. Brown: None. C.V. Buhusi: None. M. Buhusi: None.

Poster

323. Stress and Cognition: Animal Studies

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Title: The pregnane x receptor is implicated in the therapeutic mechanism of finasteride

Authors: *L. J. MOSHER^{1,2}, J. L. STAUDINGER², M. BORTOLATO¹ ¹Pharmacol. and Toxicology, Univ. of Utah, Salt Lake City, UT; ²Pharmacol. and Toxicology, Univ. of Kansas, Lawrence, KS

Abstract: Tourette syndrome (TS) is a neurodevelopmental disorder characterized by recurring motor and phonic tics, which are preceded by a sense of discomfort known as a premonitory urge. TS symptoms are contributed to by an inability of the brain to filter out irrelevant sensory information. As such, these patients have deficits in sensorimotor gating processes, which aim to discriminate between relevant and irrelevant stimuli and can be measured by prepulse inhibition (PPI) of the startle reflex. TS symptoms are underpinned in part by hyperdopaminergic systems within the cortico-striato-thalamo-cortical loop. In accordance, dopamine (DA) agonists have been extensively studied in preclinical models of TS to replicate various aspects of the disorder. For example, D1 receptor agonists induce PPI deficits and stereotyped behaviors in mice. Current therapies for TS are often not effective or are associated with side effects that decrease patient compliance. To address this issue, our lab has been studying the contribution of

neurosteroids (NS) as potential therapeutic candidates. Converging evidence supports a role for NS in modulating the DA system and clinical data supports the hypothesis that NS contribute to TS pathogenesis and symptom fluctuations. One of the most important clinical observations in support of this role is the heightened stress sensitivity observed in these patients, which implicates allopregnanolone (AP). We have previously demonstrated that both stress and systemic AP administration exacerbate TS like symptoms, including PPI deficits, in animal models. In addition, we have also shown that blocking the production of AP with the 5α -reductase inhibitor finasteride alleviates symptoms in both adult males with TS and ablates PPI deficits induced by D1 receptor agonists in mice. However, the contributing molecular mechanisms of these findings remain unclear.

Previous preliminary research from our lab has indicated that the mechanism of AP does not directly involve the GABA_A receptor so we have expanded our studies to other potential receptors. Recent data has demonstrated that AP can bind to the pregnane X receptor (PXR) and has highlighted a function for this receptor in DA-AP interactions within the brain. We found that mice deficient of PXR are resistant to AP (15mg/kg)-induced PPI deficits. In addition, we found that finasteride (50mg/kg) is not effective in these mice to ablate the PPI deficits induced by the D1 agonist SKF 82958 (0.3mg/kg). These data suggest that PXR mediates the effects of AP in the regulation of sensorimotor gating; however, more research is needed to determine how this receptor modulates DA signaling.

Disclosures: L.J. Mosher: None. J.L. Staudinger: None. M. Bortolato: None.

Poster

323. Stress and Cognition: Animal Studies

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 323.08/KK34

Topic: F.04. Stress and the Brain

Title: Altered stress adaptation, glucose metabolism, and increased susceptibility for mood disorders in a mouse model with decreased mitochondrial complex I function

Authors: *T. L. EMMERZAAL¹, E. VASILEIOU¹, B. GEENEN¹, K. SCOTT², B. H. GRAHAM³, W. J. CRAIGEN³, E. MORAVA², R. RODENBURG¹, T. L. KOZICZ⁴ ¹Radboudumc, Nijmegen, Netherlands; ²Tulane Univ., New Orleans, LA; ³Baylor Col. of Med., Houston, TX; ⁴Anat., Radboud Univ. Nijmegen Med. Ctr., Nijmegen, Netherlands

Abstract: An increasing body of evidence points toward the involvement of suboptimal mitochondrial function (SMF) in depression and other mood disorders. Several clinical and preclinical studies show that depression is associated with altered mitochondrial structure and function. Furthermore, stress, a major risk factor for depression, directly influences mitochondrial function. There is a surprising high association between mitochondrial disease and

depression as well, which is estimated to be around 50%. This is significantly higher than the expected 16% depression prevalence in the general population. These findings suggest that SMF is causal in depression, however, this notion has not been substantiated yet. In this study, we tested the hypothesis that SMF influences the animals' stress response and impacts various biological domains linked to the pathobiology of depression. To test this hypothesis, a new genetically engineered mouse model was used. These animals have a deficiency of the NDUFS4 protein (Ndufs4def mice), a structural protein of complex I (CI), an essential component of the electron transport chain and oxidative phosphorylation. This deficiency leads to a 25% reduction of CI activity in the brain compared to WT mice. Despite this reduction, Ndufs4def mice exhibited no differences in body weight and temperature, physical activity (distanced travelled and velocity in the open filed), and motor coordination (Rotarod-test) compared to their WT littermates. After exposure to a chronic variable stress protocol, a well-validated animal model for depression, Ndufs4def mice showed increased anxiety like behavior (open field) as well as a disturbed day-night rhythm compared to WT animals, a symptom that can also be seen in individuals with depression. Furthermore, preliminary results suggest an altered glucose metabolism and stress response in the Ndufs4def mice. To provide mechanistic insights, we assessed the activation of brain nodes implicated in the pathobiology of depression. The expression of the protein FOS, a surrogate marker of neuronal activation, revealed distinct activation of several brain regions of WT and Ndufs4def mice. These results indicate a difference in functional coupling within and an altered balance between major brain networks, a well-established phenomenon in individuals with depression. In conclusion, here we report on distinct chronic stress-evoked responses in various biological domains in Ndusf4def and WT mice, supporting our hypothesis that suboptimal mitochondrial function mediates the impact of stress on mental health.

Disclosures: T.L. Emmerzaal: None. E. Vasileiou: None. B. Geenen: None. K. Scott: None. B.H. Graham: None. W.J. Craigen: None. E. Morava: None. R. Rodenburg: None. T.L. Kozicz: None.

Poster

323. Stress and Cognition: Animal Studies

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 323.09/KK35

Topic: F.04. Stress and the Brain

Support: NSFCAREERIOS1552416

Title: Corticotropin releasing factor in the medial septum impairs spatial learning in rats

Authors: *K. WIERSIELIS, M. SALVATORE, H. LEFEBO, H. JANG, A. CERETTI, V. CANTORAL, D. BANGASSER Temple Univ., Philadelphia, PA

Abstract: Stress can disrupt a variety of cognitive processes, including learning and memory. Previous studies in rodents have demonstrated that central infusions of the stress-neuropeptide, corticotropin releasing factor (CRF), can disrupt mnemonic processes. However, where CRF is working within the brain to regulate cognition is largely underexplored. A candidate region for direct CRF regulation is the medial septum (MS), because this forebrain cholinergic nucleus is critical for spatial learning and CRF receptors are found on cholinergic neurons therein. Here we assessed whether administering CRF directly into the MS impaired spatial learning in male and female rats. Specifically, we infused different doses of CRF or vehicle into the MS prior to testing on an object location task, which tests spatial learning, and a novel object recognition task, which does not test spatial learning. On the object location task, we found that CRF in the MS reduced time spent exploring the displaced object compared to the familiar object, suggesting that this manipulation impairs spatial learning. In addition, males were more sensitive to this effect than females, such that a low dose of CRF in the MS that had no effect in females disrupted object location learning in males. In the novel object recognition task, CRF in the MS did not decrease preference for the novel object in either sex, suggesting that the effects of CRF in the MS are specific to spatial learning. Future experiments will examine the influence of circulating ovarian hormones in regulating sensitivity of the MS to CRF. Collectively, these studies reveal that CRF in the MS selectively impairs spatial learning, especially in males, highlighting an unexplored mechanism by which stress can regulate cognition. Clinically, these findings suggest that drugs which block the effects of CRF represent a viable therapeutic option to treat cognitive deficits that characterize certain stress-related psychiatric disorders.

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Poster

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Program#/Poster#: 323.10/KK36

Topic: F.04. Stress and the Brain

Support: NSFCAREERIOS1552416

Title: Chronic stress regulation of sustained attention and cholinergic dendritic morphology in rats

Authors: *A. TELENSON¹, B. WICKS², J. BERGMANN³, M. SHORE³, N. NEWCAMP³, A. CERETTI³, S. ECK³, A. HALL³, K. WIERSIELIS⁴, J. TUCCI³, D. A. BANGASSER⁵ ¹Psychology, ²Psychology and Neuroscienc Program, Temple Univ., Philadelphia, PA; ³Temple Univ., Philadelphia, PA; ⁵Dept. of Psychology and Neurosci. Program, ⁴Temple Univ., Philadelphia, PA

Abstract: Chronic stress can impair attention and exacerbate symptoms in disorders with cognitive deficits as a key feature, including schizophrenia, attention deficit hyperactivity disorder, and bipolar disease. However, the neurobiological mechanisms by which chronic stress disrupts attention remain largely unknown. Using a 6 day chronic variable stress procedure for rats, we tested how chronic stress affects attention and neurons critical for attentional processes. To test attention, we used a sustained attention task, where rats are trained to distinguish signal from non-signal trials. We found that chronic variable stress impaired performance on signal trials, especially in male rats when attentional demands were high. It has been previously shown that accuracy on signal trials require cholinergic neurons in the nucleus basalis of Meynert (NBM) in the basal forebrain. In other brain regions, such as the amygdala and hippocampus, chronic stress is thought to affect cognition by altering the morphology of dendrites on neurons within these regions. We wanted to determine whether chronic stress alters the morphology of dendrites on NMB cholinergic neurons. To address this question, we developed an innovative approach to label cholinergic neurons in rats by virally injecting a Cre-dependent fluorescent marker into the NBM region of rats genetically modified to express Cre-recombinase under control of the choline acetyltransferase promoter. Using this approach, our preliminary data is finding that chronic variable stress expands the morphology of NBM cholinergic dendrites, particularly at regions distal from the cell body, in male rats. Future studies are planned to test for similar morphological changes in female rats. This is the first study, to our knowledge, suggesting that chronic stress can induce hypertrophy of cholinergic dendrites. If supported with more subjects, this result would suggest that chronic stress impairs attention by affecting inputs into the NBM via the regulation of the morphology of cholinergic dendrites.

Disclosures: A. Telenson: None. B. Wicks: None. J. Bergmann: None. M. Shore: None. N. Newcamp: None. A. Ceretti: None. S. Eck: None. A. Hall: None. K. Wiersielis: None. J. Tucci: None. D.A. Bangasser: None.

Poster

323. Stress and Cognition: Animal Studies

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Program#/Poster#: 323.11/LL1

Topic: F.04. Stress and the Brain

Support: NSF CAREER IOS-1552416

Title: The effects of corticotropin releasing factor in the nucleus basalis of meynert on sustained attention in male and female rats

Authors: *S. ECK, B. WICKS, N. DUNCAN, M. SALVATORE, S. COHEN, J. BERGMANN, A. CERETTI, A. HALL, D. BANGASSER Temple Univ., Philadelphia, PA

Abstract: Attention is disrupted in a variety of disorders, including schizophrenia, attention deficit hyperactivity disorder, and post-traumatic stress disorder. One characteristic that all of these disorders share is their sensitivity to stress. Despite these connections, the neurobiological mechanism by which stress modulates attentional processes remains largely underexplored. We aim to fill this gap in the literature by studying the effects of corticotropin releasing factor (CRF) on performance on a sustained attention task (SAT) in rats. In this task, rats are trained to discriminate signal from non-signal trials. Previously, our lab has shown that central administration of CRF causes a dose-dependent impairment in performance on SAT in both male and female rats. Now, we are trying to determine where exactly in the brain CRF is exerting this effect. Previous studies have shown that accurate performance on signal trials depends on cholinergic neurons in the nucleus basalis of Meynert (NBM) of the basal forebrain, while accurate performance on non-signal trials is linked to GABAergic neurons in the NBM. We have shown that CRF receptors are present on both cholinergic and GABAergic neurons in this brain area, making the NBM a likely target for CRF's modulation of sustained attention. To test this idea, we infused CRF into the NBM and examined its effect on SAT. We found that intra-NBM infusions impaired performance on non-signal trials, but not on signal trials, suggesting that CRF in the NBM is altering the function of GABAergic neurons. Interestingly, the effect of CRF in the NBM appears to be more pronounced in males, suggesting a sex difference in CRF sensitivity in this region. Collectively, these results implicate novel CRF-GABA interactions in the NBM, especially in males. Moreover, these data suggest that blocking CRF signaling may be a viable therapeutic option for treating the stress-related attention impairments of various disorders.

Disclosures: S. Eck: None. B. Wicks: None. N. Duncan: None. M. Salvatore: None. S. Cohen: None. J. Bergmann: None. A. Ceretti: None. A. Hall: None. D. Bangasser: None.

Poster

323. Stress and Cognition: Animal Studies

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Topic: F.04. Stress and the Brain

Support: NIH MH092438

NSF CAREER IOS 1552416

Title: Corticotropin releasing factor differentially activates brain networks in males and females

Authors: *M. SALVATORE¹, K. WIERSIELIS², D. E. WAXLER³, D. A. BANGASSER⁴ ¹Psychology, Temple University, Philadelphia, PA; ³Dept of Psychology, ⁴Dept. of Psychology and Neurosci. Program, ²Temple Univ., Philadelphia, PA

Abstract: Women are more likely than men to suffer from psychiatric disorders that have hypersecretion of the stress neuropeptide corticotropin releasing factor (CRF) as an etiological factor, suggesting sex differences in CRF sensitivity. In rodents, sex differences in sensitivity of specific brain regions to CRF have been identified. However, regions do not work in isolation, but rather form circuits to coordinate distinct responses to stress. Here we examined whether CRF activates different circuits in male and female rats. Following central administration of CRF or vehicle, brain tissue was collected and processed to visualize the immediate early gene cFOS in stress-related areas known to contain a high density of CRF receptors. Neuronal activation in these areas was then assessed by quantifying cFOS positive cells. Functional connectivity was gauged by correlating cFOS profiles between regions and then identifying differences in correlations for aCSF-treated and CRF-treated groups. This analysis revealed baseline differences in connectivity between males and females, where activation was more highly correlated between brain regions in females than in males. Additionally, analyses showed that CRF altered the functional connectivity of different circuits in males and females. For example, CRF altered correlations with the dorsal raphe (DR) in males and correlations with the bed nucleus of the stria terminalis (BNST) in females, suggesting sex differences in stress-activated circuits controlling mood and anxiety. This study reveals sex differences in the way brain regions work together in response to CRF. These differences could drive different stress coping strategies in males and females, perhaps contributing to sex biases in psychopathology.

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Poster

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Support: R01 MH050479 (SFM)

R21 MH106817 (MVB)

T32 MH016880 (SDD)

NARSAD Young Investigator Grant (MVB)

Title: Evaluating stressor controllability effects in female rats

Authors: *I. P. FALLON¹, M. V. BARATTA¹, N. R. LESLIE¹, S. D. DOLZANI^{1,2}, L. E. CHUN¹, A. M. TAMALUNAS¹, L. R. WATKINS¹, S. F. MAIER¹ ¹Psychology and Neurosci., ²Inst. for Behavioral Genet., Univ. of Colorado Boulder, Boulder, CO

Abstract: The degree of behavioral control a subject (rat to human) can exert over an adverse life event is one of the most potent variables yet discovered that modulates the impact of that event. That is when a subject is provided with a controlling response the behavioral and neurochemical sequelae are blunted or eliminated. Prior work has established that the protective effects of behavioral control are mediated by the prelimbic region (PL) of the medial prefrontal cortex which provides top-down inhibitory regulation of stress-responsive structures such as the serotonergic dorsal raphe nucleus (DRN). However, virtually all the work has been conducted in male subjects. Here we demonstrate that behavioral control in females does not mitigate stress-induced behavioral outcomes (EXP 1) nor DRN activation (EXP 2). Furthermore, behavioral control in females does not engage DRN-projecting PL neurons (EXP 3). Pharmacological activation of the PL restored the stress-buffering effects of control (EXP 4). The present findings suggest that reduced benefit from coping responses may represent a novel approach for understanding differential sex prevalence in stress-related psychiatric disorders.

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Poster

323. Stress and Cognition: Animal Studies

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Programa de Apoyo a Investigación y Posgrado (PAIP), clave: 4500-5000.

Title: Synthesis of 1-N-substituted analogues of melatonin as potential anxiolytic-like compounds

Authors: J. T. MONTIEL-AVILÉS¹, A. ALMARÁZ-SÁNCHEZ¹, *A. S. LIRA-ROCHA², *E.-B. NARANJO-RODRÍGUEZ^{1,3}

¹Lab. of Pharmaceut. Chem., ²Lab. of Neuropharm., Univ. Nacional Autónoma De México,

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Abstract: Anxiety is a normal or adaptive response, as the fight-flight reaction, which prepares the body for an emergency, is presented by different circumstances and clinical manifestations. Its important function is related to survival, fear, anger, sadness or happiness, the treatment of anxiety is with classic anxiolytics such as benzodiazepines (BZP), which, produce several adverse effects. For this reason, new molecules have been synthesized, with lower adverse effects than those BZP. Different groups, designed, synthesized and evaluated the anxiolytic effects of melatonin analogues (MT), in particular, those with a benzyl or phenyl group in position 1 of the indole ring, with good anxiolytic-like effects in rats, these are in function of the substituents. The objectives of this work are the synthesis of a new series of 8 MT analogues, M3A.3, M7A.2, M7A.3, M8A.2, M8A.3, M8A.4, M3B-3 and M3C.3. Whose evaluation was In Vitro, rat isolated tissue and In Vivo, Marble-Buryng Test in mouse. Obtaining then, that the conformational analysis and the incorporation of the substituent in position 1 has no influence and the electronic density on the ring is not altered. In Vitro, MT analogues, produce tissue relaxation. In Vivo, compound M3A.3, shows a good anxiolytic-like effect similar to MT. These data lead us to propose that the strategy of finding prototypes by analogy, provides a greater probability of obtaining substances with the desired pharmacological activity.

Disclosures: J.T. Montiel-Avilés: None. **A. Almaráz-Sánchez:** None. **A.S. Lira-Rocha:** None. **E. Naranjo-Rodríguez:** A. Employment/Salary (full or part-time):; Employment. full, National Autonomous University of Mexico, Naranjo-Rodríguez Elia-Brosla, Employment. full, National Autonomous University of Mexico, Lira-Rocha Alfonso-Sebastián. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Contracted Research, National Autonomous University of Mexico., Montiel-Avilés Jesica Talía, Contracted Research, National Autonomous University of Mexico., Almaráz-Sánchez Aarón.

Poster

323. Stress and Cognition: Animal Studies

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 323.15/LL5

Topic: F.04. Stress and the Brain

Title: Behavioral and neurobiological effects of social housing conditions on male Long-Evans rats: Elevated plus-maze and open field

Authors: M. E. HASTINGS¹, R. M. CAIN¹, M. L. HOLLAND¹, R. J. STAMM¹, J. A. WILLNER¹, D. M. HAYES², *P. A. JACKSON¹ ²Psychology, ¹Radford Univ., Radford, VA

Abstract: Many research scenarios require animals to be singly housed for collection of individual data. However, rats are naturally social animals and thrive when allowed to engage in stimulating species-specific behaviors such as burrowing in bedding, mutual grooming, and wrestling. Animal welfare concerns suggest that rats should not be housed individually or without bedding due to known behavioral alterations, as well as increased levels of the stress hormone corticosterone. To address these concerns, new housing methods are being developed that allow for moderate social interaction without compromising the integrity of data collection. Specifically, barrier style housing utilizing a perforated cage divider allows rats to be housed in a social, but separated, manner. In this form of housing, rats can see, smell, and touch one another, but cannot have full body contact. The current study examined how this separated style of housing affected behavior and stress physiology in adult, male Long-Evans rats compared with three other common styles of housing. These included a social condition where rats were housed in pairs with bedding, and two solitary conditions where rats were housed individually either with bedding or with a wire floor. Each animal spent four weeks in its respective housing condition before behavioral testing in an elevated plus-maze (EPM) and open field (OF) apparatus to measure anxiety and locomotor activity. Rats were also tested in the Morris Water Maze (see Cain et al.). Initial analysis of data from the EPM suggested no significant differences in percent time spent in the open arms of the apparatus, but there was a trend towards increased activity in animals in the solitary-wire floor condition. Additionally, initial analysis of data from the OF suggested no significant differences in distance traveled or time spent in the center of the open field. After behavioral testing, rats were sacrificed and plasma samples, isolated from trunk blood, were collected for corticosterone analysis via enzyme-linked immunosorbent assay (ELISA). Results from this study should provide new insight into the efficacy of barrier style housing as an alternative to previous solitary housing methods.

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Poster

323. Stress and Cognition: Animal Studies

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Topic: F.04. Stress and the Brain

Title: Behavioral and neurobiological effects of social housing conditions on male Long-Evans rats: Morris water maze

Authors: R. M. CAIN, M. E. HASTINGS, M. M. GALLAGHER, J. A. SAAD, J. A. WILLNER, P. A. JACKSON, *D. M. HAYES Psychology, Radford Univ., Radford, VA

Abstract: Rats are social creatures and emerging evidence suggests that they should not be housed individually during research due to elevated stress levels, increased non-stereotypic behaviors, lack of stimulation, disturbances in sleep patterns, alterations in feeding habits, potential vulnerability to disease, and a risk to overall wellbeing. Despite their social nature, research protocols often require single housing of rats, to collect individual data or samples. New caging scenarios that allow for more social interaction without compromising data collection would therefore be useful. One such scenario utilizes a barrier through which rats can partially interact with one another (see, smell, and touch) while remaining physically separated. The current study compared this new housing condition to three other commonly used housing practices (social, solitary-bedding, and solitary-wire floor) to examine effects on spatial learning and hippocampal neurogenesis in adult, male, Long-Evans rats. Importantly, elevated stress levels are associated with decreased neurogenesis; the production of new cells in the brain. Neurogenesis involves four stages in various regions of the brain: proliferation, differentiation, maturation, and integration. In the hippocampus, neurogenesis occurs in the dentate gyrus, a region involved in spatial learning and memory. After being in the housing conditions for four weeks, animals were tested on a battery of behavioral tests (see Hastings et al.) culminating with the Morris Water Maze (MWM) task to assess various domains of spatial ability. There were no main effects of housing on latency to platform or percent time spent in thigmotaxis during the acquisition phase of the MWM task. Similarly, no differences were found for percent time spent in target quadrant during a probe trial of the MWM task. These preliminary findings suggest no differences in spatial memory across the housing conditions. After behavioral testing, brain tissue was extracted in preparation for sectioning and staining for expression of Ki67, an endogenous marker for cell proliferation. It is expected that animals housed in the solitary conditions will exhibit lower levels of cell proliferation in the hippocampus than animals in the social or separated conditions. Additionally, it is expected that the animals in the new housing condition will exhibit similar levels of cell proliferation as animals housed socially. The results of this study may be useful for making future animal husbandry decisions in research settings.

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Poster

323. Stress and Cognition: Animal Studies

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Topic: F.04. Stress and the Brain

Support: NIH Grant DK090823

Title: Sex differences in HPA and metabolic responses to early life stress in rats

Authors: *H. SHI

Miami Univ., Oxford, OH

Abstract: Sex differences exist in the regulation of energy homeostasis and the HPA axis. Stress during early life causes dysregulation of the HPA axis and metabolism. We hypothesized that chronic stress during adolescence differentially affected energy homeostasis and the stress response in male and female rats, which could extend to adulthood. Randomized, daily one-hour restraint stress was employed in 12 male and 12 female Sprague Dawley rats, starting on postnatal day (PD) 32 and lasting through PD 44. Age and metabolic profile-matched control groups (12 males and 12 females) received the same procedures as the stress groups but did not undergo restraint stress. Rats were either terminated an hour following stress on PD 44, or 20 days following stress on PD 64 by decapitation, and trunk blood samples were taken for the measurements of plasma estradiol (E2) and testosterone (T). Circulating levels of corticosterone (CORT), an indicator of HPA axis activation, was measured using serial blood samples collected before, during, and after stress or non-stress conditions. Body weight (BW), food intake (FI), and body composition were measured throughout the experiment. Both stress male and female rats significantly reduced BW and FI during the stress period. In males the degree of reduced FI was equal to and thus accounted for their reduced BW; however, in females the reduced FI did not fully account for their reduced BW. Additionally male reduction of BW, fat, and lean mass extended to adulthood even after stress was no longer present. On the first day of chronic stress, both males and females had increased CORT levels in response to stress. Stress males sustained an elevated CORT level throughout stress, whereas stress female CORT level returned to that of controls, indicating adaption to the stressor. E2 levels were similar among male groups, but were lower in stress females compared to control females during adolescence, and this E2 difference extended to adulthood. T levels were similar among female groups, but reduced in stress males compared to control males during adolescence; however, such difference did not extend to adulthood. These results suggest that when compared to females, male rats exposed to early life stress exhibit an increased sensitivity to metabolic disturbances that continue to adulthood, most likely due to HPA axis dysregulation. Further insights as to how adolescent females are able to protect themselves from stress-induced HPA dysregulation, but not gonadal dysregulation later in life, remain to be seen. Our data provides implications as to how sex differences exist in the adolescent human stress response, with consequences that impact adulthood.

Disclosures: H. Shi: None.

Poster

323. Stress and Cognition: Animal Studies

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Topic: F.04. Stress and the Brain

Support: AFOSR/14RH08COR

Title: Divergence in cognitive performance under chronic stress is associated with the hippocampal whole transcriptomic modification

Authors: *S. H. JUNG¹, M. L. BROWNLOW¹, M. PELLEGRINI², R. JANKORD¹ ¹Applied Neurosci. Br., U.S. Air Force Res. Lab., Columbus, OH; ²Dept. of Molecular, Cell, and Developmental Biol., Univ. of California, Los Angeles, CA

Abstract: Individual susceptibility determines the magnitude of stress effects on cognitive function. The hippocampus, a brain region of memory consolidation, is vulnerable to stressful environments, and the impact of stress on hippocampus may determine individual variability in cognitive performance. Therefore, the purpose of this study was to define the relationship between the divergence in spatial memory performance under chronically unpredictable stress and an associated transcriptomic alternation in hippocampus, the brain region of spatial memory consolidation. Multiple strains of BXD recombinant inbred mice went through a 4-week chronic variable stress paradigm followed by the Morris water maze (MWM) test to assess hippocampaldependent spatial memory performance and grouped animals into low and high performing groups based on the cognitive performance. Using hippocampal whole transcriptome RNAsequencing data, differential expression, PANTHER analysis, WGCNA, Ingenuity's upstream regulator analysis in the Ingenuity Pathway Analysis® and phenotype association analysis were conducted. Our data identified multiple genes and pathways that were significantly associated with chronic stress-associated cognitive modification and the divergence in hippocampal dependent memory performance under chronic stress. Biological pathways associated with memory performance following chronic stress included metabolism, neurotransmitter & receptor regulation, immune response and cellular process. The Ingenuity's upstream regulator analysis identified 247 upstream transcriptional regulators from 16 different molecule types. Transcripts predictive of cognitive performance under high stress included genes that are associated with a high occurrence of Alzheimer's and cognitive impairments (e.g., Ncl, Eno1, Scn9a, Slc19a3, Ncstn, Fos, Eif4h, Copa, etc.). Our results show that the variable effects of chronic stress on the hippocampal transcriptome are related to the ability to complete the MWM task and that the modulations of specific pathways are indicative of hippocampal dependent memory performance. Thus, the divergence in spatial memory performance following chronic stress is related to the unique pattern of gene expression within the hippocampus.

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Poster

323. Stress and Cognition: Animal Studies

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Topic: F.04. Stress and the Brain

Support: NIH Grant R01 MH050479 (SFM)

NIH Grant R21 MH106817 (MVB)

NARSAD Young Investigator Grant (MVB)

Title: Investigating the fear-reducing effects of controllable stress with a robust activity marking system

Authors: *N. R. LESLIE¹, M. V. BARATTA¹, A. T. SØRENSEN², B. J. KEDL¹, L. R. WATKINS¹, Y. LIN³, S. F. MAIER¹

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Abstract: The degree of behavioral control over some aspect of an adverse life event potently modulates the outcome of that event. Prior work has demonstrated that controllable stress impacts subsequent fear-related processes. Behavioral control a) blunts later fear expression; b) facilitates fear extinction; and c) prevents the spontaneous recovery of fear. The fear-reducing effects of behavioral control require activation of the medial prefrontal cortex, specifically the infralimbic region (IL), during both the stress experience (Time A) and later fear testing (Time B), suggesting that control potentiates the IL response to later fear-related stimuli. Here we use a Robust Activity Marking (RAM) system to determine if specific ensembles of neurons within the IL are activated during both Time A and Time B. We used an AAV-based RAM system containing a synthetic activity-regulated promoter that is strongly induced by neuronal activity, and a modified Tet-Off system for temporal control. In EXP1, male rats were injected with AAV-RAM-mKate2 into the IL and maintained on doxycycline-treated chow (Dox) until 96 h before stress treatment. Subjects received controllable, uncontrollable, or no stress, and the IL neuronal ensemble activated by stress exposure was labeled by RAM. Animals were placed back on Dox and subjected to auditory tone conditioning 48 h later. Following the tone test subjects were sacrificed and brain sections were stained for the immediate early gene Fos in order to label IL ensembles triggered by the tone test. Subjects previously exposed to controllable stress

exhibited reduced fear expression to the conditioned tone compared to uncontrollable stress and no stress controls. Correspondingly, co-labeling of RAM (Time A) and Fos (Time B) in the IL was increased by prior behavioral control. Surprisingly, when the above experiment was conducted in females (EXP 2) we found no effect of controllability on fear expression nor IL RAM and Fos co-labeling. These results indicate that, in males, the activation of IL neuron populations during controllable stress may potentiate activity of those same cells during later fear-related stimuli, effectively reducing expression of the fear response. In females the protective effect of behavioral control was absent suggesting that the neural processing of coping responses differs between the sexes.

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Poster

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Topic: F.04. Stress and the Brain

Support: Gildor Chair, Elton Lab, AMN Foundation, ISF, MAFAT

Title: The autism-mutated ADNP is a risk factor for post-traumatic stress: Protection with the regulatory neuropeptide PACAP

Authors: *I. GOZES, S. SRAGOVICH Sackler Sch. Med/Tel Aviv Univ., Tel Aviv, Israel

Abstract: Trauma may occur as a result of life-threatening events such as combat, terrorist attacks, accidents, and natural disasters. Following the stressful event, most survivors of trauma naturally resume their normal lives. However, some trauma victims suffer from long-lasting stress reactions that may worsen over time, and could develop into post-traumatic stress disorder (PTSD).

Activity-dependent neuroprotective protein (ADNP) discovered and first characterized in our laboratory as vital for mammalian brain formation was found to be one of the leading genes mutated *de novo* and causing an autistic syndrome. ADNP is regulated by vasoactive intestinal peptide (VIP), as well as by pituitary adenylate cyclase-activating peptide (PACAP), which change its content toward neuroprotection. In this respect, PACAP was identified as a known master regulator of stress response, and has been tightly associated with PTSD in a sex-dependent manner. Interestingly, the PACAP-regulated ADNP is also sexually dimorphic. Furthermore, children with autism may be at increased risk for both encountering traumatic events and developing traumatic sequelae.

In the current study, the impact of the Adnp genotype and the efficacy of PACAP pre-treatment were tested in a unique mouse model of Adnp-haploinsufficiency subjected to stressful conditions. Mice were pre-treated twice daily with PACAP for one month, followed by a 48 hour period of solitude in a clean cage under constant bright illumination (stressful conditions). Anxiety levels were tested using the elevated plus maze, and complemented by cognition and social activity tests. Our results revealed that impairments displayed in stress-challenged Adnp^{+/-} mice in the novel object recognition and the social memory tests were normalized to the Adnp^{+/+} phenotype by PACAP treatment. Odor discrimination test revealed that the affected olfaction in challenged mice was partially restored by PACAP. The assessment of anxiety-related behavior in the elevated plus maze showed that challenged mice exhibited altered behavior, also normalized by PACAP. Interestingly, significant sex differences were observed with Adnp^{+/-} males more susceptible to stress in the object and social recognition tests.

Our findings suggest a correlation between ADNP levels and PTSD. Thus, low ADNP transcript expression level may indicate a worse response to stressful events, which can be successfully ameliorated by PACAP treatment. Altogether, this could establish ADNP as a possible biomarker, identifying people who are either prone to develop PTSD or already suffering from its symptoms.

Disclosures: I. Gozes: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Coronis Neurosciences. S. Sragovich: None.

Poster

323. Stress and Cognition: Animal Studies

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 323.21/LL11

Topic: F.04. Stress and the Brain

Support: APHIS to GDH

Title: Nosema parasitism in the honey bee brain: A neuroethology approach toward a honey bee stressor implicated in colony losses

Authors: *S. L. GAGE^{1,2}, C. KRAMER³, S. CALLE⁴, A. RODRIGUES⁴, M. CARROLL⁴, M. HEIEN³, G. DEGRANDI-HOFFMAN^{4,2}

¹Carl Hayden Bee Res. Center, USDA-ARS, Tucson, AZ; ²Entomology, ³Chem. and Biochem., Univ. of Arizona, Tucson, AZ; ⁴Carl Hayden Bee Res. Ctr., USDA-ARS, Tucson, AZ

Abstract: *Nosema* sp. is an internal parasite of the honey bee, *Apis mellifera*, and one of the leading contributors to colony losses worldwide. This parasite is found in the honey bee midgut, and has profound consequences on the host's physiology. *Nosema* sp. impairs foraging

performance in honey bees, but whether this is due to effects on the bee's neurobiology is unclear. In these studies, we ask three main questions: (1) Does *Nosema* sp. affect brain physiology, (2) is there a behavioral effect, (3) and if there are neurobiological consequences, can they be mitigated through nutrition? To address these questions, we infected newly emerged bees with *Nosema ceranae* spores. At approximate nurse and forager ages, we measured amino acid and biogenic amine levels in the brain, and evaluated learning and memory with an odorassociative conditioning assay using the proboscis extension reflex. We detected significant effects of *N. ceranae* infection on amino acid concentrations, some of which were age-specific; as well as altered serotonin, octopamine, dopamine, and L-dopa concentrations. In the behavioral assays, nurse-aged bees infected with *N. ceranae* significantly outperformed controls in odor learning and memory—suggestive of precocious foraging; but by forager age, infected bees were slower to learn and showed memory impairment. These findings suggest *N. ceranae* parasitism affects the brain of the honey bee and behavioral tasks may be compromised. These results yield new insights into the host—parasite dynamic of honey bees and *N. ceranae*, as well as the neurochemistry of odor learning and memory in healthy and parasitized individuals.

Disclosures: S.L. Gage: None. C. Kramer: None. S. Calle: None. A. Rodrigues: None. M. Carroll: None. M. Heien: None. G. DeGrandi-Hoffman: None.

Poster

323. Stress and Cognition: Animal Studies

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 323.22/LL12

Topic: F.04. Stress and the Brain

Support: MH-095972

Title: Evidence that chronic stress-induced prefrontal dendritic spine loss and working memory impairment are not sexually-differentiated in adult rats

Authors: *R. M. ANDERSON¹, M. MAHANNA¹, S. ROMIG-MARTIN¹, J. J. RADLEY^{1,2} ¹Psychology Dept, Program in Neurosci., ²Interdisciplinary Dept. of Neurosci., Univ. of Iowa, Iowa City, IA

Abstract: Stress exposure is widely implicated in psychiatric illnesses, although in recent years attention has been directed toward the fact that females show a higher incidence of stress-related affective disorders than males. Hippocampal and prefrontal dysfunction are widely implicated in stress-related disorders, and laboratory rodent models of chronic stress have advanced our understanding of the resultant structural and functional impairments in these cortical regions. Paradoxically, much of the rodent literature shows intact, and in some cases even enhanced cognitive functioning in females following chronic stress. Work and others have previously

shown that chronic stress in male rats leads to dendritic spine attrition in the medial prefrontal cortex (mPFC) and accompanying working memory impairments, however, we have not previously critically evaluated these effects in female rats. Here we examine dendritic spine morphological alterations in the prelimbic region (PL) of mPFC, and working memory functioning, in both female and male rats exposed to 14 days of chronic variable stress (CVS; daily exposure to different stressors at unpredictable times over 14 days). In the first experiment, cohorts of male and female rats were subjected to CVS or unstressed control conditions, and then were perfused for neuronal morphological analyses on day 15. Pyramidal neurons in layers II/III of PL were selected for intracellular fluorescent dye-filling, followed by high-resolution 3D imaging and analysis of spine density and morphometry. CVS-exposure decreased spine density on PL pyramidal neurons in both males and females as compared to respective within-sex control treatments (by 16% and 11%, p < 0.05). In the second experiment, cohorts of male and female rats were trained on the delayed alternation task using a *T*-maze to measure spatial working memory. After training, a subset of male and female rats was subjected to 14 d CVS, and all rats were submitted to behavioral testing on days 15-18. Both groups of CVS-treated male and female rats showed impairments in working memory performance and increased corticosterone levels as compared with same-sex unstressed controls (p < 0.05 for each). These studies extend our previous work showing that the adverse effects of chronic stress on prefrontal structural and functional plasticity have broad effects that are not well-differentiated between adult male and female rats, and suggest that cognitive resilience shown in female rodents following prolonged stress exposure does not generalize to the prefrontal endpoints examined here.

Disclosures: R.M. Anderson: None. M. Mahanna: None. S. Romig-Martin: None. J.J. Radley: None.

Poster

324. Energy Metabolism and Blood Brain Barrier

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 324.01/LL13

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant NS085282

Title: Molecular and biochemical characterization of the rare mitochondrial respiratory disorder Leber's hereditary optic neuropathy associated with multiple sclerosis-like illness

Authors: *A. E. CHIARAMELLO¹, M. N. UITTENBOGAARD², C. A. BRANTNER³, A. GROPMAN⁴

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Abstract: Mitochondrial respiratory disorders are characterized by a chronic energy deficit due to mutations in the nuclear or mitochondrial genome affecting the oxidative phosphorylation (OXPHOS) responsible for ATP synthesis. Leber's hereditary optic neuropathy (LHON) is a rare and intractable maternally inherited disorder characterized by severe loss of bilateral vision and permanent blindness. Most mutations map in mitochondrial-encoded subunits of the rate-limiting OXPHOS complex I, leading to impaired ATP production, loss of retinal ganglion cells and degeneration of optic nerve axons. Among the three primary mitochondrial mutations associated with LHON, the m.11778A>G mutation is the most severe and affects the mitochondrialencoded subunit ND4 of complex I. Intriguingly, an association between the m.11778A>G mutation in LHON and multiple sclerosis has been predominantly observed in female patients and is referred to as LHON-MS. To understand the underlying molecular pathogenesis of LHON-MS, we performed a skin biopsy on a 34-years old female patient carrying the m.11778A>G mutation. She presented with vision loss at age 27 affecting both near and distant vision, which progressed to headaches, VA of 20/280 OD, 20-160 OS, some color desaturation on Ishihara plates, and bilateral ceocentral scotoma on visual field testing. At age 31, she developed additional symptoms inconsistent with LHON, including urinary retention, bladder spams, loss of sensation, otalgia, muscle and joint and radicular symptoms of pain and numbness in the arms, which led to the diagnosis of LHON-MS. Our live-cell mitochondrial respiratory studies revealed that the proband's fibroblasts exhibited a substantial bioenergetic deficit with a decreased basal consumption oxygen rate and ATP-linked respiration, when compared to agematched healthy fibroblasts. Diseased fibroblasts had a severe decrease of spare respiratory capacity, which stunted their ability to respond to a surge in ATP demand resulting in an energy crisis. We performed a mitochondrial morphometric analysis by transmission electron microscopy, which revealed small mitochondria with sparse and short cristae in the proband's fibroblasts. They rarely exhibited a branched mitochondrial configuration, indicative of abnormal mitochondrial connectivity and dynamics. Thus, our mitochondrial ultrastructural analyses are congruent with our findings from our functional studies on mitochondrial bioenergetics parameters, revealing the ATP crisis as one of the mechanisms leading to selective cell death in patients affected with LHON-MS.

Disclosures: A.E. Chiaramello: None. M.N. Uittenbogaard: None. C.A. Brantner: None. A. Gropman: None.

Poster

324. Energy Metabolism and Blood Brain Barrier

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 324.02/LL14

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant NS085282

Title: Epigenetic modifiers induce an energy metabolic shift accompanied by increased mitochondrial biogenesis in differentiating neuroprogenitor cells

Authors: *M. N. UITTENBOGAARD¹, C. BRANTNER³, J. S. LIU⁴, A. E. CHIARAMELLO² ²Anat & Regenerative Biol, ¹George Washington Univ. Med. Ctr., Washington, DC; ³Nanofabrication and Imaging Ctr., George Washington Univ., Washington, DC; ⁴Ctr. for Neurosci., Children's Natl. Med. Ctr., Washington, DC

Abstract: A hallmark of brain metabolism is the tight coupling between energy demand and supply, which involves a constant regulation of mitochondrial homeostasis and bioenergetics at all stages of brain development. The brain, a highly energy-demanding organ, is highly dependent on mitochondrial homeostasis for ATP production via oxidative phosphorylation (OXPHOS). Thus, the question of how mitochondrial biogenesis is regulated becomes of paramount importance in order to maintain a tight coupling between energy demand and supply to insure proper neural development and survival. Our previous studies have shown that the neurogenic basic Helix-Loop-Helix (bHLH) transcription factor NeuroD6 increases the number of bioenergetically competent mitochondria at the onset of neuronal differentiation. The main question addressed in this study is the interplay between epigenetics and mitochondrial energy metabolism in a neuronal context, which has remained largely unexplored. It is well established that pan-HDAC inhibitors induce differentiation of neuronal progenitor cells. We investigated whether sodium butyrate (NaBt) could modulate mitochondrial bioenergetics and biogenesis and subsequently enhance neuronal differentiation. To address this question, we took advantage of our engineered neuroprogenitor-like PC12-NeuroD6 (PC12-ND6) cells and E17.5 hippocampal neurons expressing high levels of NeuroD6. Our collective findings show that: 1) NaBt increases the mitochondrial biomass by stimulating mitochondrial biogenesis via the TFAM-NRF-1 axis; 2) NaBt induces a mitochondrial ultrastructural remodeling accompanied with increased cristae, consistent with increased bioenergetics parameters; 3) NaBt induces a metabolic shift toward OXPHOS for ATP synthesis, resulting in enhanced mitochondrial respiration and spare respiratory capacity; 4) modulation of the mitochondrial biomass using gain-and loss-offunction-like pharmacological assays has exposed mitochondrial biogenesis as a key event to enhance the neuronal progenitor competency to differentiate. In conclusion, our study provides evidence for a novel developmental epigenetic layer coupling mitochondrial energy metabolism to neuronal differentiation.

Disclosures: M.N. Uittenbogaard: None. C. Brantner: None. J.S. Liu: None. A.E. Chiaramello: None.

Poster

324. Energy Metabolism and Blood Brain Barrier

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 324.03/LL15

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: BBSRC

Title: Assessing the impact of glucose and lactate for presynaptic ATP generation during sustained transmission at the calyx of Held

Authors: *S. J. LUCAS¹, C. B. MICHEL², V. MARRA¹, J. L. SMALLEY¹, M. H. HENNIG³, B. P. GRAHAM², I. D. FORSYTHE¹ ¹Univ. of Leicester, Leicester, United Kingdom; ²Univ. of Stirling, Stirling, United Kingdom; ³Edinburgh Univ., Edinburgh, United Kingdom

Abstract: The brain has high energy demands which increase during intense neuronal activity. There is an established relationship between increased brain activity, blood flow and cognition in which energy provision for synaptic transmission is crucial. Presynaptic terminals have particularly high metabolic demands causing local activity-dependent metabolic constraints on physiological transmission and contributing to pathological injury during stroke, traumatic brain injury or neurodegeneration. We have used high frequency stimulation and changes in energy substrates to induce metabolic stress at the calyx of Held/MNTB synapse in the auditory pathway. Energy depletion was achieved under conditions of saturated oxygen and without pharmacological block of respiration, by washout of extracellular metabolic substrates, while measuring glutamatergic synaptic transmission. Patch clamp recording from MNTB neurons in mouse brainstem slices showed that glucose depletion enhanced EPSC depression during high frequency stimulation and slowed recovery of EPSC amplitude. Depletion of presynaptic ATP (achieved by patching the presynaptic terminal to allow diffusion with the patch pipette) also impaired transmitter release in an analogous manner. Computational modelling of these experimental data demonstrated that the impaired transmission was caused by a reduction in the number of functional release sites and slowed vesicle pool replenishment, rather than a sustained change in release probability. Lactate maintained presynaptic function in the absence of glucose. When glucose was perfused in the aCSF as the sole energy substrate, lactate generated from glial cells did contribute to the maintenance of synaptic transmission, but that the terminal was also using glucose directly. We conclude that lactate contributes to supporting presynaptic function, but that glucose is a major presynaptic energy substrate. Presynaptic metabolism is a physiological constraint on synaptic transmission; exploring these physiological limits will give insights into preserving brain function and cognition following hypoxic insults.

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Poster

324. Energy Metabolism and Blood Brain Barrier

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 324.04/LL16

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: ZIADA000566

Title: Rapid fluctuations in nucleus accumbens oxygen levels induced by arousing stimuli: Relationships with changes in brain glucose and metabolic neural activation

Authors: *E. A. KIYATKIN, K. T. CAMERON-BURR, E. SOLIS, Jr. Behavioral Neurosci Br., NIDA-IRP, NIH, DHHS, Baltimore, MD

Abstract: Proper entry of oxygen from arterial blood into the brain is essential for maintaining brain metabolism under normal conditions and during functional neural activation. However, little is known about physiological fluctuations in brain oxygen and their underlying mechanisms. To address this issue, we employed high-speed amperometry with platinum oxygen sensors in freely moving male rats. Recordings were conducted in the nucleus accumbens (NAc), a critical structure for sensorimotor integration. Rats were exposed to different arousing stimuli (brief auditory tone, a 1-min novel object presentation, a 3-min social interaction with a conspecific, and a 3-min tail-pinch). We found that all arousing stimuli increased NAc oxygen levels. Increases were rapid (4-10-s onset latencies), modest in magnitude (1-3 µM or 5-15% over baseline) and duration (5-20 min), and generally correlated with the arousing potential of each stimulus. Two strategies were used to clarify the mechanisms underlying the observed increases in NAc oxygen levels. First, we showed that NAc oxygen levels phasically increase following intra-NAc microinjections of glutamate that excite accumbal neurons. Therefore, local neural activation and subsequent local vasodilation are involved in mediating physiological increases in NAc oxygen induced by arousing stimuli. Second, by employing oxygen monitoring in the subcutaneous space, a highly-vascularized area with no metabolic activity, we determined that physiological increases in NAc oxygen also depend on a rise in blood oxygen levels caused by respiratory activation. Due to the co-existence of different mechanisms governing oxygen entry into brain tissue, NAc oxygen responses differ from fluctuations in NAc glucose, which, within a normal behavioral continuum, are regulated exclusively by neuro-vascular coupling due to the highly stable levels of glucose in the blood. Finally, we discuss the relationship between physiological fluctuations in NAc oxygen and glucose, and metabolic brain activation assessed by intra-brain heat production.

Disclosures: E.A. Kiyatkin: None. K.T. Cameron-Burr: None. E. Solis: None.

Poster

324. Energy Metabolism and Blood Brain Barrier

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 324.05/LL17

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Assessing affective behaviors in mice lacking the creatine transporter

Authors: *Z. I. ABDULLA¹, J. L. PENNINGTON², K. C. UDOBI¹, N. LATUSHKA², M. R. SKELTON¹

¹Neurol., Cincinnati Children's Res. Fndn., Cincinnati, OH; ²Univ. of Cincinnati, Cincinnati, OH

Abstract: Changes in bioenergetics are seen in a variety of mental illnesses. While most of the work has focused on changes in mitochondrial function, the role of energy buffers such as creatine (Cr) have not been thoroughly investigated. Cr is a nitrogenous amino acid that acts as a spatial and temporal ATP buffer in cells with a high-energy demand. We have developed Cr transporter (Crt) knockout (Slc6a8-'y) mice to better understand how the loss of Cr affects brain function. Slc6a8-/y mice have elevated levels of serotonin (5-HT) in the hippocampus and prefrontal cortex. Increased 5-HIAA, a metabolite of 5-HT, is found in the hippocampus and neostriatum of these mice. Additionally, *Slc6a8*-/y mice are hypersensitive to parachloroamphetamine, a 5-HT mediated stimulant. It is well established that 5-HT activity is a crucial factor in affective behaviors, and it is important to note that Cr has been shown to influence these types of behaviors as well. An acute dose of Cr produced an equally antidepressive effect as fluoxetine in the tail suspension test (TST). Additionally, it was demonstrated that Cr supplementation reduced depression-like measures in the forced-swim test in females, but exacerbated effects in males. If 5-HT is important for affective behaviors and depleting Cr from the brain results in augmentations to 5-HT, will a lack of Cr result in an altered affective phenotype? To address this, we tested wild-type and $Slc6a8^{-/y}$ mice in measures of anxiety, sociability, and depression. Elevated zero maze revealed a trend toward decreased latency to enter the open arms in KO males and heterozygous females (HET), and increased head dips in HETs. Social preference revealed an interesting sex dependent result, in which the presence of cellular Cr was associated with increased sociability in females, but decreased sociability in males. No differences were observed in TST, which reveals a lack of sensorimotor deficits in the $Slc6a8^{-/y}$ mice.

Disclosures: Z.I. Abdulla: None. J.L. Pennington: None. K.C. Udobi: None. N. Latushka: None. M.R. Skelton: None.

Poster

324. Energy Metabolism and Blood Brain Barrier

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Program#/Poster#: 324.06/LL18

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: FAPESP 2014/11752-6

CAPES/Proex

Title: Leptin absence in early life causes long-term disturbances in energy balance that can be restored by early intervention

Authors: *A. M. RAMOS LOBO, P. S. TEIXEIRA, I. C. FURIGO, A. M. LIMA, J. DONATO, Jr

Physiol. and Biophysics, Univ. of Sao Paulo, Sao Paulo, Brazil

Abstract: The adipocyte-derived hormone leptin is essential for the regulation of energy balance. However, some studies have suggested that in early life leptin functions are associated with neuronal development and dependent of a critical neonatal period.

To determine the effects of the early absence of leptin signaling in adulthood and whether these effects can be reversed by reactivation of leptin signaling in adult life or weaning, a LoxP-flanked transcription-blocking cassette was inserted in the Lepr gene to generate mice *null* for the leptin receptor. They were bred with animals expressing Cre-ERT2 fusion protein under the human ubiquitin C promoter sequence. Consequently, tamoxifen injections can induce Cre Recombinase activity and restore *Lepr* gene expression in adult (10 weeks) or young (4 weeks) Ubi-LepRNull mice.

As a validation of the model we assessed the number of leptin-responsive cells in the brain through an acute leptin injection. LepRNull mice exhibited no leptin responsive cells and the neural projections from the arcuate nucleus (ARH) to the paraventricular nucleus were disrupted, and were both restored in Ubi-LepRNull mice, as was their response to leptin.

Adult LepRNull and Ubi-LepRNull mice were morbidly obese and hyperphagic. Six weeks after tamoxifen treatment, adult Ubi-LepRNull mice restored normal food intake but were heavier and had larger adiposity, they also displayed lower energy expenditure and locomotor activity than Ubi mice. Ubi-LepRNull mice had lower glycaemia as well as higher glucose and pyruvate tolerance than Ubi mice, although they remained insulin resistant. Their brain weight was partially recovered compared to Ubi mice.

We next examined the parameters that were not restored after adult LepR reactivation in young mice. After tamoxifen injections young Ubi-LepRNull mice recovered normal body weight and food intake, together with normal energy expenditure and locomotor activity, as well as their glycemic homeostasis by early reactivation of LepR, although their brain mass remained lighter.

These results suggest that lack of leptin signaling in early life causes long-term changes in energy balance and glucose homeostasis regulation, although ARH projections can be normalized after *Lepr* reactivation in adults, and that weaning is a critical period for leptin's role.

Disclosures: A.M. Ramos Lobo: None. P.S. Teixeira: None. I.C. Furigo: None. A.M. Lima: None. J. Donato: None.

Poster

324. Energy Metabolism and Blood Brain Barrier

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Program#/Poster#: 324.07/LL19

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: BBS Research Enhancement Funds

Clark Foundation

Title: High fat diet impairs hippocampal intrinsic excitability and memory and sex-dependently alters insulin signaling in hippocampus

Authors: *N. TANDON, L. T. THOMPSON Univ. of Texas At Dallas, Richardson, TX

Abstract: Modern western diet with excess energy-dense fats disturb metabolic homeostasis promoting obesity and type-2 diabetes. Cognitive deficits have been clinically observed in humans suffering from obesity and diabetes, and intensely studied in animal models fed high fat diets (HFDs) from weaning. However, sex-differences are rarely systematically analyzed and compared. Previous studies from our lab with an outbred Long Evans (LE) rat HFD model showed impaired spatial memory in both sexes, correlating with significantly reduced intrinsic excitability of hippocampal CA1 pyramidal neurons (enhanced post-burst AHPs). Notable sexdifferences in hormone signaling were observed, wherein HFD induced clinically relevant symptoms of type 2 diabetes in males (obesity, loss of blood glucose control, elevated insulin), but not in females (normal weight and blood glucose control, reduced insulin). Sex-differences were also found in insulin-dependent intrinsic excitability of CA1 neurons; HFD male neurons lost all insulin sensitivity while HFD female neurons had increased sensitivity. Our objectives are to study the effects of HFD in the CA1 region: 1. on proteins involved in Ca2+-dependent intrinsic excitabilitySK2 channels and the Ca2+ sensors calmodulin and hippocalcin; and 2. to understand the induced sexual dimorphism in insulin sensitivity and on proteins involved in insulin signaling pathways. Age-matched HFD or control LE rats were used. Rat brains were either flash frozen for western blotting or perfused for IHC/IFC analysis of protein expression in CA1. Our results show diet-dependent increases in SK2 channel and hippocalcin protein

expression, consistent with increases in medium- and slow-AHPs in CA1 neurons of HFD rats and with impaired spatial memory our laboratory previously reported. Only HFD females showed upregulation in insulin signaling pathways, i.e. increased expression of insulin receptor protein and upregulation of Akt protein phosphorylation. HFD sex-dependently alters insulin signaling in the hippocampus of females but not males. Considering these profound differences, treatment strategies designed to ameliorate cognitive deficits caused by obesity and type-2 diabetes must be sex specific.

Disclosures: N. Tandon: None. L.T. Thompson: None.

Poster

324. Energy Metabolism and Blood Brain Barrier

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 324.08/LL20

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Nutrient shuttling in hypothalamic astrocytes is linked to the whole body energy metabolism

Authors: *J. KIM¹, J. LEE², B. PARK³

¹Div. of Life Sci., Incheon Natl. Univ., INCHEON, Korea, Republic of; ²Div. of Life Sci., ³Incheon Natl. Univ., Incheon, Korea, Republic of

Abstract: Astrocytes are the most abundant cells in the central nervous system, yet they have often been relegated to a less than prominent role in the control of complex brain functions supported by neuronal circuits. The regulation of food intake and energy expenditure is tightly linked to the hypothalamic neural circuits. However, the direct contribution of astrocytes in this system is ill defined. Nutrients availability in the brain is crucial for neuronal functions in which astrocytes have also been deeply implicated. In this study, we identified active roles of hypothalamic astrocytes as a sensor of nutrient concentration and a primary modulator to maintain the whole body energy metabolism. We found that the impairment of nutrient shuttling from the blood vessel to the neurons led to the abnormality of energy intake and glucose production utilizing genetic mouse model in which peroxisome proliferator-activated receptor gamma (PPAR gamma) genes are time-specifically ablated in astrocytes in the pathogenesis of obesity. Collectively, we argue an important role of hypothalamic astrocytes in the regulation of the whole body energy homeostasis.

Disclosures: J. Kim: None. J. Lee: None. B. Park: None.

Poster

324. Energy Metabolism and Blood Brain Barrier

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 324.09/LL21

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIDDK Intramural Research

Title: Activation of Adenosine A2A or A2B receptor sub-types causes hypothermia in mice

Authors: ***J. CARLIN**¹, S. JAIN², C. XIAO¹, J. A. AUCHAMPACH³, K. A. JACOBSON², O. GAVRILOVA², M. L. REITMAN² ²NIDDK, ¹NIH, Bethesda, MD; ³Dept. of Pharmacol., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Background: Adenosine has been implicated in mediating torpor in small mammals during hibernation or fasting. Adenosine has been known to elicit hypothermia for over 80 years. One mechanism of adenosine-induced hypothermia is proposed to be via adenosine A_1 receptors (A_1AR) in the nucleus of the solitary tract. In mice, adenosine also acts peripherally on A_3 receptors (A₃AR) on mast cells, causing hypothermia from activation of histamine H₁ receptors during a shock response. However, we observed that adenosine-induced hypothermia still occurs in mice lacking both A₁AR and A₃AR. Therefore, we tested if the other two adenosine receptors, A2AAR or A2BAR can mediate hypothermia. A2A agonists cause vasodilation and both A2AAR and A_{2B}AR RNA are widely expressed, but there is little information on the possible role of A_{2A}AR or A_{2B}AR in regulating body temperature set point or causing hypothermia. Methods: Adenosine agonists were administered intraperitoneally (i.p.) or intracerebroventricularly (i.c.v.) and core body temperature and physical activity were monitored by telemetry in freely active mice. Wild type mice, mice lacking mast cells (Kit^{W-sh/W-sh}), and mice lacking $A_{2A}RAR$, $A_{2B}AR$, or $A_1AR + A_3AR$ were studied. Results: We found that A_{2A}AR agonists CGS-2160 (0.5mg/kg ip) and PBS-0777 (3mg/kg, i.p) are cause hypothermia in WT, but not and Adora2a1 -/-, Adora3 -/- mice. A2AR agonist hypothermia was accompanied by severe hypometabolism and hypoactivity, resembling, a torpor-like state. CGS-2160 hypothermia is intact in *Kit^{W-sh/W-sh}* mice, demonstrating that mast cells are not required, and associated with reduced energy expenditure, reduced activity, and behavior in a temperature gradient test. PBS-0777 (0.03mg/kg i.c.v) caused hypothermia in Adora1 -/-, Adora3 -/- mice and suggests a centrally mediated mechanism. A2BAR agonist BAY60-6583 (1mg/kg ip i.p) causes hypothermia in WT, but not Adora2b-/- miceand Adora1-/-, Adora3-/- mice via A_{2B}AR. BAY60-6583 hypothermia is intact in Kit^{W-sh/W-sh} a mice and associated accompanied by with rreduced energy expenditure and , reduced aactivity, and behavior in a temperature gradient test. BAY60-6583 (0.004mg/kg icv i.c.v) caused hypothermia

equivalent to a 250-larger ip dose, in Adora1 -/-, Adora3 -/- mice and suggests consistent with a

centrally mediated mechanism. BAY60-6583 activated neurons in temperature and metabolism related regions of the brain as were visualized with c-fos immunohistochemistry. Conclusion: Adenosine agonists can cause hypothermia via $A_{2A}AR$ or $A_{2B}AR$. Thus, agonists for each of the four adenosine receptors can independently elicit hypothermia in mice.

Disclosures: J. Carlin: None. S. Jain: None. C. Xiao: None. J.A. Auchampach: None. K.A. Jacobson: None. O. Gavrilova: None. M.L. Reitman: None.

Poster

324. Energy Metabolism and Blood Brain Barrier

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 324.10/LL22

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: College of Human Sciences Seed Grant

Title: The impact of running on the expression of brain adenosine receptors

Authors: *E. BAUER, B. A. BAUSTAIN, A. BELL, M. R. CARLSON, P. J. CLARK FSNH, Iowa State Univ., Ames, IA

Abstract: Regular participation in physical activity is associated with a reported decrease in central-mediated fatigue, however, the precise mechanisms are not well understood. Adenosine is a product of cellular adenosine triphosphate (ATP) consumption and can act as a neuromodulator during metabolic demand. Adenosine signaling in the brain is known to play a significant role in the modulation of fatigue. Previous work from our group found that 6 weeks of wheel running potently reduces the adenosine 1 (A_{R1}) & 2a (A_{R2A}) receptor mRNA throughout the rat striatum. The purpose of this study was to determine if wheel running also reduces the expression of adenosine receptor protein in the striatum, as well as other brain areas where adenosine modulates fatigue behavior including the hippocampus, prefrontal cortex, and hypothalamus. Therefore, adult male C57BL/6J mice were individually housed with access to running wheels or in standard cages for 3 or 8 weeks. Following 3 and 8 weeks, mice were transcardially perfused with saline and paraformaldehyde. Immunohistochemistry was performed on thin brain sections to detected A_{R1} and A_{R2A} . Brain sections will be imaged and densitometry will be performed to semi-quantitatively measure the density of adenosine receptors in brain regions. Data collection is underway. We hypothesize A_{R1} and A_{R2A} expression will decrease across the all brain regions, as a result of peripheral increases of adenosine entering the brain during running. A widespread reduction in brain adenosine receptors may contribute to reduced sensations of fatigue in individuals that regularly exercise, and may have implications for the pro-cognitive and anti-depressant effects of exercise.

Disclosures: E. Bauer: None. B.A. Baustain: None. A. Bell: None. M.R. Carlson: None. P.J. Clark: None.

Poster

324. Energy Metabolism and Blood Brain Barrier

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 324.11/LL23

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIHR21 HD83389

Saudi Arabian Cultural Mission

Title: Lateral but not medial hypothalamic AMPK activation occurs at the hypoglycemic nadir in insulin-injected male rats: Impact of caudal dorsomedial hindbrain catecholamine signaling

Authors: *H. N. ALHAMAMI, K. P. BRISKI

Univ. of Louisiana At Monroe, Monroe, LA

Abstract: The hypothalamic energy sensor adenosine 5'-monophosphate-activated protein kinase (AMPK), an important regulator of counter-regulatory responses to hypoglycemia, responds to pharmacological manipulation of hindbrain AMPK activity. Dorsomedial hindbrain A2 noradrenergic neurons express hypoglycemia-sensitive metabolo-sensory biomarkers, including AMPK. Here, adult male rats were pretreated by intra-caudal fourth ventricular administration of the selective neurotoxin 6-hydroxydopamine (6-OHDA) to determine if catecholamine signaling from the aforesaid site controls hypothalamic AMPK activation during insulin-induced hypoglycemia (IIH). Animals were sacrificed at neutral protamine Hagedorn insulin-induced hypoglycemic nadir (coincident with A2 AMPK activation and regulation of dorsomedial hindbrain noradrenergic input to the hypothalamus) for micro-punch dissection of arcuate (ARH), ventromedial (VMH), paraventricular (PVH), dorsomedial (DMH) nuclei and lateral hypothalamic area (LHA) for Western blot analysis of AMPK, phospho-AMPK (pAMPK), and relevant metabolic neuropeptides. IIH elevated LHA and reduced VMH pAMPK protein, profiles that were respectively unchanged or increased by 6-OHDA. PVH and ARH pAMPK was resistant to IIH, but augmented in ARH of neurotoxin- plus insulin-treated rats. ARH neuropeptide Y (NPY) and pro-opiomelanocortin (POMC) proteins were correspondingly increased or refractory to IIH; 6-OHDA pretreatment normalized NPY and increased POMC expression after insulin injection. Results demonstrate site-specific (ARH/VMH) inhibition of pAMPK by dorsomedial hindbrain catecholamine signaling, causing normalized sensor activity in hypoglycemic male rats. It is unclear if these examples of sensor deactivation reflect enhanced local energetic stability by this hindbrain-to-hypothalamus communication. Catecholaminergic suppression versus stimulation of IIH-associated hypothalamic catabolic (ARH POMC/VMH

steroidogenic factor-1) or anabolic (ARH NPY) neuropeptide profiles, respectively, may involve local AMPK-dependent against -independent mechanisms.

Disclosures: H.N. Alhamami: None. K.P. Briski: None.

Poster

324. Energy Metabolism and Blood Brain Barrier

Location: Halls A-C

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Program#/Poster#: 324.12/LL24

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH R21 HD083389

Title: Role of hindbrain adenosine 5'-monophosphate-activated protein kinase (AMPK) in hypothalmic AMPK and neuropeptide adaptation to recurring insulin-induced hypoglycemia

Authors: *S. K. MANDAL, K. P. BRISKI

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Abstract: Glucose counter-regulatory dysfunction correlates with impaired activation of the hypothalamic metabolic sensor adenosine 5'-monophosphate-activated protein kinase (AMPK). Hypothalamic AMPK is controlled by hindbrain energy status; we examined here whether hindbrain AMPK regulates hypothalamic AMPK and metabolic neurotransmitter maladaptation to recurring insulin-induced hypoglycemia (RIIH). Brain tissue was harvested after single versus serial insulin dosing for Western blot analysis of AMPK, Phospho-AMPK (pAMPK), and relevant biosynthetic enzyme/neuropeptide expression in micro-punch dissected arcuate (ARH), ventromedial (VMH), dorsomedial (DMH) nuclei and lateral hypothalamic area (LHA) tissue. The AMPK inhibitor compound c (Cc) or vehicle was administered to the caudal fourth ventricle ahead of antecedent NPH injections. RIIH caused site-specific elevation (ARH, VMH, LHA) or reduction (DMH) of total AMPK protein versus acute hypoglycemia; Cc respectively exacerbated or attenuated this response in the ARH and VMH. Hindbrain AMPK correspondingly inhibited or stimulated LHA and DMH pAMPK expression during RIIH. RIIH elicited Cc-reversible augmentation of VMH glutamate decarboxylase profiles, but stimulated (ARH pro-opiomelanocortin; LHA orexin-A) or decreased (VMH nitric oxide synthase) other metabolic neurotransmitters without hindbrain sensor involvement. Results demonstrate acclimated up-regulation of total AMPK protein expression in multiple hypothalamic loci during RIIH, and document hindbrain sensor contribution to amplification of this protein profile in the VMH. Concurrent lack of net change in ARH and VMH tissue pAMPK implies adaptive reductions in local sensor activity, which may/may not reflect positive gain in energy state. It remains unclear if 'glucose-excited' VMH GABAergic and/or ARH pro-opiomelanocortin

neurons exhibit AMPK habituation to RIIH, and whether diminished sensor activation in these and other mediobasal hypothalamic neurotransmitter populations may contribute to HAAF.

Disclosures: S.K. Mandal: None. K.P. Briski: None.

Poster

324. Energy Metabolism and Blood Brain Barrier

Location: Halls A-C

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Program#/Poster#: 324.13/LL25

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: FACEPE Grant APQ-0164-4.05/15

Title: Fluoxetine-induced mitochondrial and molecular effects in the hypothalamus of overfed rats

Authors: *G. FEITOZA, S. C. SILVA, C. M. FREITAS, A. A. PEDROZA, A. I. DA SILVA, C. J. LAGRANHA

Federal Univ. of Pernambuco, Vitória de Santo Antão, Brazil

Abstract: Obesity strongly contributes to the onset of chronic diseases as well as to higher morbidity and mortality worldwide. Specially at early ages, obesity have taken epidemic proportions. The energy imbalance linked to obesity is particularly mediated by mitochondrial dysfunction that represents one of the most inducers of the obesity pathophysiologic process. Several central regulators are involved in mitochondrial function like mitochondrial uncoupling protein 2 (UCP 2), that plays a key role in the central homeostatic mechanisms and reactive species (RS), and AMP-activated kinase (AMPK), that stimulates cellular catabolic pathways and mitochondrial biogenesis. Although obesity treatment with antidepressants like fluoxetine (also known as Prozac) has becoming clinically relevant, there are no scientific reports related to mitochondrial effects. With this our aim was to evaluate the effect of fluoxetine (Fx) treatment during 21 days (from 39 to 59 days of age) on mitochondrial and molecular parameters in the hypothalamus of overfed rats. To induce neonatal overnutrition, the primary litter size was reduced on the third day of life to 3 pups per litter (Small litters group-SL; n =8). In normal litters, the size was adjusted to 9 neonates per mother (Normal litters group-NL; n =9). The Fxtreatment started with 39 days of age, using 10mg/Kg B.W. (i.p. injection) in male Wistar rats of both groups. At 60 days of age was evaluated mitochondrial oxygen consumption (MOC) at different states (St2-basal respiration; St3-ADP-stimulated respiration; St4-resting respiration; Vmax-uncoupled respiration) and the respiratory control ratio (RCR) with complexes I or II substrates; RS production with complex II substrates, AMPK and UCP 2 mRNA expression. Related to MOC analysis, the neonatal overnutrition induces reduction in St 3 (30%), increase in St 4 (44%) and reduction in RCR (59%) with complex I substrates. In addition, overnourished

rats showed increased St 2 (92%) and St 4 (59), and reduction in St 3 (30%) and in RCR (36%) with complex II substrates. Fx-treatment reverted the effects of neonatal overnutrition in MOC with complex II substrates: decreased St 2 (32%), increased St 3 (46%), decreased St 4 (20%) and increased Vmax (53%); with improvement of RCR (75%) and tendency to reduction in RS production. Fx also increased AMPK and UCP 2 expression in overfed rats (170% and 146%, respectively). Our results suggest that Fx-treatment improves mitochondrial oxygen consumption in obese rats mainly by enhanced phosphorylation capacity and regulation of central energy metabolism. The present study received financial support from FACEPE.

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Poster

324. Energy Metabolism and Blood Brain Barrier

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 324.14/LL26

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: KAKENHI Grant JP15H02718

AIST

Title: Non-monotonic effect of temperature on cortical-evoked potentials

Authors: *M. GOTOH^{1,2}, K. NAGASAKA^{1,2,3}, I. TAKASHIMA^{1,2}, S. YAMAMOTO¹ ¹AIST, Tsukuba, Japan; ²Univ. of Tsukuba, Tsukuba, Japan; ³JSPS Res. fellow, Tokyo, Japan

Abstract: Body temperature interacts with various biological activities and shows fluctuations around the normal temperature. Although temperature plays an important role in the brain via numerous energy exchanges, few studies have investigated whether and how temperature modulates neural information processing. Given that the activity of proteins is monotonically (and mostly positively) correlated with temperature, it has been assumed that neuronal activity changes monotonically with the temperature. However, the brain is composed of various types of neurons (e.g. excitatory and inhibitory neurons) that interact with each other in a complex manner. Therefore, determining whether the effect of temperature on neural processing *in vivo* follows the previous assumption is important.

To answer this question, we performed an *in vivo* rat experiment to examine how the temperature in a local brain area affects local field potentials. We delivered electrical stimulation to the ventral tegmental area (VTA) and measured the evoked potentials from the prefrontal cortex (PFC) using a silver ball electrode placed on the cortical surface. To control the temperature of the PFC, we developed a water heating/cooling system. A coil-shaped stainless steel tube was embedded inside the chamber on the PFC, and then temperature-controlled water flowed through the stainless pipe, regulating the temperature of the cortical surface via thermal conduction as the saline filled the chamber. During the experiments, we measured the temperature using a thermocouple thermometer inserted at a depth of 1 mm. The temperature prior to thermal regulation was ~27°C (initial temperature).

In contrast to the previous assumption, temperature affected the amplitude of the evoked potentials non-monotonically. The effect on the amplitude was negatively correlated around the normal initial temperature (~20-35°C). The amplitude decreased as the temperature increased, whereas it increased as the temperature decreased. The amplitude was maximized at a temperature ~8°C lower than the initial temperature, and further cooling further decreased the amplitude. Administering Gabazine (a GABA_A receptor antagonist) into the chamber eliminated the non-monotonic correlation effects on the amplitude, revealing the monotonically positive correlation.

Our data suggest that the net amplitude of the evoked potentials decreases at higher temperatures because both not only excitatory but also inhibitory neurons are affected in a positively correlated manner. These findings suggest that a mediator exists to regulate the brain temperature and control neural activity.

Disclosures: M. Gotoh: None. K. Nagasaka: None. I. Takashima: None. S. Yamamoto: None.

Poster

324. Energy Metabolism and Blood Brain Barrier

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Program#/Poster#: 324.15/LL27

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant DK107900

Title: Courtship changes in a Drosophila model of classic galactosemia

Authors: V. BAGGETT¹, A. KEHRER², J. FRIDOVICH-KEIL⁴, *T. ZARS³ ¹Biol. Sci., ²Div. of Biol. Sci., ³Univ. of Missouri, Columbia, MO; ⁴Human Genet., Emory Univ., Atlanta, GA

Abstract: Classic Galactosemia (CG) is a rare genetic disorder affecting about 1 in 60,000 newborns annually, with no treatment other than immediate removal of dietary galactose. Even with dietary restrictions, individuals with the loss of galactose-1-phosphate uridyltransferase (GALT) have many associated chronic complications. Chronic complications include cognitive disorders, ataxia, and decreased fertility. The current *Drosophila melanogaster* model of CG (GALT^{AP2}) has allowed us to investigate behavioral consequences of the loss of GALT. In an

attempt to more closely mimic the genetic changes in the human disease, we have generated two additional alleles (GALT³ and GALT⁴) in *Drosophila* through removal of the catalytic domain of GALT using CRISPR/Cas9. To better understand the connection between loss of GALT and reduced fertility, we investigated the courtship behavior of GALT^{AP2} and GALT^{C2} control flies. We continue to investigate GALT³ and GALT⁴ behavioral changes. We found a decrement in copulation latency in GALT^{AP2} flies. This provides a model for genetic and pharmacological intervention strategies in a genetic model of CG.

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Poster

324. Energy Metabolism and Blood Brain Barrier

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Program#/Poster#: 324.16/LL28

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: FAPESP 2015/26190-6

FAPESP 2014/50457-0

Title: Central role of melatonin on food intake and energy balance

Authors: *D. C. BUONFIGLIO, R. PARTHIMOS, R. CERQUEIRA, F. G. AMARAL, J. A. DA SILVA, A. M. RAMOS-LOBO, R. A. MATOS, J. S. DA SILVA, Jr, L. CLEMENTE, J. CIPOLLA, Neto Physiol and Biophysics, Univ. of Sao Paulo, Sao Paulo, Brazil

Physiol. and Biophysics, Univ. of Sao Paulo, Sao Paulo, Brazil

Abstract: Feeding behaviour is under the control of several hypothalamic nuclei, many hormones and neurotransmitters regulate food intake. Melatonin (Mel), a hormonal mediator of photoperiodic information to the central nervous system, promotes energy homeostasis through the establishment of a proper energy balance. Its administration reduces food intake, body weight and abdominal fat in rodent models. *In vitro*, rhythmic Mel treatment synchronizes leptin secretion by adipocytes. However, the mechanism through which Mel reduces body weight is unclear. We aimed to investigate how Mel regulates energy intake and energy expenditure, promoting a proper energy balance.

Male *Wistar* rats were assigned to Control, Control+Mel, Pinealectomized (PINX) and PINX+Mel groups. Mel (1mg/kg) was added to the drinking water exclusively during the dark phase for 13 weeks. Rats were subjected to a 24-hour fasting test, an acute leptin responsiveness test, cold challenge and circadian euthanasia. Western Blot and qPCR analyses were performed in hypothalamus and Brown Adipose Tissue (BAT).

Mel treatment reduced food intake, body weight and adiposity. Response to fasting was

unaltered in all groups. In the leptin responsiveness test, PINX rats showed no response on body weight, food intake or hypothalamic phosphorylation of STAT3 after acute leptin injection. mRNA analysis revealed a reduction of *Agrp* and *Orexin* expressions in the PINX+Mel group and an increase of *Agrp*, *Npy* and *Orexin* expressions in the PINX group. PINX rats had lower UCP1 protein levels in the BAT and lower thermogenic response to cold challenge. We showed that melatonin absence led to impaired acute response to leptin, pointing to a possible leptin resistance. On the other hand, in addition to reducing energy expenditure in BAT, melatonin treatment reduces orexigenic genes expression which leads to reduced food intake, body weight gain and adiposity in Mel treated rats, that may be mediated by a sensitization of leptin signalling pathway by melatonin. Funding: FAPESP 2015/26190-6

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Poster

324. Energy Metabolism and Blood Brain Barrier

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: BBS Research Enhancement Funds

Clark Foundation

Title: Assessing sex-differences in the effects of intranasal insulin on spatial memory impairments of young LE rats on a chronic high-fat diet

Authors: *N. DOS SANTOS¹, L. T. THOMPSON²

¹Sch. of Behavior and Brain Sci., ²Behavioral & Brain Sci., Univ. of Texas At Dallas, Richardson, TX

Abstract: High fat diets (HFDs) produce physiological and cognitive impairments, with symptoms including hyperglycemia, hyperinsulinemia and memory deficits. Most studies, however, fail to include female subjects, which prevents sexual differences to be characterized. Previous research from our lab has shown that unlike males, female rats fed a HFD do not develop hyperglycemia, and remain sensitive to exogenous insulin both peripherally and centrally (on CA1 pyramidal neurons, critical for memory consolidation). At the same time, endogenous concentrations of insulin are lower in HFD females than in controls, higher in HFD males than in controls, leading to development of insulin resistance. While CA1 neurons in both

male and female rats have larger post-burst AHPs (reduced intrinsic excitability), male HFD neurons are insulin-insensitive, while female neurons maintain insulin sensitivity. Insulin is critical not only for transport of glucose and fatty acids, but insulin-signaling pathways also contribute to cognitive functioning and to memory. To assess potential sex-dependent reversal of cognitive deficits by intranasal insulin (or saline control), LE rats were fed a HFD chronically for 10 or 15 wk from weaning, and spatial memory was assessed in an unrewarded spontaneous alternation task (SAT) after intranasal treatment. After 10 wk on the HFD, less severe memory deficits were observed in both male and female rats than after the longer duration on the diet. At 10 wk, intranasal insulin treatment reversed these mild cognitive impairments in both male and female rats. By 15 wk on the HFD, when significant differences in circulating insulin and in insulin sensitivity are shown, intranasal insulin treatment is expected to improve cognitive function in females only. Differences in fasted blood glucose and in insulin-tolerance responses (time series measures of blood glucose pre- and post-intranasal insulin) are being assessed in control and HFD male and female cohorts after 10 or 15 wk on their respective diets, and corticosteroid and insulin concentrations in both plasma and cerebrospinal fluid are being measured to assess other treatment effects on the rats, on their behavior, and on their response to treatment.

Disclosures: N. Dos Santos: None. L.T. Thompson: None.

Poster

324. Energy Metabolism and Blood Brain Barrier

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Title: Neuronal stimulation triggers neuronal glycolysis and not lactate uptake

Authors: C. DÍAZ-GARCÍA¹, R. MONGEON¹, C. LAHMANN¹, D. KOVEAL¹, H. ZUCKER¹, *G. YELLEN^{2,1} ¹Neurobio., ²Harvard Med. Sch., Boston, MA
Abstract: Brain metabolism supports a variety of neuronal activities, ranging from neurons that constitutively fire action potentials, to neurons that remain silent most of the time but burst at very high frequencies upon stimulation. However, the metabolic pathways allowing neurons to cope with these large dynamic variations in energy demand are not fully understood. During brain excitation, the increase in cerebral blood flow and glucose consumption is almost ten times higher than the increase in oxygen consumption in the activated area. This mismatch indicates that the glycolytic conversion of glucose to lactate exceeds the rate of mitochondrial fuel oxidation. Although the increased energy demand occurs mainly within neurons, some have suggested this glycolysis occurs mainly in astrocytes, which then shuttle lactate to neurons as their primary fuel. Using the metabolic biosensor Peredox, expressed in dentate granule neurons from acute hippocampal slices, we find that neurons increase the cytosolic NADH/NAD⁺ ratio in response to synaptic or antidromic stimulation. These metabolic responses do not depend on astrocytic stimulation by glutamate release, nor do they require neuronal uptake of lactate, as revealed by an increased metabolic response in the presence of blockers for monocarboxylate transporters or the enzyme lactate dehydrogenase. Inhibition of glycolysis at the level of the enzyme GAPDH, significantly decreases the metabolic responses to stimulation. Our results suggest that this transient of neuronal glycolysis acts as a metabolic "first responder" to meet the increased energy needs of the stimulated neuron.

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Poster

324. Energy Metabolism and Blood Brain Barrier

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: PICTO-Glaxo Grant 2013-0065

Title: Ghrelin signaling regulates GABA neurons of the nucleus of the solitary tract

Authors: *M. P. CORNEJO¹, P. N. DE FRANCESCO¹, G. GARCIA ROMERO¹, E. PORTIANSKY², M. REYNALDO¹, J. M. ZIGMAN³, M. PERELLO¹ ¹IMBICE, La Plata, Argentina; ²Facultad de Ciencias Veterinarias, UNLP, LAI, La Plata, Argentina; ³Intrnl. Med. - Psychiatry, Universityof Texas-Southwestern Med. Ctr., Dallas, TX

Abstract: The nucleus of the solitary tract (NTS) is a major sensory nucleus in the dorsal medulla that receives cardiovascular, visceral, respiratory, gustatory and orotactile information. It forms part of the dorsal vagal complex, which mainly regulates autonomic neural circuits. Ghrelin is a stomach-derived peptide hormone and its receptor, the growth hormone

secretagogue receptor (GHSR), is a G protein-coupled receptor highly expressed in the brain. The NTS is known to express GHSR, but the identity and the physiological role of GHSRexpressing neurons of the NTS are uncertain. In this study, we used a transgenic mouse model in which the enhanced green fluorescent protein (eGFP) is expressed under the control of the GHSR promoter (GHSR-eGFP mice) to perform a neuroanatomical and functional characterization of GHSR-expressing neurons of the NTS. We first mapped the neuroanatomical distribution of eGFP neurons within the NTS. Then, we explored the phenotype of eGFP neurons of the NTS using IHC against specific markers of neuronal populations known to be present in this brain area. In order to explore the physiological role of GHSR-expressing neurons of the NTS, we exposed GHSR-eGFP animals to different experimental protocols known to activate the NTS and to involve ghrelin signaling, and then we examined the pattern of expression of the marker of cellular activation c-Fos in eGFP positive cells. Overall, we found that GHSRexpressing neurons were located throughout the extension of the NTS, but preferentially forming two clusters: one rostral, mostly comprising the ventral subnucleus, and one caudal, mostly involving the parvicellular subnucleus. We also found that a population of GHSR-expressing neurons is GABAergic. Finally, eGFP neurons of the NTS failed to show an increase in c-Fos in response to the experimental protocols performed to GHSR-eGFP mice.

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Poster

324. Energy Metabolism and Blood Brain Barrier

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: FACEPE Grant APQ-1026-4.09/12

FACEPE Grant APQ-0164-4.05/15

Title: Sex-related differences in neonatal fluoxetine treatment in brainstem's mitochondrial bioenergetics

Authors: *T. SILVA¹, G. F. B. BRAZ², S. C. A. SILVA², C. M. FREITAS², A. I. DA SILVA², C. J. LAGRANHA²

¹Federal Univ. of Pernambuco, Vitória De Santo Antão, Brazil; ²Federal Univ. of Pernambuco-CAV, Vitória de Santo Antão, Brazil

Abstract: Mitochondria are dynamic organelles that perform several interconnected actions. In eukaryotic cells, mitochondria represent the most important ATP producer site and is the major

source of Reactive Species (RS). In the brain, the high O_2 consumption combined to its specialized characteristics, such as, prevalence of polyunsaturated fatty acid, amine content, RS production by monoamine oxidase and the presence of excitotoxic amino acids, make this tissue particularly vulnerable to RS damage. Hefty evidences have demonstrated that mitochondria dysfunction is the hub of several neuronal disorders. Studies suggest that mitochondria dysfunction in brainstem is involved in cardiovascular diseases (CVDs) due to the dysregulation of cardiovascular homeostasis and systemic blood pressure control by modulating the sympathetic vasomotor tone. Although equally harmful for both genders, mitochondrial dysfunction seem to be less severe on females. Evidence that estrogen plays a crucial role in protection is in line with the antioxidant function attributed to sex hormones. Previous study from our laboratory have shown that neonatal fluoxetine treatment may improve mitochondrial bioenergetics in different areas of the brain; however, until now there is a lack in the literature concerned to sex-related differences in mitochondrial bioenergetics and neonatal fluoxetine treatment. The treatment was carryout during the lactation time using 10mg/Kg B.W. (i.p. injection) in female and male Wistar rats. Was evaluated body weight and brainstem mitochondrial oxygen consumption with complex I substrates, respiratory control ratio (RCR), RS production and Krebs cycle enzyme (i.e citrate synthase-CS activity). Our results showed that male has higher basal oxygen consumption, RS production and CS activity, but a tendency to show lower RCR. However, neonatal fluoxetine treatment significant increases ADP phosphorylation capacity, RCR, CS activity, in addition to a remarkable decrease in RS production. Our results suggest that neonatal treatment with fluoxetine positively modulates male's mitochondria, decreasing the main factor responsible to mitochondrion and cellular damage, the reactive species in brainstem. The present study receives financial support from FACEPE.

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Poster

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Title: Fed and fasted brain glucose metabolism determined by imaging mass spectrometry

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Abstract: Glucose is the primary energy source for the brain and crucial for normal brain function. While in vivo imaging techniques may be used to assess where in the brain glucose is delivered, they do not provide information on how that glucose is metabolized. Knowledge of how glucose is utilized in specific brain regions is critical for understanding the mechanisms of diabetes-associated cognitive dysfunction, which is presumably being driven in part by recurrent hyper- and hypoglycemia. To generate a more comprehensive view of regional glucose metabolism we applied several molecular biology techniques to dissected brain regions, including immunohistochemistry, qPCR, western blotting and enzyme assays. In addition, measurement of multiple glucose metabolites across the brain, at a resolution of 100 microns, was performed using imaging mass spectrometry (IMS). By combining these techniques, we show clear differences in the regional fate of glucose in the brain, which are altered by the fasted state. Glucose can be metabolized by the glycolytic or pentose phosphate pathways. We show that there are low levels of hexose bisphosphate (a glycolytic intermediate) and high levels of the pentose phosphate pathway enzyme glucose-6-phosphate dehydrogenase (G6PD) in myelinated structures, such as thalamus. Conversely, the neuron-rich amygdala and cortex have low levels of G6PD, and high hexose bisphosphate as evidenced by IMS. The regional differences in glycolysis were not evident when comparing expression or activity of phosphofructokinase, the glycolytic enzyme responsible for generating hexose bisphosphate. Interestingly, ATP, the energy product primarily generated by glycolysis coupled to the TCA-cycle, was highest in several myelinated structures including the corpus callosum and internal capsule. Comparison of mice in the fed versus fasted state demonstrated similar regional differences in hexose bisphosphate and ATP but, a significant increase in lactate production in the cortex and amygdala was observed in the fasted state. These data demonstrate the importance of direct measurement of multiple metabolic intermediates across brain regions to determine the ultimate fate of glucose in health and disease states. IMS provides a powerful new tool for investigating the impact of changing glucose levels on brain homeostasis.

Disclosures: H.A. Ferris: None. A. Kleinridders: None. M.L. Reyzer: None. M. Rath: None. M. Soto: None. J. Spraggins: None. R.M. Caprioli: None. C.R. Kahn: None.

324. Energy Metabolism and Blood Brain Barrier

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 324.22/MM1

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Alpha-tocopherol exposure effect over weight and dyslípidaemia of obese infant rats induced by perinatal sucrose rich diet

Authors: *I. ZARCO DE CORONADO¹, M. A. HERRERA², M. J. GARCÏA³ ²BIOLOGIA CELULAR, ³FARMACOLOGÏA, ¹UNAM, Mexico DF, Mexico

Abstract: Perinatal administration of high rich sucrose diet (HSD) is associated with obesity, dyslipidemia and hippocampal damage in rat offsprings. Oxidative stress has been associated with these alterations. Alpha tocopherol is a well known antioxidant. The present project focused on the effects of this antioxidant over the high rich sucrose diet exposed infant rats. Two weeks pregnant Wistar rats were supplied with plane water (Control) or 20% sucrose solution divided in three experimental groups: E1 (born in time) and E2 (2 days delayed births). The third group E3 was supplied with sucrose solution with Alpha tocopherol (400U/100ml). Food, liquid solution ingested and body weight of dams and offsprings were determined twice by week. At 25 days of life the glucose, triglycerides and cholesterol were measured on blood samples. The controls animals drank around 40% liquid and the experimentals around 70%. The weight of the E3 offsprings was the same than controls and the E1, E2 groups were elevated (P=0.05). Experimental offsprings were hyperglycemic but not to a significant level. The triglycerides showed not significant increment in experimental offsprings. The cholesterol was elevated in the experimental groups with statistical significance (females P=0.05 and males P=0.001). These results suggest an antioxidant activity of alpha tocopherol blocking obesogenic effect of sucrose solution but not dyslipidaemia. We considerate that the differences may be a result of some placental, mammary gland or blood brain barrier factors.

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Poster

324. Energy Metabolism and Blood Brain Barrier

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 324.23/MM2

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: The PACAP and VIP receptor, VPAC2R, regulates glucose and fat metabolism at rest and during psychogenic stress in adult female C57BL6 mice

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Abstract: Several lines of evidence implicate PACAP as an important regulator of central and peripheral components of the stress axes (Hashimoto et al, 2011). PACAP also acts as an important hormonal regulator of lipid and carbohydrate metabolism (Giodanetto et al, 2013; Gray et al, 2001; Tomimoto et al, 2004, 2008). PACAP proves to act through a PACAP-specific receptor PAC1R and VIP/PACAP shared receptors, VPAC1R and VPAC2R. Unlike VPAC1R and PAC1R, VPAC2R is found in adipose tissue (Wei et al, 1996; Delgado et al, 1999). PACAP effects, mediated via VPAC2R signaling, produces lipolysis (Akesson et al, 2003) and improves glucose-dependent insulin secretion and insulin sensitivity (Ma et al, 2015). However, the results of insulin tolerance testing (ITT) in VPAC2R-deficient (VPAC2R KO) mice, which appear different in males and females, are inconclusive (Asnicar et al, 2002). Therefore, VPAC2R KO and wildtype (WT) female mice were used to further study the role of VPAC2R in glucose and fat metabolism in female C57B16 mice (Harmar et al, 2002, Tan et al, 2015). Mean basal glucose values were significantly greater in VPAC2R KO vs WT after 8 hr ON fast but not after 5 hr morning and 11 hr ON fast. Results from GTT (11 hr fast ON; 2.0 g/kg glucose) and weak ITT stimulation (5 hr morning fast, 0.75 UI) showed no effect of genotype. However, with stronger ITT (8 hr fast, 0.5 UI), VPAC2R KO females showed less insulin sensitivity at 60 min postinjection. Peak stress-induced hyperglycemia (immobilization, 1.5 hr) was more robust in VPAC2R KO mice vs WT: 320.6 and 229.8 mg/dL for KO and WT, respectively (p=0.019). The absence of group differences in stress-induced corticosterone levels suggests similar psychogenic stimulation however, VPAC2R KO but not WT showed greater stimulated plasma epinephrine (p<.05). The results suggest that abnormal VPAC2R gene expression may contribute to a diabetic phenotype that may be associated with increased sympathetic activity which has also been associated with PACAP signaling via PAC1R. The significance of intestinal permeability that may contribute to diabetic profiles was also examined (Turner, 2009). Mice received 60 mg/kg FITC-dextran. VPAC2R KO and WT mice showed moderate but similar gut integrity. Therefore, altered gut function and/or permeability are not likely the cause of abnormal glucose metabolism in VPAC2R KO mice. At sacrifice, mean normalized levels of liver lipids were no different in VPAC2R KO vs WT but the former showed an apparent reduction in normalized brown adipose fat wt (BAT, p=.083). Liver Oil Red O was noticeably less in VPAC2R KO mice. These results indicate that VPAC2R activity in female mice may participate in glucose and fat metabolism.

Disclosures: E. Kozlova: None.

324. Energy Metabolism and Blood Brain Barrier

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 324.24/MM3

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Neuroenergetic metabolome adaptation upon glucose and oxygen reduction

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Abstract: The brains energy demand is high as it accounts for about 20% of the body's energy consumption, however only for about 2% of the total body weight. In humans glucose is the brains obligatory energy substrate and is almost fully oxidized. Oxygen and glucose are essential for the mitochondrial energy metabolism in order to generate energy in form of adenosine triphosphate through the oxidative phosphorylation.

Cerebral hypoperfusion is characterized by a diminished blood flow through a tissue and strongly correlates with accelerated aging and advanced neurodegeneration. One of the most prominent features of hypoperfusion is glucose and oxygen reduction. Ischemia, tissue damage and poorly vascularized cancer can restrict oxygen supply leading to metabolic stress and potentially apoptosis.

Enhanced autophagic activity buffer metabolic stress and also contribute in maintaining the cellular energy balance. Thus, changes in glucose and oxygen availability induce an adaptive response that may allow for extended survival.

In our present study, we aimed to analyze the crosstalk between the energy homeostasis with autophagy in primary cortical neuronal culture. *In silico* pathway analyses revealed that several pathways including glycolysis, citric acid cycle, protein biosynthesis and mitochondrial electron transport chain are affected, apparent by changes not only in metabolite levels but also connected metabolite level ratios. In addition, cortical astrocytes contribute in the cellular adaptation towards glucose and oxygen reduction by enhancing glucose transporter protein levels. Moreover, the neuroenergetic needs are also counter balanced by significant changes in the autophagic degradation activity.

Taken together, our results implicate that in primary neuronal cultures the neuroenergetic needs are compensated by altered major energy metabolism pathways associated with autophagy.

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325. Blood Flow: Assessment and Basic-Translational Relevance

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

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Scholar Award from the McKnight Endowment Fund for Neuroscience

Title: Dissecting neuronal cell-type specific control of neurovascular coupling using DREADDs

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Abstract: Changes in local neural activity drive vasodilation and vasoconstriction of the cerebral vasculature, and understanding the cellular mechanisms of this control is important for interpreting hemodynamic signals. Previous work has suggested that activity of interneurons is the primary drivers of arteriole diameter changes (Cauli 2004; Uhlirova et al. 2016). To test the role of neural activity in manipulating vascular tone, we virally expressed DREADDs (Designer Receptors Exclusively Activated by a Designer Drug) pan-neuronally, or exclusively in excitatory neurons in the somatosensory cortex of awake mice, allowing us to bi-directionally manipulate neural activity. We assayed resting vascular tone and vascular responses to voluntary locomotion (Gao and Drew 2016; Gao, Greene, and Drew 2015) using two-photon microscopy. Suppressing the activity of all neurons caused a decrease in the resting diameter and a decreased hemodynamic response relative to vehicle injections, while suppressing the activity of only excitatory neurons had no effect. This work suggests that interneuron activity controls hemodynamic tone and responses during natural behaviors.

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325. Blood Flow: Assessment and Basic-Translational Relevance

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Title: Optical coherence tomography reveals spatio-temporal dynamics of capillary stalls in awake mice

Authors: *E. ERDENER¹, J. TANG¹, A. SAJJADI¹, C. B. SCHAFFER², D. A. BOAS¹ ¹Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp., Charlestown, MA; ²Biomed Engin., Cornell Univ., Ithaca, NY

Abstract: Objective: Optical coherence tomography (OCT) allows label-free capillary imaging with high spatio-temporal resolution and can be utilized to understand the details of capillary dysfunction and small vessel disease. Capillary stalls were previously shown to occur in myeloproliferative disease models with increased circulation of red blood cells. In this work, using OCT microangiography, we studied the stall dynamics within cerebral microcirculation in awake healthy mice. Methods: C57BL/6 mice (4-6-month-old) with chronic cranial windows were trained to stay attached to a cradle. Imaging was done in trained mice without anesthesia and during rest. In each mouse, 600x600 µm areas were imaged, using a spectral-domain OCT system (Axial and transverse resolution: 3.5 µm, 10x objective). For each area, 60 angiograms were acquired with ~9 second interval. Raw data were processed to yield the maximum intensity projection images of capillaries. ~200 segments were observed in each region, 150-250 µm below the cortical surface. We manually counted stalling segments indicated by abrupt drops in intensity to get their prevalence, frequency and duration. Capillary RBC velocities were measured using dynamic light scattering OCT in CD1 mice (2-3 month-old) with acute windows, under isoflurane anesthesia. Results: In awake mice (n=7), 7.5 \pm 2.5% of all capillaries within the imaging area were stalling during any given 9 minute imaging period, each stall lasting for 15±5 seconds. At each time point, 0.5+0.4% segments in a region were stalled. In areas with a higher prevalence of stalls, each segment was stalling more frequently and/or for a longer duration $(R^2=0.42)$. When imaging was extended to 18 min, more stalling segments could be detected,

with ~70% of all stalling segments detectable within the first 9 min. When imaging was repeated after one month, stall incidence in the same cortical area remained constant and 45-50% of stalling capillaries were coincident in the repeated sessions. Segments with a higher stall frequency in the first session were more likely to be detected to stall during the next session (P<0.001). More than 80% of stalling segments were at the venous side of the microcirculation, with lower RBC velocities than nonstalling segments in the same cortical region (P<0.001). Conclusion: These data suggest that capillary stalls in the brain in awake mice are very frequent and consistent observations. These events can be imaged and quantified by OCT angiography in an extremely efficient manner. Investigation of the frequency, distribution and duration of capillary stalls can be a promising tool for a greater understanding of diseases of microcirculation.

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Poster

325. Blood Flow: Assessment and Basic-Translational Relevance

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: CDMRP (DoD): W81XWH-15-1-0138

Title: Microvascular effects following traumatic ocular injury

Authors: *A. M. MELCHIADES NOZIMA¹, J. I. CASTRO², M. LEDO², M. HARPER⁵, S. MARTINEZ-CONDE³, S. L. MACKNIK⁴

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Abstract: Blast mediated injuries from Improvised Explosive Devices are strongly associated with severe ocular morbidity and visual impairment and are the leading cause of combat-related injuries in Operation Enduring and Iraqi Freedom. Eye-related injuries cost an estimated \$2.2B in medical care per year. Residual physical symptoms reported after blast exposure includes headache, nausea, light and noise sensitivity and visual disturbances, and visual outcomes from a direct closed-globe eye injury, or complications resulting from the Traumatic Brain Injury, are understudied. In this study, we documented both *in vivo* microvascular dysfunction in the retina as a function of a blast, but also explored the effects of a therapeutic intervention to halt/reserve the degeneration. The objective of this study was to determine if clinically available blood flow regulating drugs-currently not used to treat retinal damage-may serve to ameliorate retinal

degeneration in those who experienced blast-related traumatic injuries. We used 80 mice split into three cohorts related to how long after the blast (7 days, one month or four months) they were studied and a control group. Our hypothesis is that when arterial flow is pathologically reduced, for example by ocular injury, all of the flow in the downstream capillary beds is reduced uniformly. We tested this hypothesis with Confocal Laser Endomicroscopy (CLE) *in vivo* imaging to examine the blood flow through the arteriolar vessels and into capillary beds. We used the same techniques that we have used before when studying epilepsy models in mice. This technique also identifies mural cells such as pericytes, which have the ultimate active control point for blood flow and regulate non-uniform blood flow in capillary beds. Dysfunctional mural cell-driven non-uniform blood flow can then lead to cell death due to the failure of local oxygenation gradients within the capillary bed. Our hypothesis concerning the mechanistic pathway of action of nitric oxide precursors (i.e. L-Arginine) is that they improve capillary blood flow and prevent ischemia/hypoxia by dilating arterioles and microvessels that would otherwise vasospasm or restrict due to TOI. CLE revealed a significantly different vasospasm rate among cohorts, which are postulated as deriving from mural cell dysfunction in TOI.

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Poster

325. Blood Flow: Assessment and Basic-Translational Relevance

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American Heart 16SDG27190001 (KZ)

Title: Sex differences in changes in the vasculature of the subventricular zone niche with age

Authors: *K. L. ZULOAGA¹, C. S. BJORNSSON², E. WAIT³, W. MANKOWSKI³, Y. WANG², A. R. COHEN³, S. TEMPLE²

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Abstract: In the adult brain, neurogenesis occurs in the subventricular zone (SVZ) lining the lateral ventricles, but declines markedly with aging. Blood vessels within the SVZ play a vital

role in the neural stem cell niche by promoting proliferation, neurogenesis and guiding the chains of migrating neuroblasts. Here we sought to determine how the structure of this vascular stem cell niche differs between sexes and how it changes with age. Mouse SVZ wholemounts at 2, 18 and 22 months were immunostained for laminin to label blood vessels and doublecortin to label neuroblasts. Using 3D computer-based image analysis, we assessed changes in vessel diameter, vessel tortuosity and organization of the neuroblast chains. In addition, we used uptake of EdU, a thymidine analog, to assess progenitor cell proliferation. We found that vessel tortuosity increases slightly with age, yet sex differences do not emerge until 22 months of age, when males have more torturous vessels than females. Vessel diameter in the SVZ changes with age in a sex-dependent manner. Young females have smaller vessel diameters than males, however, their vessel diameters increase between 18 and 22 months of age. Conversely, in males, vessel diameters steadily decrease with age. Thus, although young males start with larger vessels than females, by 18 months the males have smaller vessels, a difference that is exacerbated by 22 months. With regard to the progenitor cells, the chains of migrating neuroblasts become disorganized and less linear at 18 months but surprisingly this organization improves by 22 months, especially for the females. While both sexes show a decline in proliferation with age, as expected, females have significantly less proliferation than males at 18 months. In conclusion, we have found complex, sex-dependent changes in the SVZ niche vasculature with age that correlate with changes in proliferation of progenitor cells.

Disclosures: K.L. Zuloaga: A. Employment/Salary (full or part-time):; Albany Medicial College. 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.; National Institue of Aging. **C.S. Bjornsson:** A. Employment/Salary (full or part-time):; Zeiss. **E. Wait:** A. Employment/Salary (full or part-time):; Drexel University. **W. Mankowski:** None. **Y. Wang:** A. Employment/Salary (full or part-time):; Neural Stem Cell Institute. **A.R. Cohen:** A. Employment/Salary (full or part-time):; Drexel University. **S. Temple:** A. Employment/Salary (full or part-time):; Drexel University. **S. Temple:** A. Employment/Salary (full or part-time):; Drexel University. **S. Temple:** A. Employment/Salary (full or part-time):; Drexel University. **S. Temple:** A. Employment/Salary (full or part-time):; Drexel University. **S. Temple:** A. Employment/Salary (full or part-time):; Drexel University. **S. Temple:** A. Employment/Salary (full or part-time):; Drexel University. **S. Temple:** A. Employment/Salary (full or part-time):; Drexel University. **S. Temple:** A. Employment/Salary (full or part-time):; Drexel University. **S. Temple:** A. Employment/Salary (full or part-time):; Drexel University. **S. Temple:** A. Employment/Salary (full or part-time):; Drexel University. **S. Temple:** A. Employment/Salary (full or part-time):; Drexel University. **S. Temple:** A. Employment/Salary (full or part-time):; Drexel University. **S. Temple:** A. Employment/Salary (full or part-time):; Drexel University. **S. Temple:** A. Employment/Salary (full or part-time):; Drexel University. **S. Temple:** A. Employment/Salary (full or part-time):; Drexel University. **S. Temple:** A. Employment/Salary (full or part-time):; Drexel University. **S. Temple:** A. Employment/Salary (full or part-time):; Drexel University. **S. Temple:** A. Employment/Salary (full or part-time):; Drexel Universit

Poster

325. Blood Flow: Assessment and Basic-Translational Relevance

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Title: Optogenetic stimulation of pericytes lacking alpha smooth muscle actin produces a decrease in capillary blood flow *In vivo*

Authors: *D. A. HARTMANN¹, R. I. GRANT¹, S. A. HARRILL², T. NOONAN², A. Y. SHIH¹ ¹Neurosciences, Med. Univ. of South Carolina, Charleston, SC; ²Col. of Charleston, Charleston, SC

Abstract: Vascular mural cells surrounding the cerebrovasculature are responsible for modulating blood supply in accordance with the metabolic demands of the brain. It is widely accepted that vascular smooth muscle cells on arterioles can regulate blood flow. However, whether pericytes can control blood flow at the level of capillaries remains highly debated. To test the hypothesis that pericytes throughout the capillary bed have the capacity to influence blood flow, we stimulated individual pericytes in vivo using two photon excitation of channelrhodopsin (ChR2), as done previously (Hill, et al. Neuron, 87(1):95-110, 2015). Although the PDGFRBeta-Cre promoter was used to drive ChR2 expression in both smooth muscle cells and pericytes, two photon optogenetics enabled spatially-precise stimulation of pericytes on capillaries and not vascular smooth muscle cells of upstream arterioles. Simultaneously, with the same two photon laser used for pericyte excitation, we measured capillary diameter and red blood cell velocity after labeling the blood plasma with intravenous fluorescent dextrans. We found that pericyte excitation for 60 seconds produced, on average, a ~20% reduction in capillary diameter and blood cell velocity throughout the cortical microvasculature (1-9 branch points distal to the penetrating arteriole), which included territories that lacked alpha smooth muscle actin, a key component of canonical vascular contractile machinery. In addition, we found that blood flow remained halted in a large proportion of vessels (~40%) even 1 minute after stimulation. Importantly, identical stimulation parameters did not produce hemodynamic changes in control mice expressing cytosolic YFP or membrane-bound GFP in pericytes. Further, the observed decrease in vessel diameter and blood cell velocity in response to pericyte ChR2 stimulation was inhibited when we applied fasudil, a Rho kinase inhibitor and clinically-used vasodilator, to an intact dura. Our results suggest that pericytes, even those in territories without alpha smooth muscle actin, have the capacity to modulate blood flow. Considering that 20% diameter decreases and sustained stoppages in capillary flow are not typically seen with normal brain capillaries in vivo, our findings may provide new insight into how pericytes respond during pathologies such as stroke or traumatic brain injury (Yemisci, et al. Nat Med 15(9):1031-7, 2009; Hall, et al. Nature 508(7494):55-60, 2014). In these and other disease states, excessive depolarization of pericytes throughout the capillary bed may lead to poor brain perfusion.

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Poster

325. Blood Flow: Assessment and Basic-Translational Relevance

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Title: Oxygen-extractor, an efficient software to measure partial pressure of oxygen with 2-photon phosphorescence lifetime imaging

Authors: *E. CHAIGNEAU¹, M. ROCHE¹, M. BARRETT², B. WEBER², S. CHARPAK¹ ¹Inserm U1128, Paris, France; ²Inst. of Pharmacol. and Toxicology, Zurich, Switzerland

Abstract: During the last decade, several sensors based on oxygen-dependent quenching of phosphorescence have been developed to measure brain oxygenation. Some of them can be combined to 2-photon microscopy to measure partial pressure of oxygen (pO₂) at micrometer resolution *in vivo*.We used 2-photon phosphorescence lifetime microscopy (2PLM), in the olfactory bulb of anesthetized rodents, to measure vascular pO₂ and flow in control conditions and during odor stimulation. The high spatial resolution of 2PLM revealed the presence of erythrocyte associated transients (EATs) in capillaries (Lecoq et al. 2011, Parpaleix et al. 2013) and allowed to determine the physiological values of pO₂ in brain vessels and neuropil of awake mice (Lyons et al. 2016). All our data were acquired and analyzed with a custom-built software named Oxygen Extractor and written in LabVIEW and MATLAB.

We describe here the experimental paradigms and the algorithms used by Oxygen Extractor to acquire data and calculate red blood cell (RBC) flow, hematocrit and pO_2 at a chosen set of points in the brain. We have developed two data acquisition methods. The first one is a sequential method, where the 2-photon beam is parked at one point for a given acquisition time, and then moved to another point for the same acquisition time, and so on, until the whole set of points has been explored. The second one is a "quasi-synchronous" method that we call the "kinetic mode". In this mode, the beam is moved from one acquisition point to another, every 250-500 μ sec, the whole sequence of points being repeatedly explored during the acquisition time. In addition to that, the laser power can be adjusted at each individual point. The analysis functions developed allow measuring the mean pO_2 as well as pO_2 as function of time or of the

distance to individual RBCs (required to calculate EATs). Experimental data stress the importance of using pO_2 calibration curves that accurately match the experimental *in vivo* conditions. Oxygen Extractor allows to incorporate such calibration curves, either provided by the chemists or built on site, i.e. below the objective. Finally, the Oxygen Extractor software will be accessible on our laboratory web site with all the information required to realize accurate pO_2 measurements in the rodent brain.

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Poster

325. Blood Flow: Assessment and Basic-Translational Relevance

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Coupling of dentate granule cell activity and micro-vessel hemodynamics *In vivo* regulates adult hippocampal neurogenesis

Authors: *J. SHEN¹, D. WANG², G. KIRSCHEN¹, Q. XIONG¹, J. XIA², S. GE¹ ¹The Dept. of Neurobio. & Behavior, State Univ. of New York At Stony Brook, Stony Brook, NY; ²Dept. of Biomed. Engin., Univ. at Buffalo, Buffalo, NY

Abstract: The adult hippocampus continuously generates new dentate granule cells in response to ongoing neuronal activity within its circuits. A variety of external factors known to enhance adult neurogenesis, such as exposure to enriched environments (EEs), are also known to elevate the activity of mature dentate granule cells (DGCs). However, how hippocampus-related stimuli and behavior impact adult neurogenesis remains largely unknown. Given that the generation and integration of new dentate granule cells is metabolically costly, and that local blood flow dictates nutrient and oxygen supply, we hypothesized that DGC activity would directly recruit an increased local blood supply to support EE-induced enhanced neurogenesis. Here, we used a miniature microscope to detect both neuronal activity and local blood flow in micro-vessels simultaneously in the dentate gyrus (DG) of freely behaving mice. We discovered that the activity of DGCs is coupled to local micro-vessel hemodynamics. Interestingly, we observed a temporal delay of blood flow velocity elevation in comparison to the onset of increased neuronal firing. Furthermore, we found that the increase in blood flow paralleled the potentiation in neuronal activity, albeit with a more prolonged kinetic profile. Importantly, this increase of micro-vessel flow velocity was attenuated by both chemogenetic and pharmacological approaches, which preliminary evidence suggests may abolish experience-induced enhanced neurogenesis. Together, our results reveal a new mechanism whereby the adult hippocampal

circuit regulates its local metabolic environment in conjunction with its neuronal activity to promote neurogenic homeostasis.

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Poster

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Title: Statistical modeling of cerebral vessel morphometry and blood flow in wildtype and tissue-plasminogen activator deficient mice

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Abstract: BACKGROUND: The serine protease tissue-plasminogen activator (tPA), classically known for its role regulating fibrinolysis, is also expressed on the abluminal side of the vasculature in the CNS. Though tPA has been implicated in a variety of neurological processes, its intimate association with the vasculature and its activity-dependent release properties were shown to be coupled in a study demonstrating tPA^{-/-} mice to have an attenuated functional hyperemia response following whisker-barrel stimulation. Recently, however, an analysis of the cerebral vasculature in adult tPA^{-/-} mice demonstrated significant reorganization of vessel architecture, compared to their C57BL/6J wildtype littermate controls, with tPA^{-/-} mice having a reduction in larger smooth-muscle covered vessels and an increase in smaller capillary blood vessels.

OBJECTIVE: Therefore, to determine if the previously reported attenuated neurovascular coupling response is an indirect consequence of architecture or a direct consequence of tPA's action, a statistical approach to modeling the cortical capillary morphometry and blood flow in wildtype and tPA^{-/-} mice was taken.

METHODS: To confirm the report of aberrant cerebrovasculature architecture in tPA^{-/-} mice and

to gather the necessary statistics for blood flow modeling, a more comprehensive vessel analysis was done using SeeDeepBrain (SeeDB) clearing technology. 500uM thick slabs of brain tissue were cleared from tPA^{-/-} mice and their wildtype counterparts and vessel architecture was analyzed after doubly labeling the vasculature with a FITC-gelatin cast and a FITC-tomato lectin glycocalyx intercalating dye.

To model blood flow, instead of using specific measured capillary cortical networks, statistics on capillary diameter, length, and blood flow were reproduced by creating an ensemble of bifurcating branching networks.

RESULTS: In a preliminary analysis of vessel architecture, tPA^{-/-} mice were found to have significantly more blood vessels than wildtype mice. In addition, baseline cortical blood flow measurements using laser speckle revealed tPA^{-/-} mice to have a significantly higher basal rate of blood flow than their wildtype controls, which is consistent with the observed differences in vascular architecture and suggests that the denser capillary bed in tPA^{-/-} mice results in a functionally elevated basal blood flow rate.

CONCLUSIONS: This data will provide important insights into how the cerebrovasculature architecture can affect blood flow regulation, which has implications for the role attributed to tPA in neurovascular coupling.

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Poster

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Endo Pharmaceutical Investigator Initiated Grant

Title: Early progressive and preferential vulnerability and reorganization among subsets of cutaneous microvascular innervation in diabetic monkeys and humans especially involving different populations of sensory fibers that contain the vasodilatory calcitonin gene-related peptide

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Abstract: Major complications of diabetes include peripheral neuropathy and a breakdown in cutaneous microvascular circulation particularly affecting distal limbs (i.e. "stocking/glove") resulting in loss of sensation, often accompanied by chronic pain, and in gangrenous deterioration requiring amputation. Yet, surprisingly little is known about the normal components and distribution of cutaneous microvascular innervation and how it affected during the onset and progression of diabetes. Herein, we used large skin specimens from the hands of aging Rhesus monkeys to conduct multi-molecular immunofluorescence microscopic profiles of the microvasculature and its innervation in normal monkeys and those that naturally developed type 2 diabetes. The non-vascular tactile innervation had previously been profiled in the same specimens (see Paré et al., J. Comp. Neurol. 2007, 501:543 for methodological details). Having profiled neurovascular pathologies in these large specimens, we used the same multi-molecular "ChemoMorphometric Analyses" (INTiDYN CMA) to profile the microvasculature and its innervation in small 3mm punch biopsies taken without consequence from the lateral margin of the foot in cohorts of nondiabetic human volunteers (n=16) and diabetic patients with painful (n=29) and non-painful (n=12) diabetic neuropathy (PDN vs NPDN) who had no imminent loss of skin integrity. Procedures were approved by the Albany Medical College IRB. Cutaneous microvascular arterioles, precapillary aretioles, and even capillaries have a well known extensive C-fiber sensory innervation that contains the potent vasodilatory peptides CGRP and Substance P whose role has mostly been attributed to normal anti-inflammatory responses. Otherwise, most normal vasoregulation has focused on vasoconstrictive action of noradrenergic sympathetic innervation, with vasodilatation largely viewed as passive under reduced sympathetic activity. In nearly all of the diabetic monkey and humans, nearly all of the CGRP innervation was absent first on the AVS and then on the arterioles regardless of the diabetes duration, beginning perhaps as early as metabolic syndrome. By contrast, the sympathetic innervation persisted even in long term diabetes. Moreover, the CGRP innervation increased among capillaries in the papillary dermis while a *de novo* innervation with cholinergic characteristics gradually appears among the precapillary arterioles. These results indicate that there are distinct functional subsets of CGRP that are uniquely vulnerable or persistent during the onset and progression of diabetes that could be targeted to prevent subsequent loss of skin integrity.

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Poster

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: The problem of inversion of coupling function-metabolism-blood flow in the brain

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Abstract: On the basis of analysis the results of own acute and chronic experiments on rats and the published data the issues of adequate local blood flow changes in the brain, caused by changes in functional activity - a phenomenon known as coupling function - metabolism - blood flow is described. In particular, the issues of regulation of local cerebral blood flow in condition of so-called nonworking brain, in cases of sensory stimulation, functional loading, changes in emotional tension and motor activity. The correlation of local blood flow and electrical activity of the brain is also analyzed. The special attention is paid to the problems of maintenance of a homeostatic range of cerebral blood supply and caused by its breach the inversion of mentioned coupling and its transformation into a new one - blood flow - metabolism - function with possible disturbances in the functioning of the brain.

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Poster

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

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We would like to acknowledge David Kleinfeld, Philbert Tsai and Pablo Blinder for kindly sharing their anatomical data with us.

Title: Structural and hemodynamic comparison of synthetic and anatomical cerebral capillary networks

Authors: *A. SMITH, M. PEYROUNETTE, A. LARUE, V. DOYEUX, Y. DAVIT, S. LORTHOIS

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Abstract: A computational method is presented for generating 3D synthetic, random capillary networks which match the topological, geometrical and functional properties of the cerebral

microcirculation. This enables production of larger capillary networks than can currently be extracted using high-resolution imaging modalities. These networks can then be coupled to lower-resolution data sets of whole-brain vasculature (capillaries unresolved) to model blood flow and mass transport, and to validate equivalent continuum/hybrid models. Another motivation is to reveal the dominant structural features of cerebral capillary networks, enabling us to tune these features to model different brain regions or pathological states such as Alzheimer's disease. Previous works (Linninger et al, Ann Biomed Eng, 2013; Su et al, Microcirc, 2012) lacked physiological basis, and although resulting networks conformed to expected global morphometric properties, they were not subjected to thorough topological or functional analysis.

In contrast, our approach is based on the physiological assumption that the maximum separation of tissue cells from the nearest capillary is limited by the diffusion distance of oxygen (Lorthois & Cassot, J Theor Biol, 2010). Previously, synthetic, space-filling 2D networks were constructed by placing one point randomly in each cell of an $n \times n$ grid; from this set of points, Voronoi diagrams were extracted with the edges producing a 2D capillary network with mainly three capillaries per vertex, a characteristic feature of cerebral capillary networks. Here, we present a 3D extension of this approach and compare the resulting structural and hemodynamic properties to those of anatomical cerebral capillary networks.

In 3D, Voronoi diagrams produce polyhedrons with many capillaries at each vertex. To derive a network with only bifurcations, clusters of vertices were systematically merged and capillaries were then randomly removed. The resulting network structures were compared to capillary regions extracted from human and mouse anatomical data sets (Cassot et al, Microcirc, 2006; Tsai et al, J NeuroSci, 2009; Blinder et al, Nat Neurosci, 2013), showing excellent agreement. Geometrical metrics included the mean/S.D. of capillary lengths and edge/length/vertex densities. To measure the interconnected network topology, capillary loops were identified and the mean number of edges per loop, loop length, and number of loops per edge were compared. The spatial arrangement of capillaries was compared by studying the distribution of extravascular distances. Finally, the permeability was computed as a hemodynamic measure of blood flow conductivity.

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Poster

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: ERC Consolidator BrainMicroFlow 615102

CALMIP Projet P1541

Title: How do capillary occlusions impact brain microcirculation in Alzheimer's disease? Numerical Simulations and Experimental validation

Authors: *M. BERG¹, M. PEYROUNETTE², J. C. HERNANDEZ³, O. BRACKO³, M. HAFT JAVAHERIAN³, V. DOYEUX², A. SMITH², Y. DAVIT², M. QUINTARD², N. NISHIMURA³, C. B. SCHAFFER³, S. LORTHOIS²

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Abstract: The most common form of dementia is Alzheimer's disease, making it a major health problem. While studied intensively, no cure or even clear diagnostic procedures have been found yet (Nelson JNEEN 2012). A recent study (Iturria-Medina Nat Com 2016) has shown that a significant decrease in blood flow arises in the onset of Alzheimer's disease. In vivo two photon microscopy of cortical vasculature in APP/PS1 mouse models suggests that the mechanism underlying this blood flow reduction is capillary occlusions due to leucocytes adhering to inflamed blood vessel walls (Cruz-Hernandez Neuroscience meeting 2016). However, these techniques generate massive amounts of anatomical and functional data that are difficult to interpret without proper theoretical and numerical frameworks. Here, we develop a model describing blood flow in large microvascular networks that accounts for complex blood rheology (hematocrit-dependent viscosity and non-linear phase separation at bifurcations, Pries Microvasc Res 1989). We validate it by comparison with in vivo measurements in mice and extrapolate the model to humans. We then quantify the impact of capillary occlusions on regional perfusion for both species.

The numerical simulations, based on an iterative algorithm derived from Newton's fixed point method (Lorthois, Neuroimage 2011) are run in 4 post-mortem mice anatomical networks with more than 10,000 vessels each acquired by two-photon imaging (Tsai J Neuroscience 2009). For human we use a dataset composed of 300 μ m thick slices (over 20,000 vessels per slice) acquired by confocal microscopy (Cassot Microcirculation 2006). We correct post-mortem vessel variations in both dataset using shape-preserving transformations based on available in vivo measurements.

We validate our model and corrections by direct comparison with in vivo red blood cell velocity measurements performed in mice (Santisakultarm AJPHCP 2012, Taylor JCBFM 2016). We then investigate, for both humans and mice, the impact of an increasing percentage of capillary occlusions on regional perfusion, defined as the sum of flows in all arterioles supply the studied domain. We observe a clear linear dependence in both species, with similar slopes and no threshold effect. Our results imply that even a small percentage of occluded vessels (2-4%) yield a drastic reduction of regional perfusion (5-12 % in mice and humans), which is of the same order of magnitude as observed experimentally in APP/PS1 mice. Finally, we show that these slopes are controlled by the capillary network connectivity and spatial distribution of occlusions across the network, while only slightly affected by uncertainties on vessel diameters.

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Poster

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

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Title: Hypoxia-induced vascular responses in the adult mouse brain

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Abstract: Mild continuous hypoxia is a phenomenon observed in several central nervous system (CNS) conditions including diabetic retinopathy, chronic hypoperfusion, and Alzheimer's disease (AD). Pericytes, mural cells that cover capillaries, become dysfunctional and degenerate in ischemic stroke, diabetic retinopathy and AD. Pericytes are vital orchestrators of key neurovascular functions including blood-brain barrier (BBB) integrity, cerebral blood flow (CBF), and angiogenesis. Increased CBF and angiogenesis are physiological responses in brain elicited by a mild hypoxic state. The transcriptional regulation of these structural/functional hypoxic responses has never before been investigated in the adult brain, nor has its corresponding impact on brain microvascular health. Adult wild-type mice exposed to mild continuous hypoxia up to 21 days show a 60% increase in capillary diameter at day 3 and 53% increase in red blood cell velocity by day 7 in the somatosensory cortex using longitudinal multiphoton imaging. Immunohistochemistry reveals an increased regional rate of microvascular density, suggesting that some brain regions are more susceptible to mild hypoxia. Additionally, RNA-sequencing analysis of differentially expressed genes from brain microvascular cells after mild continuous hypoxia reveals significantly upregulated expression of the Gene Ontology categories including cytoskeleton reorganization, cellular response to stress, cell division, etc. Furthermore, since pericyte-deficient mice are reported to have disrupted oxygen availability and BBB dysfunction, pericyte deficiency is hypothesized to impair the normal physiologic response to mild continuous hypoxia in adult brains. Altogether, elucidating the transcriptional, structural and functional responses to mild continuous hypoxia in the adult brain is vital to inform therapeutic efforts to combat hypoxic insults and/or microvascular dysfunction in numerous CNS disorders such as diabetic retinopathy, chronic hypoperfusion, and AD.

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Poster

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Title: Perivascular macrophages mediate the cerebrovascular and cognitive dysfunction in DOCA-salt hypertension

Authors: *M. M. SANTISTEBAN, G. FARACO, G. RACCHUMI, J. ANRATHER, C. IADECOLA Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY

Abstract: Hypertension (HTN) is a leading risk factor for stroke and dementia. About half of patients with HTN are salt-sensitive, and a high sodium diet is an additional risk factor for these devastating neurovascular diseases. Deoxycorticosterone (DOCA)-salt HTN is a recognized model of HTN driven by sodium retention and brain renin-angiotensin system (RAS) activation. However, it is unknown whether essential mechanisms regulating the cerebral circulation are altered in DOCA-salt mice, and, if so, whether these alterations are associated with cognitive impairment. To this end, C57BL/6 mice were implanted with 50mg DOCA pellets SQ and received 0.9% NaCl drinking water for 3 weeks. Cerebral blood flow (CBF) was measured in the somatosensory cortex by laser-Doppler flowmetry through a cranial window. DOCA-salt increased systolic blood pressure (BP; 148 \pm 3 vs 112 \pm 3 mmHg in controls; p<0.01), and attenuated the CBF increase induced by whisker stimulation (WS; 16.0 \pm 1.1 vs 22.4 \pm 0.6 %; p<0.01) or by neocortical application of acetylcholine (ACh; 13.5 \pm 0.9 vs 22.8 \pm 1.1 %; p<0.01), without affecting the response to the smooth muscle relaxant adenosine (p>0.05). This cerebrovascular dysfunction was associated with cognitive impairment shown by decreased

exploration of the novel object (52.7 ± 1.7 vs 71.6 ± 1.2 % total exploration time; p<0.01) and time spent in the target quadrant during Barnes Maze (37.8±2.8 vs 49.9±1.6 sec; p<0.01). Perivascular macrophages (PVM) express AT1 receptors and Nox2, and, as such, may be a key source of radicals mediating the cerebrovascular effects of brain RAS overactivity. To test this hypothesis, brain PVMs were depleted by icv administration of clodronate (CLO) liposomes. BP was not affected by CLO in either control or DOCA mice (p>0.05). PVM depletion improved both novel object exploration time (p<0.01) and time spent in the target quadrant of Barnes Maze (p<0.05), while also restoring the CBF responses to both WS (DOCA-CLO 19.6±0.9%; p<0.05) and ACh application (DOCA-CLO 20.0±1.7%; p<0.05). Next, we tested whether reactive oxygen species (ROS) are involved in the cerebrovascular dysfunction. We observed a 45% upregulation in gp91 mRNA expression in isolated cerebral vessels from DOCA mice, which was prevented by PVM depletion (p<0.05). Furthermore, application of the ROS scavenger MnTBAP rescued CBF responses to both WS ($20.3\pm0.9\%$; p<0.05) and ACh ($19.0\pm0.8\%$; p<0.05) in DOCA-salt HTN. We conclude that PVMs play an important role in the cerebrovascular and cognitive dysfunction of DOCA-salt HTN. PVMs may have a unique role in mediating the detrimental effects of HTN on the brain, and could therefore become a novel cellular target for therapy.

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Poster

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

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Center for Healthy Aging, Copenhagen University

Rigshospitalet Glostrup, Dept of Clinical Neurophysiology

Title: Increased cerebral blood flow response due to specific optgenetic stimulation of parvalbumin positive interneurons

Authors: M. K. DAHLQVIST, *K. J. THOMSEN, M. J. LAURITZEN TransNeurogroup, Univ. of Copenhagen, Copenhagen, Denmark

Abstract: Fast spiking parvalbumin positive (PV+) interneurons spike with a frequency up to 200 Hz. The gamma activity in the brain (30-90 Hz) is an interaction between PV+ interneurons

and pyramidal neurons, where synchronization of PV+ interneurons allows the pyramidal cells to fire within specific time intervals. Fast spiking is energy consuming and is therefore greatly dependent upon energy substrates supplied by the blood. We hypothesise that gamma activity per se induces cerebral blood flow (CBF) increases during states of increased neuronal activity and energy demand.

3-5-month-old transgenic mice with channelrhodophsin-2 (ChR2) -expressing PV+ interneurons were used. The mice were stimulated in three ways: by whisker pad stimulation (15 s, 2 Hz, 1.5 mV, 1 ms pulses), optogenetic laser light (473 nm, 15 s, 100 Hz, 2 mW, 7.5 ms pulses), and both simultaneously. Tissue partial pressure of oxygen, total extracellular electrical activity and CBF responses were monitored. Blockers of neuronal signalling were applied in the following order: MK-801 (300 μ M) (N-methyl-D-aspartate receptor (NMDAR) blocker) + NBQX (200 μ M) (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) blocker); Gabazine (20 μ M) (synaptic gamma-aminobutyric acid receptor (GABAR) blocker); and lastly, TTX (20 μ M) (tetrodoxin).

Optogenetic stimulation of PV+ interneurons resulted in a CBF response independent of pyramidal neuron activation. MK-801 + NBQX abolished the CBF response to whisker pad stimulation as expected, but did not affect the CBF response to light stimulation. It also abolished gamma activity during all three stimulation types. Subsequent synaptic GABAR blockade augmented the CBF response to light stimulation. CMRO₂ (cerebral metabolic rate of oxygen) responses to optogenetic stimulation paralleled the CBF responses. All responses were abolished when TTX was applied. Light stimulation alone induced much less gamma activity than either whisker pad stimulation or simultaneous light + whisker pad stimulation. We found that CBF and CMRO₂ responses to light stimulation of PV+ interneurons are dependent on neuronal events, but are not dependent on NMDAR and AMPAR activation or on gamma activity and thereby the interaction with PV+ interneurons. When synaptic GABA was blocked, CBF and CMRO₂ responses to light and light + whisker pad stimulation increased, possibly due to decreased inhibitory tone. We conclude that PV + interneuron activation but not gamma activity is necessary and sufficient to induce CBF and CMRO₂ responses.

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Poster

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: SUVN-I6107: A novel muscarinic M1 receptor positive allosteric modulator (M1-PAM) addresses cholinergic side effects

Authors: *A. K. SHINDE, S. YATHAVAKILLA, A. VUYYURU, R. MEDAPATI,, G. VENKATA RAMALINGAYYA, J. TADIPARTHI, N. GANUGA, N. MUDDANNA, S. DARPELLI, V. UTHUKAM, D. SISODAYA, N. PATIBANDLA, M. SRIRANGAVARAM, V. MEKALA, R. SUBRAMANIAN, V. GOYAL, K. BOJJA, S. KOMMINENI, S. MANCHINEELLA, R. NIROGI Suven Life Sci., HYDERABAD, India

Abstract: Improving the cholinergic neurotransmission reduces the symptoms of AD. Thus, modulating the cholinergic system through muscarinic receptors could be a viable option for developing new treatments for Alzheimer's disease (AD). Recently published literature suggests that positive allosteric modulator of muscarinic M1 receptor causes cholinergic side effects, which may limit their clinical use. SUVN-I6107 is one of our lead M1-PAM. It was evaluated for its binding potential at the orthosteric and allosteric site of muscarinic receptors and its ability to potentiate the effect of acetylcholine in the reporter gene assay. The pharmacokinetics and brain penetration was evaluated in rodents. The efficacy of SUVN-I6107 was evaluated in animal models of cognition. The effect on the modulation of soluble amyloid precursor protein- α $(sAPP-\alpha)$ was studied in rat cortex. Effect on the neuronal oscillations was evaluated in combination with donepezil. Cardiovascular safety was assessed using the patch clamp technique. The cholinergic side effects were evaluated in mice and dogs (gastro intestinal transit and salivation). SUVN-I6107 is a M1-PAM with no agonistic activity. It has good selectivity over closely related muscarinic receptor subtypes M2 to M5. It has adequate water solubility, and was found to be orally bioavailable (67%) in rats with good brain penetration and free fraction. SUVN-I6107 reversed the time as well as scopolamine induced memory deficit in the object recognition task. It also potentiated the effects of donepezil and promoted non-amyloidogenic APP processing in rats. SUVN-I6107 also enhanced the cerebral blood flow in rats. At the therapeutically effective dose, SUVN-I6107 potentiated the effects of donepezil on elicited hippocampal theta levels in rats. SUVN-I6107 did not affect the colonic/ gastro-intestinal transit in rats or dogs. No notable changes were observed in the salivation. SUVN-I6107 was found to be safe when tested in hERG patch clamp assay and in early stage animal toxicity studies. SUVN-I6107 demonstrates robust efficacy in animal models without cholinergic side effects

Disclosures: A.K. Shinde: A. Employment/Salary (full or part-time):; Suven Life Sciences. S. **Yathavakilla:** A. Employment/Salary (full or part-time):; Suven Life Sciences. A. **Vuyyuru:** A. Employment/Salary (full or part-time):; Suven Life Sciences. R. Medapati,: A. Employment/Salary (full or part-time):; Suven Life Sciences. G. Venkata Ramalingayya: A. Employment/Salary (full or part-time):; Suven Life Sciences. J. Tadiparthi: A. Employment/Salary (full or part-time):; Suven Life Sciences. N. Ganuga: A. Employment/Salary (full or part-time):; Suven Life Sciences. N. Muddanna: A. Employment/Salary (full or part-time):; Suven Life Sciences. S. Darpelli: A. Employment/Salary (full or part-time):; Suven Life Sciences. V. Uthukam: A. Employment/Salary (full or part-time):; Suven Life Sciences. D. Sisodaya: A. Employment/Salary (full or part-time):; Suven Life Sciences. N. Patibandla: A. Employment/Salary (full or part-time):; Suven Life Sciences. N. Patibandla: A. Employment/Salary (full or part-time):; Suven Life Sciences. N. Patibandla: A. Employment/Salary (full or part-time):; Suven Life Sciences. N. Patibandla: A. Employment/Salary (full or part-time):; Suven Life Sciences. N. Patibandla: A. Employment/Salary (full or part-time):; Suven Life Sciences. N. Patibandla: A. Employment/Salary (full or part-time):; Suven Life Sciences. **R. Subramanian:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **V. Goyal:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **K. Bojja:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **S. Kommineni:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **S. Manchineella:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **R. Nirogi:** A. Employment/Salary (full or part-time):; Suven Life Sciences. **R.**

Poster

325. Blood Flow: Assessment and Basic-Translational Relevance

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 325.17/MM20

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: IBS-R015-D1

Title: Acute administration of haloperidol increases evoked CBF and BOLD fMRI to the somatosensory stimulation in anesthetized rats

Authors: *Y. KIM¹, K. LEE¹, S. HAN¹, J. SON¹, S.-G. KIM^{1,2} ¹Ctr. for Neurosci. Imaging Res., Inst. for Basic Sci. (IBS), Suwon, Korea, Republic of; ²Biomed. Engin., Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: Dopaminergic neurotransmission modulates sensory process, but the effects of the antipsychotics which is known as a dopamine antagonist on the sensory modulation were still controversial. Since the fMRI research in schizophrenia field increases rapidly, it is critical to examine the effect of commonly-used antipsychotics haloperidol to neurovascular coupling and BOLD fMRI. Haloperidol was introduced into rats either acutely (0.2mg/kg, I.V) or chronically (consecutive 21 days 0.2mg/kg per day, I.P), and electrical forepaw stimulation (1.0-1.5mA, 10Hz, 0.1ms, 2-5sec) was performed under isoflurane anesthesia (~1.5%). We measured evoked CBF (cerebral blood flow) response using LDF (laser Doppler flowmetry) in the primary somatosensory cortex (S1), BOLD fMRI to determine blood oxygen level changes. To eliminate the effects of physiological changes due to drug administration, physiological measurements including body temperature, blood pressure, and heart rate were observed carefully during entire experiments. No significant physiological change was detected. Acute haloperidol administration did not change baseline CBF in S1, but increased evoked CBF in S1 to the somatosensory stimulation. Interestingly, evoked CBF response in the chronic group was not increased as much as that in the acute group. Similar observation was detected in the fMRI BOLD experiment. To determine whether the change in vascular responses observed in acute haloperidol administration is related to neural activity, we are currently measuring local field potentials in S1. Our initial results show that the effect on the sensory modulation of dopamine antagonist haloperidol is closely dependent on the frequency of drug usage, as implicated in the previous

electrophysiology studies. BOLD fMRI in schizophrenia should be carefully interpreted in the context of type, dose, and frequency of antipsychotic drugs.

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Poster

325. Blood Flow: Assessment and Basic-Translational Relevance

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01NS078168

NIH Grant R01NS079737

Scholar Award from the McKnight Endowment Fund for Neuroscience

Title: Noradrenergic modulation of neurovascular coupling in awake behaving mice

Authors: *Q. ZHANG¹, K. W. GHERES², P. J. DREW³

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Abstract: Hemodynamic signals are widely used to infer neural activity in functional brain imaging techniques (e.g., fMRI). Our previous work (Huo et al., 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 mechanistically understand this decoupling. We hypothesized that neuromodulatory input, specifically a noradrenergic (NA) vasoconstrictory signal, interact with neural activity-driven vasodilation to shape the hemodynamic response. Here, we investigated the NA modulation of neurovascular coupling by measuring hemodynamic responses (intrinsic optical signal imaging) and neural activity (multilaminar linear electrode arrays) to voluntary locomotion in awake, head-fixed mice. Noradrenergic tone was decreased or increased by systemic administration of antagonists of a1-(prazosin) and α 2-adrenoceptors (atipamezole), respectively. Compared to vehicle controls, cerebral blood volume changes during locomotion were increased by prazosin in both the frontal and somatosensory cortices, while atipamezole decreases the amplitude the locomotion-related response in the somatosensory cortex. These results suggest that in addition to local vasodilatory signals released from neurons and astrocytes, changes in neuromodulatory tone (especially noradrenergic tone) play an important vasoconstrictory role in shaping hemodynamic signals during behavior. Furthermore, laminar neural activity distribution in the somatosensory cortex shown that increasing NA tone decreased local spiking in layer II/III, while decreasing NA tone

increased local spiking in layer II/III, both at rest and during locomotion. These results further suggest that the NA modulatory effects on hemodynamic signals are due to a cortical layer-specific modulation of neural activity.

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Poster

325. Blood Flow: Assessment and Basic-Translational Relevance

Location: Halls A-C

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Grant-in-Aid for Scientific Research (KAKENHI) (16K10716)

Title: Vasodilation induced by pinacidil are attenuated in early vascular injury after subarachnoid hemorrhage on rat cerebral penetrating arterioles

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Abstract: Early brain injury (EBI) following subarachnoid hemorrhage (SAH), which refers to the acute injuries to the whole brain before onset of delayed cerebral ischemia, is the most important contributor to poor outcome in patients with SAH. EBI is characterized by a severe reduction in cerebral blood flow suggesting alterations on the level of cerebral small vessels. The early vascular injury in small vessels after SAH demonstrated a rapid change in structure, however, vasoactive dysfunction via potassium channels is unclear. This study was therefore conducted to clarify whether SAH induced immediate potassium channels dysfunction in rat experimental SAH model. The institutional animal ethics committee in Shinshu University School of Medicine approved all experimental protocols for this study. In this study, SAH was induced using a blood injection method into the cisterna magna of male rat, and saline injection models were also made. Sham surgery rats as control received the same surgical procedures except for the injection. Rats were sacrificed at 1 hour after SAH, saline injection and sham surgery. The penetrating arterioles from the middle cerebral artery were isolated, cannulated and pressurized. Vessel diameters were recorded by computer-aided videomicroscopy. After development of vascular tone, to investigate the ATP-dependent potassium channels function, the activator pinacidil was applied. The penetrating arterioles from SAH and saline injection models developed significantly more tone compared with the arterioles from sham. The ATPdependent potassium channels activator significantly dilated the penetrating arterioles from sham surgery rats. The vasodilatory responses to the activator were attenuated in SAH rats but not saline injection rats. In conclusion, ATP-dependent potassium channels may inactivate

immediately after SAH, and the dysfunction may be induced by acute increased intracranial pressure. These results provide a background to understand the early vascular injury after SAH.

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Poster

325. Blood Flow: Assessment and Basic-Translational Relevance

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Program#/Poster#: 325.20/NN1

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R00AG047198

Title: Altered intracellular calcium reactivity of cerebral artery endothelial tubes with advancing age

Authors: M. A. HAKIM, *J. N. BUCHHOLZ, E. J. BEHRINGER

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Abstract: Intracellular Ca^{2+} ($[Ca^{2+}]_i$) signaling in cerebral artery endothelial cells coordinates smooth muscle cell relaxation facilitating cerebral blood flow. However, studies of endothelial function are limited by the presence of confounding factors (e.g., perivascular nerves, blood flow, hormones) arising from intact blood vessel applications. Also, isolated cells in culture manifest significantly altered morphology and ion channel expression relative to physiological conditions. The aim of the current study was to characterize intact vascular endothelium freshly isolated (≤ 1 hr of being within the animal) from middle and posterior cerebral arteries of Young (4-7 mo), Middle (13-16 mo), and Old (24-27 mo) male and female C57BL/6 mice ($n \ge 5$; continuous superfusion of PSS at pH 7.4 & 37°C). Using Fura-2 photometry (~100 cells), we tested the hypothesis that aging increases the magnitude of cerebral endothelial $[Ca^{2+}]_i$ responses in response to a physiological G-protein coupled receptor agonist (ATP) and the oxidizing agent H₂O₂. A cerebral endothelial tube varied in length from 0.5 to 3 mm, whereby diameter was not significantly altered with age (Young: $108\pm12 \,\mu$ m, Middle: $105\pm7 \,\mu$ m, Old: $120\pm7 \,\mu$ m; mean \pm SEM) or gender. With resting $[Ca^{2+}]_i$ in the range of 100 nM to 300 nM, increases in $[Ca^{2+}]_i$ were typically up to ~500 nM during treatment with the purinergic receptor agonist ATP (100 μ M, 5 min) and highest at μ M concentrations following H₂O₂ (200 μ M, 15 min). With general trends (P>0.05) for greater increases in females vs. males across age groups, Δ [Ca²⁺]_i to ATP and H₂O₂ peaked at middle age (P<0.05; \approx two-fold vs. Young) and decreased by \approx 30% (P>0.05) in Old. These data suggest that middle age sets the stage for enhanced $[Ca^{2+}]_i$ signaling, thereby increasing the Ca²⁺ homeostasis burden of internal organelles (ER and mitochondria). Further, intracellular measurements of V_m during respective treatments demonstrated hyperpolarization of membrane potential (V_m) across groups consistent with activation of small- and intermediate Ca^{2+} -activated K⁺ channels (or SK_{Ca}/IK_{Ca}) with NS309 (ΔV_m of -5 mV to -50 mV from a resting V_m of \approx -30 mV to -40 mV). As $[Ca^{2+}]_i$ and oxidative signaling modulates SK_{Ca}/IK_{Ca} activity and vasodilation, these findings provide insight into endothelial cell regulation of cerebral blood flow with advancing age and the potential development of neurodegenerative disease. This work has been supported by NIH grant *R00AG047198* (EJB).

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Poster

325. Blood Flow: Assessment and Basic-Translational Relevance

Location: Halls A-C

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

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ARUK (ARUK_IRG-2014-10)

MRC (MR/M013553/1)

Title: Uncovering the contribution of cortical interneurons to neurovascular coupling - an optogenetic approach

Authors: *C. HOWARTH, L. LEE, C. CHRISTMAS, N. VAUTRELLE, L. BOORMAN, P. SHARP, E. BRACCI, J. BERWICK Dept. of Psychology, Univ. of Sheffield, Sheffield, United Kingdom

Abstract: Neurovascular coupling is a vital brain mechanism ensuring that neuronal energy demands are met by dynamic changes in local blood flow. This mechanism underlies functional neuroimaging techniques such as BOLD fMRI and may be dysfunctional in neurodegenerative diseases.

Inhibitory interneurons have previously been shown to induce vasodilation and constriction in the brain^{1,2,3}, thus making them a potential cellular driver of neurovascular coupling. Furthermore, interneuron dysfunction has been implicated in a number of neurodegenerative diseases^{4,5,6}.

We have combined a cell-specific optogenetic approach with 2-dimensional optical imaging spectroscopy (2D-OIS) and electrophysiology in anaesthetized mice to investigate how two populations of cortical interneurons (those expressing somatostatin or NOS) contribute to neurovascular function. Using transgenic mice expressing channelrhodopsin-2 within these neuronal subpopulations enables neuronal activation by light with high spatial and temporal specificity. Light activation was performed with and without somatosensory stimulation. Evoked

cortical surface haemodynamic changes were measured concurrently with neural activity as a function of cortical depth, allowing us to define the roles of somatostatin-expressing and NOS-expressing interneurons in neurovascular coupling in the healthy brain.

By understanding the influence of these cells on normal network physiology and haemodynamic correlates we can not only begin to decipher how interneurons may contribute to disease processes but also improve our ability to interpret perfusion-based neuroimaging signals. References:

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- 3. Uhlirova H., et al. (2016) Elife 5: e14315
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Poster

325. Blood Flow: Assessment and Basic-Translational Relevance

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: MRC Grant MR/M013553/1

ERUK Grant P1501

Title: Insights into anti-seizure mechanisms of focal cerebral cooling through multi-modal assessment of the interaction between cortical temperature and neurovascular function

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Abstract: Active manipulation of brain temperature is routinely used for cerebral protection during brain surgery and is being investigated as a therapeutic intervention for various pathologies, such as to reduce the effects of seizures in drug-resistant epilepsy or for the prevention of seizures following traumatic brain injury. However little is known of the effects of temperature changes on brain physiology, especially perfusion-related mechanisms which are often disrupted in neuropathological conditions. To address this research gap, we used a multi-

modal neuroimaging approach to investigate neurovascular coupling in the brain of the urethaneanesthetized rat under normothermia and hypothermia. Intrinsic cortical temperature changes were assessed initially during normothermia, with neural and hemodynamic changes evoked through sensory stimulation (16s, 5Hz, 1.2mA), graded hypercapnia (5 and 10%) and recurrent acute focal neocortical seizures induced by infusion of 4-aminopyridine (4-AP, 15mM, 1µl). Concurrent neural activity was recorded alongside cortical temperature, tissue oxygenation, blood flow and hemoglobin concentration. Cortical temperature was found to increase significantly (p < 0.01) during sensory stimulation (0.19±0.02°C), 5% hypercapnia (0.89±0.11°C), 10% hypercapnia (1.46±0.19°C), and recurrent seizures (1.78±0.08°C). A novel system was developed to accurately manipulate the temperature of the cortical surface via superfusion of fluid across a thinned cranial window overlying the somatosensory cortex. Temperatures were maintained above and below normal physiological levels (6 to 40°C), while simultaneously recording neural and hemodynamic changes. Early findings suggest a complex interplay in stimulus evoked neuro-vascular coupling responses across different temperatures. A slowing of the onset of stimulus evoked functional hyperaemia was observed for smaller reductions in cortical temperature ($\sim 20^{\circ}$ C), with attenuation of neural and hemodynamic responses observed during greater levels of cooling (~10°C). Ongoing work will involve the induction of seizures by infusion of 4-AP, followed by cortical hypothermia at a range of temperatures. These findings uncover both the temperature shifts occurring in response to neurovascular changes and the neurovascular alterations which occur during active temperature manipulation. This offers insight into the route of function of various hypothermic therapies and will be relevant to the interpretation of pre-clinical functional neuroimaging data in health and disease.

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Poster

326. Metabolism Control and Obesity

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 326.01/NN4

Topic: F.10. Food Intake and Energy Balance

Title: Functional connectivity of the pituitary gland associated with body mass index

Authors: *T. IKUTA¹, P. RUCKER²

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Abstract: The pituitary gland (PG) plays the central role in the endocrinological function of the central nervous system. The hormones secreted by the PG include somatotrophin (HGH) and oxytocin, which are associated with growth and body weight. However, the relation between

body weight and PG-brain interaction remains unclear. Here, we aimed to isolate the functional connectivities of the PG that are associated with body weight, using resting state functional magnetic imaging (rsfMRI) data. Using enhanced Nathan Kline Institute-Rockland Sample, rsfMRI data from 495 individuals were analyzed to assess functional connectivity of the PG in voxel-wise fashion. The pituitary gland ROI was manually defined in the MNI 2mm space centered approximately at [MNI: 0, 2, -32] (Figure 1: Red). A negative correlation was found between pituitary gland functional connectivity and BMI in eleven different brain regions including posterior thalamus, lingual gyrus, precuneus, right superior temporal gyrus, right middle temporal gyrus, right pallidum, hippocampus, pons, orbitofrontal cortex, left temporal pole, and left inferior frontal gyrus (Figure 1: Blue). Our results show that disconnectivity to these regions is associated with higher BMI, implicating that the connectivity between these regions and PG may regulate body weight maintenance, including feeding behavior and growth, and may contribute to obesity.



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Poster

326. Metabolism Control and Obesity

Location: Halls A-C

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Program#/Poster#: 326.02/NN5

Topic: F.10. Food Intake and Energy Balance

Support: DK075062, DK075064

Title: Glutamatergic neurons mediate the effects of Brs3 on regulation of energy metabolism

Authors: *C. XIAO¹, *C. XIAO¹, R. A. PINOL², J. CARLIN³, A. SAHA⁴, O. GAVRILOVA³, M. REITMAN⁵

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Abstract: Great efforts are being made to understand the causal mechanisms of and treatments for obesity. Bombesin-like receptor 3 (BRS3) is an orphan G protein-coupled receptor expressed in multiple hypothalamic regions and a few other sites in the brain and in the periphery. The global Brs3 knockout mouse is obese, with increased food intake and reduced resting metabolic rate, body temperature, and heart rate. To dissect the functions of Brs3, we developed two conditional mouse models, floxed Brs3 and loxTB-Brs3, which allow selective deletion or restoration of Brs3, respectively. These mice were bred with existing mouse lines to investigate the necessity and sufficiency of Brs3 in glutamatergic (Vglut2-Cre expressing) and GABAergic (Vgat-Cre expressing) neurons for regulation of energy homeostasis. Mice with Brs3 ablation in Vglut2, but not Vgat, neurons became obese on high fat diet (HFD), and showed reduced effect of the BRS3 agonist MK-5046 to inhibit food intake and increase metabolic rate. Conversely, mice with selective restoration of Brs3 in Vglut2 neurons had normalized food intake and wildtype levels body weight on the HFD. Restoration of Brs3 in Vgat neurons did not alter the obese phenotype, resembling that of the global knockout. Similarly, restoration of Brs3 in Vglut2, but not Vgat, neurons, recovered the ability of MK-5046 to inhibit food intake and increase resting metabolic rate. The effects of Brs3 deletion and restoration in chow-fed mice were generally consistent with, but milder than those observed in HFD-fed mice. The mice with increased adiposity typically had worse glucose tolerance and insulin resistance, consistent with an effect of the increased fat mass. These data demonstrate that the Brs3 located in Vglut2 neurons is both necessary and sufficient to maintain normal food intake, body weight and adiposity, metabolic rate and insulin sensitivity. In contrast, Brs3 in Vgat neurons (and other places) appears to be neither necessary nor sufficient for regulation of these aspects of energy homeostasis.

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Poster

326. Metabolism Control and Obesity

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Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant 5R01-MH096875-02

Title: Investigating the genetic structure of complex phenotypes in free-ranging rhesus macaques

Authors: *S. MADLON-KAY¹, M. J. MONTAGUE¹, N. SNYDER-MACKLER², J. E. HORVATH³, L. J. N. BRENT⁴, J. SKENE⁵, M. L. PLATT¹ ¹Univ. of Pennsylvania, Philadelphia, PA; ²Univ. of Washington, Seattle, WA; ³North Carolina Central Univ., Durham, NC; ⁴Univ. of Exeter, Exeter, United Kingdom; ⁵Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: Free-ranging populations of non-human primates (NHPs), like humans, live in dynamic social environments where managing both resources and relationships is crucial for survival. Because such environments are difficult to reproduce in a laboratory setting, freeranging NHP populations provide a potentially powerful model for understanding how genetic and environmental factors shape complex phenotypes. However, genetic variation in many complex phenotypes is driven by small contributions across many genetic variants, which require large sample sizes to estimate reliably. Free-ranging NHP populations for which genetic information is available may be too small to identify the effects of individual genetic variants using traditional approaches. This poses an obstacle for identifying specific neurobiological genetic pathways that contribute to a particular phenotype. Here we take an alternative approach by using recently developed computational methods that increase statistical power by aggregating effects across large numbers of genetic variants within pathways or gene sets of interest. These methods are sometimes known as "realized relatedness" analyses, as they use genomic data to examine overall genetic similarity between individuals and compare it to phenotypic similarity. By allowing genetic similarity in different regions of the genome to contribute differentially to a phenotype, it is possible to identify specific gene sets or pathways that contribute to the phenotype.

We investigated whether this approach could be used to uncover neurogenetic pathways that contribute to complex phenotypes in a large, free-ranging population of rhesus macaques (*Macaca mulatta*) on Cayo Santiago Island, for which large amounts of genetic, behavioral, and morphological data are readily available. We focused on obesity-related morphological phenotypes, including height, weight, and abdominal and subscapular skinfold thickness, as obesity is a pathology with neurological influences that is highly heritable in humans. In order to measure genome-wide and pathway-specific genetic similarity, we generated whole genome sequences for 217 individuals and identified over 19 million single-nucleotide variants. We assessed overall heritability of these morphological phenotypes as well as the specific contributions of neurobiological gene sets that have been implicated in obesity in humans, namely gene sets involving neurotrophin, hedgehog, and glutamate signaling (Locke et al, 2015). These methods may provide a path towards using free-ranging NHP populations to study the neurobiological basis of complex behaviors such as sociality.

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326. Metabolism Control and Obesity

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Topic: F.10. Food Intake and Energy Balance

Support: NIH-R01-DA012976

NIH-U19-DA027990

Title: Nicotinic acetylcholine receptors containing the β 2 subunit regulate body weight in mice

Authors: *G. DEZFULI, T. OLSON, B. SIEGARS, N. SAHIBZADA, R. GILLIS, K. J. KELLAR, 20057 Pharmacol. and Physiol., Georgetown Univ., Washington, DC

Abstract: Nicotine, the primary psychoactive component of tobacco smoke, has been well documented to have weight-reducing properties in rats and mice, and similar effects are seen with other drugs that target the nicotinic acetylcholine receptor (nAChR). Moreover, we recently reported that drugs that selectively desensitize the nAChR containing the ß2 subunit, such as Sazetidine-A (SAZ-A) exert robust weight reducing effects in rats and mice (Hussmann et al., 2014; Dezfuli et al., 2016). One purpose of the present study was to further test the involvement of B2-containing nAChRs as a major receptor-mediated mechanism by which nicotine lowers body weight and suppresses feeding. To this end, we investigated whether or not chronic nAChR blockade (functionally akin to chronic receptor desensitization) via the use of the selective $\alpha 4\beta 2$ nAChR competitive antagonist dihydro-beta-erythroidine (DHBE) produces weight-reducing effects similar to SAZ-A. An additional goal of the present study was to test the hypothesis that endogenous tonic activity of nAChRs containing the ß2 subunit drives a state of positive energy balance characterized by weight gain. To test this hypothesis, we characterized the body weight phenotype of 8-week old male mice with conventional knockout of the β 2 subunit (β 2^{-/-}) in comparison to littermate controls ($\beta 2^{+/+}$) over a 7-week monitoring period. Results from the present studies demonstrate: (a) DHBE robustly suppresses weight gain in C57BL6/J mice fed a high fat diet, in comparison to saline treated controls [F (10,150) = 2.26, p<0.05] without any concomitant change in dark-cycle locomotor activity, and (b) 8 week-old male $\beta 2^{-/-}$ mice have significantly reduced weight gain in early adulthood compared to littermate controls [F (2,115) = 6.47, p<0.01]. Together, these findings indicate a potentially prominent role for nAChRs containing the ß2 subunit as intrinsically active regulators of body weight. Furthermore, these studies highlight the nAChR containing the β 2 subunit as a potential new drug target site for obesity pharmacotherapy.

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Poster

326. Metabolism Control and Obesity

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 326.05/NN8

Topic: F.10. Food Intake and Energy Balance

Support: University of Zurich

Title: Transient and selective overexpression of dopamine 2 receptors in striatal medium spiny neurons promotes diet-induced obesity

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Abstract: Abnormal dopaminergic signaling in the striatum has been repeatedly associated with obesity. Clinical investigations have outlined changes in dopamine 2 receptor (D2R) availability within the striatum in obese individuals, although the direction of such changes remains controversial and may depend on the severity of the obesity phenotype. In addition, numerous animal investigations have shown that obesity and/or high-fat diet (HFD) feeding can cause a reduction in striatal D2R binding and expression. Yet it remains unclear whether preexisting changes in striatal D2R levels can per se promote the development of obesity. Recent work has shown that D2R knock-out in indirect-pathway medium spiny neurons (MSNs) reduces locomotion but does not lead to obesity, nor does it affect food intake or energy expenditure in mice, even when fed HFD. In our study, we made the opposite hypothesis, postulating that an upregulation of D2R in MSNs may increase vulnerability to obesity. This was based on recent clinical work suggesting that genetic propensity for greater dopamine signaling may predict future weight gain in lean or modestly obese individuals. We used a transgenic mouse model where D2R levels were specifically upregulated within the striatum; in all MSNs, but not in cholinergic neurons or interneurons. We showed that D2R-overexpression (D2R-OE) robustly increased body weight gain and body fat when fed high fat diets as compared to control mice. We also identified multiple metabolic impairments in D2R-OE mice in the form of glycemic intolerance, hyperlipidemia and hyperinsulinemia. Because striatal D2Rs have been implicated in abnormal reward behavior and eating, we speculated that obesity vulnerability in D2R-OE mice may be due to increased food intake. Surprisingly, D2R-OE mice were hypophagic. They did, however, demonstrate early reductions in energy expenditure following HFD feeding, which were followed by later decreases in daily locomotor activity, supporting the idea that energy

output, rather than input, was causing obesity. We then wondered whether increased obesity in D2R-OE mice was due to ongoing elevated activity of striatal D2Rs in adulthood or whether it may emerge as a result of developmental overexpression of D2Rs. We therefore restricted overexpression of striatal D2Rs to development, and showed that this manipulation was in fact sufficient to promote obesity. Overall, these findings indicate that striatal D2R upregulation may be causally implicated in the development of obesity, and may do so through developmental mechanisms. This work should provide a better understanding of the clinical observations linking striatal D2Rs and obesity.

Disclosures: M.A. Labouesse: None. C. Kellendonk: None. U. Weber-Stadlbauer: None.

Poster

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Topic: F.10. Food Intake and Energy Balance

Support: NSERC

Title: Investigating the role of ghsr signalling in the dmh and pmv on energy homeostasis

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Abstract: Ghrelin is a gut peptide hormone that increases food intake and adiposity. The orexigenic effects of ghrelin depend on binding to its endogenous receptor, the growth hormone secretagogue receptor (GHSR). The GHSR is highly expressed throughout the peripheral and central nervous system, and in particular within the hypothalamus, a region of the brain important in homeostatic processes. Two regions within the hypothalamus, the dorsomedial hypothalamus (DMH) and ventral premammilary nucleus (PMV), show GHSR expression, yet the effects of ghrelin on these regions remains to be elucidated. The DMH is important for many energy balance processes, such as regulating brown fat thermogenesis, food intake, circadian rhythms, and the generation of the stress response, while the PMV is thought to be an integrative centre, owing to its responsiveness to reproductive, metabolic, and social signals. The current experiments aim to identify how altering GHSR signaling in the DMH, or the PMV, alters metabolic processes in mice. In experiment 1, male C57BL6J mice were equipped with a minipump filled with either saline, the GHSR antagonist JMV 2959 (2 µg/day/28days) or ghrelin (10 µg/day/28days) attached to a cannula aimed at the DMH (AP 1.55mm, ML 0.25mm, and DV 5.25 mm). Following surgery, food intake and body weight was monitored for a week before animals were housed in metabolic chambers for 48 hours to determine differences in metabolism

using indirect calorimetry. They were then allowed a recovery period before being tested for glucose clearance using the glucose tolerance test (GTT). The same design was used in Experiment 2, but this time, cannulae were aimed at the PMV (AP 2.45mm, ML 0.3mm, and DV 5.4mm). Our results show that infusions of ghrelin into the PMV but not the DMH affects fuel utilization, while infusions of ghrelin into the DMH but not the PMV affects body weight and fat accumulation. Overall, these results suggest that while GHSR signaling is important for energy homeostasis in both the DMH and PMV, specific metabolic effects are selectively mediated in a region-specific manner.

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Poster

326. Metabolism Control and Obesity

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Program#/Poster#: 326.07/NN10

Topic: F.10. Food Intake and Energy Balance

Title: p75^{NTR} involvement in metabolic control pathways

Authors: *T. P. REMCHO¹, L. M. SIPE², D.-A. JOHNSON², J. EPHREM², C. DEPPMANN¹ ¹Biol., ²Univ. of Virginia, Charlottesville, VA

Abstract: Obesity levels present an urgent public health challenge. The extensive sympathetic innervation of adipose tissue provides a target for treatment. This innervation regulates energy liberation from adipocytes. We have identified a putative protein, integral in the regulation of lipolytic events, the p75 neurotrophin receptor (p75^{NTR}). This protein is expressed in several tissue types and binds a wide variety of neurotrophins. A lack of p75^{NTR} was previously shown to reduce weight gain in response to a high fat diet by increasing the rate of energy liberation from fat stores. Here, we show that mice deficient in p75^{NTR} lose less weight due to restricted caloric intake. This data suggests p75^{NTR} modulates energy homeostasis and does not solely upregulate lipolysis. We aim to understand the mechanism by which p75^{NTR} maintains energetic homeostasis. To elucidate the cell-specific function of p75^{NTR}, we subjected conditional knockouts of p75^{NTR}, in sympathetic neurons and adipocytes, to caloric restriction and analyzed their weight loss. Additionally, the rate of norepinephrine turnover was shown to differ in mice deficient in p75^{NTR}; this begins to elucidate the mechanistic role of p75^{NTR} in our weight-loss resistant phenotype. The understanding of neural mechanisms underlying energetic homeostasis in adipose tissue suggests the possibility for neuromodulation as an avenue for weight control via alteration to energetic homeostasis.

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Poster

326. Metabolism Control and Obesity

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Title: Selective activation of regionally distinct catecholamine neurons in the ventrolateral medulla is sufficient for elicitation of key glucoregulatory responses in normoglycemic rats

Authors: *S. RITTER, Q. WANG, A.-J. LI

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Abstract: Acute glucose deficit is a potentially lethal condition. Hindbrain catecholamine (CA) neurons are required for elicitation of protective responses to acute glucose deficit, as shown by selective immunotoxin lesions, localized gene silencing and localized nanoliter injections of the glucoprivic agent, 5-thioglucose. However, whether selective activation of CA neurons is sufficient to elicit these responses is not known. In addition, questions remain regarding the regional distribution of neurons that mediate specific glucoregulatory responses. To address these questions, we virally transfected a Cre-dependent designer receptor exclusively activated by a designer drug (DREADD) construct, AAV2-DIO-hSyn-hM3D(Gq)-mCherry, at one of 4 rostrocaudal levels of the VLM in Th-Cre transgenic rats: rostral C1 (C1r), middle C1 (C1m), the area of A1 and C1 overlap (A1/C1) and A1. Transfection was highly selective for CA neurons at each injection site. We measured feeding, blood glucose, glucagon and corticosterone (Cort) responses to activation of transfected CA neurons following systemic injection of the DREADD receptor agonist, clozapine-N-oxide (CNO). Glucagon was not significantly elevated by CNO in any transfected rat. Feeding was increased in rats transfected at C1r, C1m or A1/C1 sites. Cort secretion was elicited most effectively in rats transfected at A1/C1 or C1m. Neither feeding nor Cort responses were elicited from A1 transfected rats. Blood glucose was not increased in any of these single-site transfected rats, but was increased in rats transfected simultaneously at two adjacent sites (C1m and C1r). Consistent with the blood glucose results, CNO increased c-Fos expression in the spinal intermediolateral column and adrenal medulla only in rats transfected at both C1m and C1r. These results reveal for the first time that selective activation of C1 CA neurons is sufficient to increase feeding, blood glucose and Cort secretion, even in the absence of glucoprivation. The results also suggest that CA neurons mediating blood glucose responses are

sparsely but widely distributed in rostral half of C1, while those mediating feeding and Cort secretion are continuously distributed along C1, but are concentrated in the caudal and middle C1.

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Poster

326. Metabolism Control and Obesity

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Topic: F.10. Food Intake and Energy Balance

Support: CAPES

CNPq

INCT-INNT

Title: The glucose sensitivity of mesencephalic cells: Effect on tyrosine hydroxylase regulation

Authors: *A. C. REGO¹, Y. PAES COLLI², D. BECKMAN³, L. E. SANTOS⁴, S. T. FERREIRA¹, F. DE MELLO², I. E. DE ARAUJO⁵, R. D. REIS² ¹Lab. of Neurochemistry, Rio De Janeiro, Brazil; ²Federal Univ. of Rio de Janeiro, Rio de Janeiro, Brazil; ³California Natl. Primate Res. Ctr., UC Davis, Davis, CA; ⁴UFRJ, Rio de Janeiro, Brazil; ⁵The J. B. Pierce Lab. and Yale Univ. Sch. of Med., New Haven, CT

Abstract: Different lines show that the expression of preferences for highly palatable foods depends on the gastrointestinal and/or metabolic effects produced by caloric foods. It remains to be determined how the central nervous system converts the sensing of metabolic signals into behavioral preferences. Previous studies show that the midbrain dopaminergic system is a central mediator of food preference formation, given its critical role in the expression of normal feeding behaviors. It is thus of great interest the identification of the physiological pathways allowing the midbrain dopaminergic system to sense metabolic post-ingestive effects. Our results show that cultured dopaminergic neurons are highly sensitive to glucose levels. Exposing cultured murine mesencephalic neurons to hypoglycemic media, or to the glucose anti-metabolite 2-DG, suppressed dopamine levels. Moreover, we show in adult mice that intraperitoneal injections of glucose, but not of 2-DG, 30 minutes after were sufficient to increase the phosphorylation of tyrosine hydroxylase residues Ser40 (2.4-fold) and Ser31 (1.5-fold), molecular events critical for dopamine synthesis. Finally, increases in Ser40 phosphorylation levels were closely associated with increases in blood glucose levels induced by the intraperitoneal injections. Our results suggest that midbrain dopaminergic regions may be activated by metabolic stimuli such as increased glycemic levels.

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Poster

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Topic: F.10. Food Intake and Energy Balance

Support: NIH R01DK102918

Brazilian National Council of Technological and Scientific Development-CNPq

Title: Long-term sucrose consumption increases excitability of NPY neurons in the arcuate nucleus of the hypothalamus

Authors: *K. O'CONNELL¹, K. ABREU², W. WEI¹, A. SMITH³, J. H. CARDOSO⁴ ¹The Jackson Lab., Bar Harbor, ME; ²Superior Inst. of Biomed. Sci., State Univ. of Ceara, Fortaleza, Brazil; ³Genetic, Genomics, and Informatics, Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; ⁴Univ. Estadual do Ceara, FORTALEZA, Brazil

Abstract: Obesity has been a major world-wide public health problem for the past 40 years associated with co-morbidities such as hypertension, type 2 diabetes, dyslipidemia, heart disease and stroke. Although caused by excessive food intake, obesity is associated with dysfunction of appetite circuits in the brain, especially in the arcuate nucleus of hypothalamus (ARH), that regulate energy balance. We previously demonstrated that high fat diet (HFD) is associated with increased spontaneous firing in ARH NPY neurons, independent of weight gain. High sugar intake is also associated with obesity and diabetes. This study investigated the effect of high sugar intake on both mouse metabolic parameters and the excitability of ARH NPY neurons in acute brain slices using whole cell current clamp. Male hrGFP-NPY mice were maintained on control chow diet, and given either regular drinking water or water containing 10% sucrose (w/v) starting at 6 weeks of age and continuing for 12 weeks (long term) or 2 days (short term). Analysis of food and water intake in the 12 week sucrose cohort revealed that although these mice drank significantly more water, there was no difference in food intake, resulting in an overall increased calorie intake, although this was not associated with a significant increase in body weight. In both the glucose and insulin tolerance tests, the sucrose group had elevated blood glucose compared to controls, consistent with a pre-diabetic state. To determine the impact of increased sucrose consumption on neuronal excitability, we used whole-cell current clamp to assess the electrical properties of ARH NPY neurons. We found that 12 weeks of sucrose water was associated with depolarization of the membrane from -65.82 ± 7.31 mV (control) to $-55.94 \pm$ 1.44 mV (sucrose) with no change in input resistance (995.1 \pm 109.5 M Ω (control) to 917 \pm 157

 $M\Omega$ (sucrose)). Sucrose intake was also associated with an increase in the spontaneous firing frequency from 0.1972 \pm 0.1929 Hz (control) to 1.128 \pm 0.2089 Hz (sucrose), with a corresponding decrease in interspike interval following long term exposure. Unlike HFD, we found that 2d of sucrose water had no impact on resting membrane potential, input resistance, or spontaneous firing rate. Taken together, these data suggest that long term high sugar intake leads to a pre-diabetic state and to a significant increase in spontaneous firing in NPY neurons without obesity.

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Poster

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Support: NIH R01 DK61935 (MJT)

Title: Estradiol modulates gut microbiome in leptin deficient female mice on a high-fat diet

Authors: X. GAO¹, E. P. BLESS¹, K. D. ACHARYA¹, J. CHEN², *M. J. TETEL¹ ¹Neurosci. Program, Wellesley Col., Wellesley, MA; ²Div. of Biomed. Statistics & Informatics & Ctr. for Individualized Med., Mayo Clin., Rochester, MN

Abstract: Estrogens act in brain and the periphery to mediate a variety of functions, including energy metabolism and reproduction. For example, lower levels of circulating estrogens in postmenopausal women are associated with increased fat weight and risk for developing heart disease and type II diabetes. These anorectic effects of estrogens have also been shown in rodents by our lab and others. Ovariectomized mice become obese when fed a high-fat diet (HFD), while treatment with 17β-Estradiol (E2) prevents this HFD-induced obesity. Leptin, a peptide hormone encoded by the ob gene, is released from adipocytes and acts as a satiety signal in the hypothalamus. Mice with a mutation of the ob gene (ob/ob) do not produce leptin and develop metabolic disorders such as obesity and diabetes. The gut microbiome, which consists of bacteria in the intestines that aid in digestion, has been implicated in obesity and disorders related to the gut-brain axis such as anxiety and depression. The present study tested the hypothesis that E2 and leptin modulate the gut microbiome of female *ob/ob* mice fed a HFD. ob/ob (obese) mice and heterozygote controls (Het; lean) were ovariectomized and implanted with capsules containing E2 (50 µg) or oil (Veh control) to form four groups: 1) *ob/ob* E2, 2) ob/ob Veh, 3) Het E2, and 4) Het Veh. After four days on a standard diet, the mice were switched to a HFD for 31 days. Throughout the study, food intake and weights were measured

and fecal samples were collected. Over the course of the experiment, E2 treated mice weighed less and consumed less food than their Veh counterparts. Additionally, *ob/ob* mice had a lower percent weight gain than Het mice, despite a greater food intake than Het mice. To explore the association between the differences in weight gain and food intake to changes in the gut microbial composition between the groups, fecal samples were sequenced for the 16S rRNA gene. We found that E2 treatment was associated with lower species evenness compared to Veh mice, while the *ob/ob* genotype was associated with lower species richness compared to Het mice. Phylum level analysis showed that *Bacteroidetes* was more abundant in E2-treated mice than Veh mice whereas *Firmicutes* was more abundant in Veh mice compared to E2-treated mice. Genotype also affected relative abundance such that the phylum *Actinobacteria* and the class *Clostridia* (phylum *Firmicutes*) were less abundant in *ob/ob* mice than Het mice. Our results reveal that both E2 treatment and the *ob/ob* genotype influence the gut microbial composition of female mice fed a HFD. Understanding the role of E2 and leptin in modulating the gut microbiota will aid in developing treatments for metabolic disorders including obesity.

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Poster

326. Metabolism Control and Obesity

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Horiba Award for Analytical Chemistry (PH)

Title: A multifaceted approach to analyze the role of serotonin in comorbid depression and obesity

Authors: *M. HERSEY¹, C. A. GRILLO², V. A. MACHT⁶, A. GREEN³, J. R. FADEL⁴, M. A. WILSON⁸, P. HASHEMI⁷, L. P. REAGAN⁵

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Abstract: Epidemiological studies estimate that greater than 60% of the adult US population may be categorized as either overweight or obese. There is a growing appreciation that the complications of obesity extend to the central nervous system (CNS) and result in increased risk for neurological co-morbidities like depressive illness. Given the hypothesized role of serotonin (5-HT) in the pathogenesis of depression, it is possible that decreases in brain 5-HT efflux are a mechanistic mediator of depressive illness in obese patients. We previously demonstrated that rodents with a phenotype that is consistent with features of the metabolic syndrome (MetS) exhibit depressive-like behaviors. To determine whether alteration in 5-HT neurochemistry may provide a potential underlying neurochemical basis for these behavioral changes, we combined two complimentary approaches, namely in vivo microdialysis and in vivo fast-scan cyclic voltammetry, to measure basal levels of 5-HT, increases in hippocampal 5-HT following administration of the selective serotonin reuptake inhibitor (SSRI) fluoxetine and hippocampal serotonin reuptake kinetics in control and obese rats. Neurochemical analyses determined that obese rats exhibited decreased basal 5-HT levels and diminished hippocampal 5-HT efflux following fluoxetine SSRI administration in the hippocampus when compared to control rats. Collectively, these data support the hypothesis that deficits in hippocampal 5-HT neurochemistry are a shared feature between depressive illness and metabolic disorders. Additionally, results identify pharmacological differences that provide a mechanistic basis for the decreased efficacy of SSRIs in the treatment of depressive illness in obese individuals.

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Poster

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Topic: F.10. Food Intake and Energy Balance

Support: R15DK108668

R15DK097644

Title: Alternate-day fasting compared to calorie restriction in lean and obesity-prone rats

Authors: ***A. E. DAVIS**¹, A. TITUS², D. MEHTA¹, L. G. KOCH⁴, S. L. BRITTON⁵, C. M. NOVAK³

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Abstract: Obesity, characterized by an excessive amount of body fat, is steadily increasing despite the many diet and exercise programs available today. This is problematic as obesity increases the risk of developing many diseases such as cardiovascular disease, cancer, and diabetes. Here, weight loss was investigated using a contrasting rat model of obesity and leanness: obesity-prone rats (low-capacity runners, LCR) and the more physically active lean rats (high-capacity runners, HCR). Our past studies found that during 50% caloric restriction, lean rats lost proportionally more fat than the obesity-prone rats. An alternative diet strategy was then investigated where 16 male rats (n=8/group) underwent 7 weeks of intermittent fasting (IF), with food being removed every-other day, and an *ad libitum* diet provided on non-fasting days. Body composition was measured weekly, and body weight and food intake were measured daily with minimal disruption of daily sleep/activity cycle. The goal of intermittent fasting was to induce loss of fat in the obesity-prone rats. With IF, the obesity-prone rats lost twice as much fat and body weight compared to the lean rats, with relatively little reduction in lean mass. Results were consistent with previous data from female rats, but contrasted with prior findings where weight loss was induced using 50% calorie restriction. Compared to calorie restriction, IF conserved lean mass while enhancing fat loss. These results suggest that IF may be a potential strategy for reducing weight in the obese and obesity-prone.

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Poster

326. Metabolism Control and Obesity

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Topic: F.10. Food Intake and Energy Balance

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Title: BMI effects on resting state networks

Authors: *B. DE CELIS ALONSO¹, S. S. HIDALGO TOBÓN², P. DIES SUÁREZ², J. M. HERNÁNDEZ LÓPEZ¹, E. MORENO BARBOSA¹, P.-W. SO³, J. BELL⁴ ¹Benemerita Univ. Autonoma De Puebla, Puebla, Mexico; ²Imagenología, Hosp. Infantil de México, Federico Gómez, Ciudad de México, Mexico; ³Neuroimaging, King´s Col. London, London, United Kingdom; ⁴Life Sci., Univ. of Westminster, London, United Kingdom

Abstract: Introduction: Obesity and excessive amounts of fat in the liver have been associated with poorer learning, memory and executive functions. The mechanisms by which this effect happens, are not well elucidated. We want to address this problem in young children. This interest lays behind the fact that disorders in initial stages of life affect adulthood. The specific objective of the work presented here was to identify changes in cognitive neuroimaging data obtained with magnetic resonance techniques (resting states and diffusion imaging) and correlate them with blood and urine chemistry as well as psychological testing and fatty liver anatomical measurements. This way the bigger question of the mechanisms underlying poor memory and learning in obese people, might be partially addressed. Materials & Methods: The brain of 160 children (ages from 7 to 9 years old) divided in three groups (normal, over weighted and obese) were studied with MRI. Two neuroimaging techniques were applied (resting states and diffusion weighted imaging). Also, fat measurements of pancreas and liver were added, in which position and amounts of ectopic fat were measured. These measurements were complemented with cognitive testing of each subject (depression, WISC-IV, WCST, Rey-Osterreith and auditory, verbal memory:word from ENI). To all these measurements we addedd blood tests in which standard measurements (cholesterol, glucose, red and white cell counts, etc.) as well as more specific inflammatory particles (cytosines) were obtained. **Results & Discussion:** Preliminary findings showed there were differences in-between children groups for the resting state networks related to reward, as well as the default mode network and the sensorimotor network. Diffusion measurements showed differences in white matter microstructure as shown by changes in fractional anisotropy measurements. As mentioned before, these are preliminary findings. When cognitive as well as blood test data are included we hope to have a broader and more complete description of the mechanisms underlaying poorer learning and memory.

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Poster

326. Metabolism Control and Obesity

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Topic: F.10. Food Intake and Energy Balance

Support: UIUC Psychology Department

Title: Chronic moderate alcohol drinking alters glucose metabolism but spared behavior on elevated plus maze, sucrose preference and novel object recognition tests

Authors: *N. G. NELSON, F. A. SUHAIDI, W. X. LAW, N.-C. LIANG Neurosci. & Psychology, Univ. of Illinois At Urbana-Champaign, Champaign, IL

Abstract: Obesity poses a great world-wide health care burden due to its associated comorbid health conditions. Health programs aimed to alleviate the menace of obesity tend to promote lifestyle changes that include the adoption of rigorous aerobic exercise regimen, and reduced intake of fatty/sugary foods. Unfortunately, not enough attention has been directed at the contribution of alcoholic beverage to the metabolic syndrome. This stems in part from the conflicting reports in the literature of how alcohol affects appetite, weight maintenance, and diabetes care. We previously reported that dose, administration route, and time course of blood ethanol concentration (BEC) dictate how alcohol affects appetite and body weight in rats. Here, we further probed the link between chronic moderate ethanol consumption, emotional-like and cognitive behaviors, and glucose metabolism in rats. We hypothesized that chronic moderate alcohol consumption will (1) be associated with depression- and anxiety-like behaviors and cognitive deficits during abstinence; and (2) impair glucose metabolism during an oral glucose tolerance test (OGTT). We exposed adolescent male and female rats to 7.5h/day (Mon - Fri) access to saccharin-sweetened 10% ethanol for 8 weeks, followed by intermittent (Mon, Wed, Fri) access to 20% unsweetened ethanol in a 2-bottle choice drinking paradigm for 6 weeks (EtOH). A free-feeding control group received water (CTL). The average body weight of a second control group that received water was matched to that of the EtOH group via mild food restriction (BW Match). During abstinence, all rats were submitted to sucrose preference, elevated plus maze (EPM) and novel object recognition (NOR) tests. BECs were measured from tail blood collected after 1 and 3 h access periods in a separate group of male and female rats given sweetened 10% ethanol. The ethanol intake dose correlated significantly with BEC. Rats attained BEC of ~ 50 mg/dL after 1h ethanol access, and the BEC was maintained around this level at the 3h sampling time point. Furthermore, EtOH rats compensated for calories from ethanol (7 kcal/g) and reduced their chow intake, and their resulting body weight did not differ from that of their respective CTL group. Performance in sucrose preference, EPM and NOR tests did not differ between the EtOH and control groups. Results of the OGTT revealed that EtOH male rats required more insulin to clear blood glucose - a sign of insulin insensitivity. Such effect was not observed in females. This landmark study showed that chronic moderate alcohol consumption can have negative metabolic consequences in the absence of overt behavioral deficits.

Disclosures: N.G. Nelson: None. F.A. Suhaidi: None. W.X. Law: None. N. Liang: None.

326. Metabolism Control and Obesity

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 326.16/NN19

Topic: F.10. Food Intake and Energy Balance

Title: Interleukin-1 β contributes to the development of hyperalgesia in overweight ovariectomized rats

Authors: *O. A. JARAMILLO-MORALES¹, J. V. ESPINOSA-JUÁREZ², F. J. LÓPEZ-MUÑOZ²

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Abstract: Several studies have reported that menopause causes a greater perception to painful stimuli and an increase in adipose tissue, which promotes a greater body weight gain compared to men. Additionally, it is known that weight gain is related to changes in the perception of pain; however, its mechanism is not clear. **Objective.** The aims of this study were to analyze the behavioral responses of hypoestrogenic overweight Wistar rats to thermal stimuli and to analyze the leptin and interleukin-1 β levels in this population. **Methods**. Animals with hypoestrogenism induced by bilateral ovariectomy were used. Animals received either a hypercaloric diet (30% sucrose) or regular water with standard laboratory food ad libitum for 4 weeks; the thermal nociception and body weight were measured during this period. Four weeks after treatment, the interleukin-1 β and leptin levels and the abdominal fat weight were measured in both groups. Nociception was assessed using the "Plantar test". Results. Overweight ovariectomized Wistar rats displayed significantly higher body weight and abdominal fat weight than did the control group. A hyperalgesic response was observed in animals fed sucrose. Thermal latency was also significantly decreased during the 4th week in these animals compared to that of controls. There were no differences in leptin levels, but the interleukin-1 β levels were altered between groups. Conclusions. Our data indicate that increased body weight, abdominal fat and increases in interleukin-1 β are associated with the hyperalgesic responses observed in ovariectomized overweight female rats.

Disclosures: O.A. Jaramillo-Morales: None. J.V. Espinosa-Juárez: None. F.J. López-Muñoz: None.

326. Metabolism Control and Obesity

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ADA Grant 1-17-ACE-31

Einstein-Mt. Sinai Diabetes Research Center Pilot and Feasibility Award

Alexander and Alexandrine Sinsheimer Scholar Award

Title: Characterizing glucose-sensing neurons in the amygdala

Authors: *K. DEVARAKONDA¹, S. STANLEY²

¹Dept. of Neurosci., ²Dept. of Medicine, Div. of Endocrinology, Diabetes and Bone Dis., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Dysfunction of blood glucose produces health problems with profound consequences. In humans, the CNS plays a vital role in glucose metabolism. The medial and basomedial amygdalar nuclei contain neurons that alter their firing in response to physiological changes in glucose concentrations and express glucokinase, an enzyme thought to act as a glucose sensor in the CNS. However, little is known about the anatomy and function of these neurons. To better understand how glucose-sensing neurons in the CNS may contribute to the regulation of metabolic function, we have identified neurochemical markers for amygdala GK neurons, characterized their response to altered glucose, and mapped their circuitry.

Disclosures: K. Devarakonda: None. S. Stanley: None.

Poster

326. Metabolism Control and Obesity

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NIH grant U01 MH105941

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Einstein-Mt. Sinai Diabetes Research Center Pilot and Feasibility award

Alexander and Alexandrine Sinsheimer Scholar Award

Title: Pancreatic projections of glucose sensing CNS neurons

Authors: *A. ALVARSSON, M. BAYNE, S. STANLEY Diabetes, Obesity and Metabolism Inst., Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: The central nervous system (CNS) plays a complex role in the control of metabolic functions. CNS glucose-sensing neurons respond to blood glucose levels and orchestrate counterregulatory responses to hypoglycemia. Although several brain regions are known to form synaptic connections with metabolic organs, the specific roles of glucose-sensing neurons in such projections have not yet been established. Glucose-sensing CNS neurons appear to constitute distinct neuronal populations that mediate different physiological functions, but previous studies have been hampered by low expression levels of the glucose sensor glucokinase (GK). However, a recently generated transgenic mouse with cre-recombinase expression in GKexpressing neurons (GK-cre/Rosa-tdTomato mice) allows mapping of GK expression. Here we performed retrograde tracing studies to identify neural pathways involving GK-expressing neurons innervating the pancreas using GK-cre/Rosa-tdTomato mice. A pseudorabies virus (PRV) expressing green fluorescent protein (GFP) was injected (5x100 nl) into the pancreas of GK-cre/Rosa-tdTomato mice via a Hamilton syringe. Four to eight days after PRV-GFP injections the mice were perfused and the brains dissected and sectioned. Immunohistochemistry was performed to amplify the GFP signal of neurons infected by the PRV. Stained brain sections were mounted for quantification using an optical microscope. The numbers of PRV-GFP infected neurons and neurons with overlapping GK and GFP labelling were quantified. GFP labelling was found throughout the hypothalamus, midbrain, hindbrain and cortex. Overlapping labelling between GK-expressing and GFP-expressing neurons identified at different time points revealed that subsets of hypothalamic glucose-sensing neurons, particularly in the lateral hypothalamus and paraventricular nucleus of the hypothalamus, form circuits with synaptic connections that project to the pancreas. These findings suggest that several brain regions, most notably in the hypothalamus, harbor glucose-sensing neurons that are potentially playing roles in the control of pancreatic functions.

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326. Metabolism Control and Obesity

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Topic: F.10. Food Intake and Energy Balance

Support: FONDECYT Grant 11150678

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CMA BIOBIO PIA-Conicyt ECM-12

Title: The estructure of GLUT2- and GLUT6-positive tanycytes is altered by acute hyperglycemia

Authors: *F. A. MARTINEZ ACUÑA¹, M. CIFUENTES³, K. A. SALAZAR⁴, N. A. JARA⁵, L. TRIGUEROS², F. J. NUALART⁶

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Abstract: The median eminence (ME) is a brain structure located at the base of the hypothalamus that is involved in integrating metabolic signals, such as glucose. To perform these functions, the ME cytoarchitecture is modulated and the low affinity glucose transporters, GLUT2 and GLUT6, are expressed. However, no study has addressed the alterations of GLUT expression in the ME caused by aging or acute blood glucose changes. We used confocal microscopy in the spectral 3D rendering mode and laser microdissection combined with qRT-PCR to characterize the cellular distribution of GLUTs in the ME. Ultrastructural modification of the ME in rats subjected to normoglycemic conditions or hyperglycemia was evaluated using transmission electron microscopy (TEM). GLUT2 and GLUT6 were both localized to β2 tanycytes, the main glial cells in the ME. At the ultrastructural level, hyperglycemia increased the arborization of $\beta 2$ tanycyte processes. An increase in vascular projections from subventricular area and the outermost region of the ME, with an increase of vacuolization and formation of blebs was also observed. Furthermore, aging had no effect GLUT2 mRNA expression, but decreased GLUT6 mRNA expression. Taken together, GLUT2 and GLUT6 expression and the structural alterations induced by hyperglycemia and aging in β^2 tanycytes is suggestive of their participation in glucose transference from the blood to the cerebrospinal fluid (CSF).

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326. Metabolism Control and Obesity

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Program#/Poster#: 326.20/NN23

Topic: F.10. Food Intake and Energy Balance

Support: Professor Milton Rosenbaum endowment fund for research in psychiatric sciences

Title: Obese mice exhibit increased motivation towards palatable food and physiological abnormalities in the ventral pallidum

Authors: *Y. M. KUPCHIK, D. INBAR, K. INBAR, L. LEVY, N. BERNAT, S. MENAHEM Dept. of Med. Neurobio., The Hebrew Univ., Jerusalem, Israel

Abstract: Obesity is a major health challenge in the modern world as obesity levels increase in an alarming rate. Obesity is mostly attributed to the lack of energy balance in the sedentary modern lifestyle but other factors, primarily the irresistible urge to eat highly-palatable energydense food, need to be taken into account. Our and others' few previous studies show that obesity entails abnormalities in the striatum, a part of the reward system, indicating a potential role for pathology in the reward system in obesity. However, it is yet to be found whether other parts of the reward system exhibit abnormalities in obesity as well. Here we used the dietinduced obesity model to induce obesity in C57 male wild-type mice. After 10 weeks of high-fathigh-sugar diet, we compared the motivation to obtain palatable food and the physiology of ventral pallidum neurons between the top third (obesity-prone) and bottom third (obesityresistant) weight gainers. We found that obesity-prone mice consistently consumed more calories compared to obesity-resistant mice. Moreover, after an overnight abstinence from the high-fathigh-sugar diet obesity-prone mice exhibited higher performance in a progressive ratio task to obtain high-fat-high-sugar precision pellets compared to obesity-resistant mice. Lastly, using whole-cell patch clamp electrophysiology we found that ventral pallidum neurons of obesityprone mice are less excitable and receive stronger inhibitory input compared with obesityresistant mice. Overall, our data indicates that the pathology in the reward system of obese mice is more widespread than previously thought and includes also the ventral pallidum. This calls for more reward-system-oriented research in trying to understand the underpinnings of obesity in modern society.

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326. Metabolism Control and Obesity

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 326.21/NN24

Topic: F.10. Food Intake and Energy Balance

Support: Hartwick Faculty Research Grant

Freedman Prize

Title: Effects of high fat diet on anxiety-like behaviors and hippocampal-dependent learning: Roles of body weight and sex differences

Authors: *K. CHAN, A. BURDICK, K. RASEFSKE, J. MCGOVERN Hartwick Col., Oneonta, NY

Abstract: Past studies have shown that high fat diets impair hippocampal-dependent learning and reduce anxiety-like behaviors in rats. This experiment extended these findings by testing the effects of high fat diet (20% lard) while controlling for body weight differences across groups. Male and female Sprague-Dawley rats received either a high fat diet or regular chow for ten weeks prior to behavioral tests. Groups were weight matched prior to the experiment and weighed and fed daily throughout the experiment to maintain similar weight gains. Half of the rats were tested in an elevated plus maze for anxiety-like behaviors and the other half received training in a spatial water maze. Subjects on the high fat diet spent a significantly longer amount of time (F(1, 16) = 7.691, p = .014) and made marginally more entries into the open arms (F(1, 16) = 3.932, p = .065) of the elevated plus maze, regardless of sex. On the other hand, a probe test conducted after the water maze training showed a significant Diet x Sex interaction on latency to target (F(1,22)=4.458, p=.046). These results are consistent with the interpretation that effects of high fat diet on anxiety-like behaviors are independent of body weight changes. On the contrary, the impairment in hippocampal-dependent learning might be related to both sex differences and body weight changes associated with the diet.

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Poster

326. Metabolism Control and Obesity

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 326.22/NN25

Topic: F.10. Food Intake and Energy Balance

Title: Energy balance regulation and obesity in mice as a function of individual rearing and western style diet exposure

Authors: L. SCHIPPER^{1,2}, S. VAN HEIJNINGEN², *E. M. VAN DER BEEK^{1,3}, G. VAN DIJK²

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Abstract: Mice are widely used for the study of obesity and cardiometabolic comorbidities. In general, rodent obesity models rely on elevated baseline activity of the hypothalamo-pituitary adrenal axis and feeding of a western style diet. This condition has been mentioned to be anxiogenic and could underlie anhedonia and depression.

We aimed at investigating the role of rearing condition in these effects by subjecting C57Bl/6J mice after weaning to individual (IND) or social (SOC, n=2 siblings/cage) housing while being kept on a low fat (AIN93G based, 20En% as fat) diet. At postnatal day (P)43, one third of the mice from the IND and SOC groups were sacrificed and the remaining groups were split and exposed to a moderate western-style diet (WSD, 40En% as fat) or kept on low fat control diet until P126. Bodyweight was monitored and energy intake and expenditure were determined using indirect calorimetry during adolescence (P40-42) and adulthood (P105-107). In addition, mice were subjected to a sucrose preference test to assess food reward mechanisms during adulthood (P74-79) and to an elevated plus maze test (EPM) to assess baseline levels of anxiety during adolescence (P39) and adulthood (P92). After dissection plasma adipokines and corticosterone were measured and body composition was determined by carcass analysis. IND mice showed lower body weight gain during adolescence than SOC mice, while energy intake and energy expenditure were increased. At P43 IND mice had reduced lean body mass, but significantly higher white adipose tissue mass compared to SOC mice. In adulthood, bodyweight gain of IND animals exceeded that of SOC mice, with elevations in both energy intake and expenditure. At P126, IND mice showed higher adiposity and a lower plasma adiponectin/leptin ratio. WSD exposure amplified these changes and reduced plasma levels of corticosterone. IND mice exposed to WSD showed the lowest sucrose preference index, indicating that food reward mechanisms were most blunted in this group. Baseline anxiety levels were surprisingly reduced by IND housing and by WSD exposure.

We conclude that individual rearing of male mice results in impaired adolescent lean mass development and an obese phenotype in adulthood. The latter effects are exacerbated by adult exposure to an obesogenic diet. These effects are associated with mild alterations in reward sensitivity and reduced baseline levels of anxiety, without elevated levels of glucocorticoids. These results suggest that individual rearing of mice can be regarded as a model for programmed adult obesity without elevated anxiety and hypercorticoidism.

Disclosures: L. Schipper: None. S. van Heijningen: None. E.M. Van Der Beek: None. G. van Dijk: None.

326. Metabolism Control and Obesity

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Program#/Poster#: 326.23/NN26

Topic: F.10. Food Intake and Energy Balance

Support: R01 DK110412-01

Title: Effects of a GLP-1 agonist on hippocampal functioning underlying the learning mechanisms associated with energy regulation

Authors: *S. JONES¹, C. H. SAMPLE¹, A. HYDE¹, S. L. HARGRAVE², T. L. DAVIDSON¹ ¹American Univ., Washington, DC; ²Natl. Inst. of Heath, washington, DC

Abstract: The hippocampus is thought to play an important role in the learned control of energy and body regulation by gating inhibitory associations between environmental food cues and postingestive consequences. The hippocampus is able to regulate intake using similar mechanisms to those underlying negative feature cues in a typical serial feature negative discrimination problem. In this problem, a target cue is followed by food reward unless it is preceded by a negative feature cue. In other words, the negative feature cue signals that the target cue will not be rewarded. Previous studies with rats have shown that manipulations which interfere with hippocampal functioning also disrupt serial feature negative discrimination performance. Exposure to Western diets (WD), high in saturated fat and sugar, for as few as 12 days disrupts this discrimination and gives rise to signs of hippocampal pathophysiology (inflammation, reduced glucose transport). In the current study, we assessed the effects of administering the glucagon-like peptide-1 (GLP-1) agonist, Liraglutide, on serial feature negative discrimination performance in male and female rats. Following the achievement of asymptotic discrimination performance, the rats were divided into two dietary conditions: WD- or chow-fed, which were further subdivided into two drug conditions: 10 µg/kg Liraglutide or vehicle control. The ability to maintain discriminative responding by utilizing the negative feature cue was tested in all groups after 4d and 12d of ad lib access to diet and daily intraperitoneal injections of Liraglutide or vehicle. After 12d of diet and drug exposure, Liraglutide significantly enhanced discrimination performance compared to vehicle. This enhancement was the result of increased inhibitory control by the negative feature stimulus. This effect could not be accounted for by any nonspecific inhibitory effect of Liraglutide on performance because responding on trials with the target cue alone did not differ dependent on drug treatment. The effects of Liraglutide did not vary as a function of sex or diet. Furthermore, Liraglutide had no significant effects on body weight. Recent reports indicate that inhibition of feeding and perhaps other behaviors depends on activation of an excitatory pathway originating in the hippocampus (Sweeney and Yang, 2015) and that activation of this pathway depends on GLP-1 signaling (Hsu et al, 2017). The present

findings indicate that administration of a GLP-1 agonist specifically promotes a form of hippocampal-dependent appetitive response inhibition, perhaps by operating on the same circuits.

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Poster

326. Metabolism Control and Obesity

Location: Halls A-C

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Program#/Poster#: 326.24/NN27

Topic: F.10. Food Intake and Energy Balance

Support: Deanship of Research, Jordan University for Science and Technology

Title: Psychological symptoms is a determinant of body mass index among patients with musculoskeletal pain: A link to vitamin D and calcium

Authors: *K. K. ABDUL-RAZZAK, M. KASSAB

Jordan Univ. of Sci. and Technol., Irbid, Jordan

Abstract: A high prevalence of psychological symptoms and vitamin D among patients with musculoskeletal pain (MSP) and obesity has been observed. People complaining of anxiety and depression are having frequent weight change compared with those without such symptoms. To date, most of the published studies, examined the co-prevalence of chronic pain, psychological symptoms and weight gain, although the loss of appetite also can occur when anxiety is high. The aim of the current study was to investigate the association between body mass index and psychological symptoms among patients with MSP in relation to vitamin D and calcium. A total of 207 participants with MSP were involved. The Hospital Anxiety and Depression Scale (HADS) were used to assess psychological symptoms. Participants' dietary habits and vitamin D were measured. The relation between their food appetite and anxiety level were recorded. Participants with MSP had a high prevalence of vitamin D deficiency (85.5%), anxiety (52.7%), and depression (39.1%) markedly. There was a significant relation between the pattern of appetite and anxiety (p=0.024), which reflected by significant changes in BMI (p=0.013).BMI tend to fluctuate (increase or decrease) depending on the influence of anxiety on appetite. Spearman correlation analyses show that anxiety is negatively associated with daily calcium intake and age ($r^2 = -0.124$, p=0.038, $r^2 = -0.227$, p=0.001, respectively), and positively associated with depression ($r^2 = -.613$, p = < 0.001).

The result of the current study showed that anxiety which negatively correlated daily calcium intake is a determinant of BMI among patients with Musculoskeletal Pain through its influence

on appetite.

Disclosures: K.K. Abdul-Razzak: None. M. Kassab: None.

Poster

326. Metabolism Control and Obesity

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Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant R01 DK056132

AHA Grant 16SDG26590000

Title: Parasympathetic output contributes to the resolution of uncontrolled diabetic hyperglycemia by vertical sleeve gastrectomy in a murine model of type 1 diabetes

Authors: C. R. BOYCHUK¹, K. C. SMITH², *B. N. SMITH³ ¹Physiol., ²Pysiology, Univ. of Kentucky, Lexington, KY; ³Dept Physiol, Univ. of Kentucky Dept. of Physiol., Lexington, KY

Abstract: Recent evidence supports the emerging hypothesis that brainstem autonomic neurons are affected by and contribute to systemic glucose regulation, particularly after bariatric surgery. Vertical sleeve gastrectomy (VSG) can result in resolution of type 2 diabetes in humans and animal models, but information about effects of VSG on type 1 diabetes is scarce. Extant data does not extend beyond 14 days post-surgery, nor does it directly investigate the contribution of parasympathetic regulation in diabetic remission after VSG. Here, we used the streptozotocin (STZ)-treated mouse model of type 1 diabetes to determine effects of VSG on uncontrolled hyperglycemia, weight, caloric intake, and markers of brainstem and peripheral glucose metabolism to test the general hypothesis that a brain-centered glucose regulatory system contributes to systemic glucose regulation after bariatric surgery. We also tested whether the contribution of autonomic regulatory systems changes at 14, 30, or 60 days after surgery. Uncontrolled hyperglycemia (>300 mg/dl) was maintained for 2 days in STZ-treated mice, at which time VSG or sham surgery was performed. After VSG, blood [glucose] was normalized shortly after surgery (<24 hrs) and could persist for 2 months; elevated blood glucose persisted in sham-operated, STZ-treated mice. Normalization of blood [glucose] in the STZ-treated VSG mice was accompanied by a normalization of food intake, similar to age-matched vehicle-VSG controls. Metabolic testing indicated that glucose tolerance was similar between STZ-treated VSG and vehicle-VSG groups 14 days after surgery. Although the glucose tolerance of the STZ-VSG group was improved compared to the STZ-naïve group, the STZ-VSG groups was less

tolerant of glucose at 2 months compared to vehicle-VSG. Examination of glucose tolerance in the presence of a parasympathetic blocker, methylscopolamine, indicated that parasympathetic output contributes to the early resolution of glucose metabolism (14 days post-VSG), but has less influence at later times (2 months). Interestingly, plasma insulin levels were unaffected by VSG surgery. Correspondingly, we examined molecular and electrophysiological markers of glucose metabolism in the dorsal vagal complex. Short (3.5 hr) exposure to elevated [glucose] in vitro resulted in elevated GLUT4, but not glucokinase (GCK), mRNA expression. After STZ-induced chronic hyperglycemia, glucose-sensitivity was significantly reduced in NTS GABA neurons, and VSG restored glucose responsiveness. These results are consistent with a role for vagal complex control of systemic glucoregulation involving a vagally-mediated gut-brain-axis.

Disclosures: C.R. Boychuk: None. K.C. Smith: None. B.N. Smith: None.

Poster

326. Metabolism Control and Obesity

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Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant MH105746

Title: The role of type 3 adenylyl cyclase in neuronal cilia

Authors: *X. CHEN^{1,2}, Y. ZHOU², L. QIU², M. STROBEL², A. STERPKA¹ ¹Dept. of Molecular, Cell. and Biomed. Sci., ²Univ. of New Hampshire, Durham, NH

Abstract: Cilia are rigid, centriole-derived, microtubule-based organelles present in a majority of vertebrate cells including neurons. They are considered the cellular "antennae" attuned for detecting a range of extracellular signals including photons, odorants, morphogens, hormones and mechanical forces. The ciliary microenvironment is distinct from most actin-based subcellular structures such as microvilli or synapses. Numerous G protein-coupled receptors (GPCRs) including odorant receptors, rhodopsin, Smoothened, and type 6 serotonin receptor are found in cilia, suggesting that these tiny processes largely depend on metabotropic receptors and their tuned signals to impact neuronal functions. The type 3 adenylyl cyclase (AC3), widely known as a cilia marker, is highly and predominantly expressed in olfactory sensory cilia and primary cilia throughout the brain. We discovered that ablation of AC3 in mice leads to pleiotropic phenotypes including anosmia, failure to detect mechanical stimulation of airflow, cognitive deficit, obesity, and depression-like behaviors. Multiple lines of human genetic evidence also demonstrate that AC3 is associated with obesity, major depressive disorder (MDD), sarcoidosis, and infertility. Here we present our recent research progress on AC3.

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Poster

326. Metabolism Control and Obesity

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Topic: F.10. Food Intake and Energy Balance

Support: CHIRP grant APG-70-3917

Title: Acetylation-based gene expression changes in the brain

Authors: N. PUTHILLATHU¹, J. R. MOFFET², J. J. K. KRISHNAN², P. ARUN², R. VENGILOTE², C. L. DALGARD², G. SUKUMAR², J. SINGH², M. WILKERSON², J. TE², *A. NAMBOODIRI²

¹USUHS, bethesda, MD; ²USUHS, Bethesda, MD

Abstract: Erythropoietin (EPO) is a glycoprotein that is released by the kidneys and liver to facilitate red blood cell production, and therefore, EPO expression is increased in response to hypoxia or blood loss. Recent studies have indicated that EPO administration can reduce or prevent some of the pathological mechanisms of secondary brain injury due to excessive inflammatory responses and oxidative damage. EPO also enhances angiogenesis. In one recent study it has been shown that acetylation of the transcription factor HIF-2a regulates EPO production during pathophysiological stress induced by hypoxia. Acetylation is necessary to recruit this transcription factor to the erythropoietin gene enhancer and increase erythropoietin gene transcription. In earlier studies we have used acetate supplementation with glyceryl triacetate (GTA), which generates acetate and glycerol on hydrolysis in vivo, to improve outcomes after traumatic brain injury (TBI). Oral GTA has been repeatedly proven to increase acetate levels in all tissues. The intracellular acetate becomes available for various metabolic and regulatory reactions through the portal of acyl-CoA short chain synthetase 2 (Acss2), the only known enzyme in the cytoplasm and nucleus of cells that can convert free acetate to acetyl-CoA. Acetate supplementation using GTA decreases expression of anti-inflammatory cytokines such as interleukin-1 β (IL-1 β) via regulating histone acetylation. Based on these earlier findings, we proposed to develop oral acetate supplementation using GTA as a way to augment acetylationbased genetic regulatory mechanisms involved in the response to and recovery from brain injury hemorrhage and hypoxia. We used gene array to begin screening for potential functional associations with Acss2 in the brain by comparing gene expression between Acss2 gene knockout mice and wild type mice. Six groups were compared using Acss2 -/- and wild type mice; normally fed, 48 hr fasted and GTA fed. The greatest number of gene expression changes was observed between 48 hr fasted wild type and Acss2 -/- mice (1579 lower expression, 1320

higher expression). In contrast, very few expression changes were observed between GTA fed wild type and Acss2 -/- mice (88 lower expression, 87 higher expression). Gene expression changes in the brain among the different groups linked Acss2 with a wide network of cellular regulatory processes ranging from signal transduction to regulation of gene expression and immune system function. These findings expand the regulatory functions of Acss2 well beyond its classical role in lipid synthesis.

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Poster

326. Metabolism Control and Obesity

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Topic: F.10. Food Intake and Energy Balance

Support: ONR Grant #N00014-16-2799

Title: Impacts of stress on glucocorticoids and energy homeostasis: Interactions between liver glycogen, corticosterone, CBG, and glucose concentrations following inescapable tail shock in rat

Authors: *M. A. CONOSCENTI, N. M. WILLIAMS, T. R. MINOR Psychology, UCLA, Los Angeles, CA

Abstract: An acute traumatic event can lead to life-long changes in stress susceptibility and result in psychiatric disease, such as Post-Traumatic Stress Disorder (PTSD). We have previously shown that access to a concentrated glucose solution for 24 hours beginning immediately after trauma decreased stress-related pathology in the learned helplessness model of PTSD and comorbid major depression. Our recent parametric studies show consistent results with previous research, suggesting a critical window of approximately three hours following trauma in which intervention may be effective in modifying stress-related behaviors. To gain further insight into the mechanism of glucose treatment in mediating stress susceptibility following trauma, we assessed the effects of post-stress glucose administration on blood glucocorticoid response and glucose levels at various timepoints including 24 hours following trauma, and before, during, and after the proposed intervention critical window. We exposed 64 male Sprague-Dawley rats to 100 inescapable tail shocks or simple restraint in the learned helplessness procedure. Rats in each stress condition had access to a 40% glucose solution or water. Animals were tested for freezing and shuttle-escape behavior 24 hours after the acute stress session. In the first experiment, we measured liver glycogen concentrations, as well as

plasma concentrations of glucose, corticosteroid-binding globulin (CBG), free corticosterone and total corticosterone, after exposure to 5 FR-1 shuttle-escape trials. We found no differences in any measure between animals that received glucose or water in the simple restraint condition. However, animals that were given access to glucose after exposure to inescapable shock exhibited decreased free corticosterone, increased CBG, and increased liver glycogen concentrations when compared to their water-drinking counterparts. In the second experiment, we measured plasma concentrations of glucose, CBG, free corticosterone and total corticosterone at four time points: pre-stress, immediately post-stress, three hours post-stress, and six hours post-stress. We hypothesized that there would be a reduction of free corticosterone beginning at three hours post-stress that would persist through six hours post-stress. We expected to see this reduction in free corticosterone correlate to increased CBG and glucose concentrations. These results imply that post-stress glucose consumption reestablishes blood glucose homeostasis, thereby upregulating CBG binding of corticosterone. This data suggest the upregulation of CBG is a mechanism by which glucose exerts its prophylactic effects.

Disclosures: M.A. Conoscenti: None. N.M. Williams: None. T.R. Minor: None.

Poster

326. Metabolism Control and Obesity

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 326.29/NN32

Topic: F.10. Food Intake and Energy Balance

Support: Joint Programming Initiative – A Healthy Diet for a Healthy Life, AMBROSIAC project.

Title: Sub-chronic Oleoylethanolamide treatment differentially afftects body weight, gut microbiota composition and cytokines expression in normal and histamine deficient mice

Authors: *G. PROVENSI¹, M. DI PAOLA¹, E. BONECHI¹, A. COSTA¹, P. BLANDINA¹, G. CLARKE³, C. BALLERINI¹, C. DE FILIPPO⁴, M. B. PASSANI² ¹Dept. di Neuroscienze, Psicologia, Area del Farmaco e Salute del Bambino, ²Dept. di Scienze della Salute, Univ. degli Studi di Firenze, Firenze, Italy; ³Univ. Col. Cork, Cork, Ireland; ⁴Inst. di Biometeorologia, Consiglio Nazionale delle Ricerche, Firenze, Italy

Abstract: Recent evidence suggests that the gut microbiota play a role in energy harvest, storage, and expenditure and its composition was shown to differ in lean and obese humans and animals and to change rapidly in response to dietary factors. Recently we demonstrated that the fat-derived satiety factor oleoylethanolamide (OEA) requires the integrity of the central histaminergic system to fully exert its hypophagic action. Here we evaluate the effects of OEA sub-chronic treatment on food intake, body weight, gut microbiota composition, plasma

tryptophan metabolites and cytokines expression in histidine decarboxylase knock out (HDC-KO) mice and wild type (WT) littermates. Male mice received daily i.p. injections of OEA (10 mg/kg) or vehicle (PEG:Tween80:saline 5/5/90) for 11 days, 1 hr before dark onset. A significant reduction in cumulative food consumption as well as in body weight was observed in OEA-treated WT mice with respect to vehicle-treated animals. Both effects were attenuated in HDC-KO mice. Amplicons containing the V3-V5 region of the bacterial 16S rRNA gene were obtained from DNAs extracted from faecal samples, sequenced on a Roche 454 GS FLX+ platform and then analyzed using bioinformatic tools. In keeping with the decrease of weight, an enrichment of the phylum Bacteroidetes and a reduction of Firmicutes emerged in the group of OEA-treated WT mice. This trend in terms of phyla, is associable to the appearance of a "lean" microbiota instead of an "obese", where the trends of the two phyla are reversed. Moreover, PICRUSt analysis revealed and enrichment of sequences associated with aminoacids metabolism. Consistently, we observed a tendency in increase of tryptophan and kynurenic acid and reduction of kynurenine plasma levels in WT but not in HDC-KO mice. OEA sub-chronic treatment also modulated the expression of cytokines from the Peyer's Patches obtained from WT animals and stimulated with faseolamine: OEA decreased the production of the inflammatory cytokines IFNy, IL-17 and IL-4, IL-6 as well CXCL-1 and CXCL-2, whereas it increases IL-10 production and Bcell activating factor (BAFF). In summary, we observed that OEA repeated administrations reduced food intake and body weight, altered the gut microbiota towards a lean phenotype and modulated the expression of pro-inflammatory cytokines. All these effects seem to require the histaminergic system. We acknowledge the Joint Programming Initiative - A Healthy Diet for a Healthy Life for support of AMBROSIAC project.

Disclosures: G. Provensi: None. M. di Paola: None. E. Bonechi: None. A. Costa: None. P. Blandina: None. G. Clarke: None. C. Ballerini: None. C. de Filippo: None. M.B. Passani: None.

Poster

327. Appetitive and Incentive Learning and Memory II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 327.01/NN33

Topic: G.01. Appetitive and Aversive Learning

Support: DoD NDSEG

NIDA K08 DA037912-01

NIDA T32 DA007281

Title: Muscarinic acetylcholine receptor antagonism decreases sign-tracking behavior in rats

Authors: *C. J. FITZPATRICK, J. D. MORROW

Dept. of Psychiatry, Univ. of Michigan, Ann Arbor, MI

Abstract: Muscarinic acetylcholine receptors (mAChRs) facilitate dopamine (DA) transmission within the mesolimbic reward system. Our laboratory uses a Pavlovian conditioned approach (PCA) paradigm to investigate this system and addiction vulnerability in rats. Briefly, rats are repeatedly presented with a conditioned stimulus (CS; a lever) followed by the responseindependent presentation of an unconditioned stimulus (US; a food pellet). Over multiple training sessions, two phenotypes can develop: sign-tracking (CS-directed behavior) and goaltracking (US-directed behavior). Sign-trackers (STs) attribute incentive-motivational value to reward-related cues and are more vulnerable to addiction-like behaviors, such as cue-induced reinstatement of drug-seeking. In addition, sign-tracking behavior requires DA transmission within the mesolimbic reward system. Therefore, we hypothesized that mAChR antagonism would attenuate the acquisition and expression of sign-tracking behavior. In agreement with our hypothesis, scopolamine (0.3-3 mg/kg; i.p.), a nonselective mAChR antagonist, decreased the acquisition of sign-tracking behavior (and increased goal-tracking behavior) during nine daily PCA training sessions. Next, during an additional PCA training session, previously vehicletreated rats received scopolamine (3 mg/kg), which reduced the expression of previously learned sign-tracking behavior in rats. Taken together, these results demonstrate that mAChRs modulate sign-tracking behavior and suggest that mAChR antagonism might be a useful component of new treatment strategies for addiction.

Disclosures: C.J. Fitzpatrick: None. J.D. Morrow: None.

Poster

327. Appetitive and Incentive Learning and Memory II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 327.02/OO1

Topic: G.01. Appetitive and Aversive Learning

Support: NHMRC #633267

MH56646

ARC DP130103965

Title: The effect of cholingergic interneuron ablation and delta-opioid receptor internalization in the nucleus accumbens shell on cue-guided choice

Authors: *A. K. MORSE¹, V. LAURENT², B. W. BALLEINE² ¹UCLA, Los Angeles, CA; ²Psychology, Univ. of New South Wales, Sydney, Australia **Abstract:** Animals can use information from their environment to guide their choices between actions. This ability can be studied through outcome-specific Pavlovian-instrumental transfer (PIT), in which a cue associated with a particular outcome biases choice between actions towards the response that earned that same outcome. The nucleus accumbens shell (NAc-S) is a neural substrate of particular interest, as it is recruited at the point of transfer, and not in encoding either Pavlovian or instrumental contingencies. Delta-opioid receptor (DOR) signaling within the NAc-S is crucial for specific PIT expression, and a plastic change in DOR expression within the NAc-S occurs during Pavlovian learning that appears to be later recruited for specific PIT. During contingent Pavlovian learning, DOR accumulates at the membrane of NAc-S cholinergic interneurons (CINs), and this accumulation is positively correlated with the magnitude of specific PIT expression.

Here we investigated the respective roles of CINs, and of DOR accumulation, within the NAc-S in specific PIT expression. In our first experiment, we selectively ablated NAc-S CINs using targeted caspase activation in ChAT-cre rats, and found that CIN ablation impaired specific PIT expression. However, incomplete ablation preserved some specific transfer. In our second and third experiments, we investigated the effect of transient reduction of membrane DOR expression in DOR-eGFP mice, and in the NAc-S in rats. SNC80 is a selective DOR agonist that triggers profound DOR internalization, and we have found that this internalization is transient in the NAc-S of trained DOR-eGFP mice. 24 hours after SNC80 exposure, membrane DOR expression was reduced, but not eliminated, on NAc-S CINs. However, 48 hours after SNC80 exposure, DOR expression returned to an accumulated state, indicating that CINs have 'memory' for this plastic change that occurs in Pavlovian learning. We therefore exploited SNC80 to investigate specific PIT expression when DOR expression was reduced, and when recovered to an accumulated state.

When SNC80 was administered systemically in DOR-eGFP mice, or infused directly into the NAc-S in rats, specific PIT expression was impaired at 24 hours, when DOR expression was reduced. Specific transfer recovered at 48 hours, when DOR had recovered to an accumulated state. These experiments, considered together, suggest that DOR accumulation at the membrane of CINs in the NAc-S is critically involved in the ability of reward-predictive cues to bias choice between actions.

Disclosures: A.K. Morse: None. V. Laurent: None. B.W. Balleine: None.

Poster

327. Appetitive and Incentive Learning and Memory II

Location: Halls A-C

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Program#/Poster#: 327.03/OO2

Topic: G.01. Appetitive and Aversive Learning

Support: NIH grant MH108924

NIH grant MH101214

NIH grant MH113316

NARSAD Independent Investigator

Title: Optogenetic probing of ventral pallidal neurons in motivated behaviors

Authors: *C. BRAVO-RIVERA, M. STEPHENSON-JONES, C. FERNANDES-HENRIQUES, B. LI Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Recently, there have been significant advances in the characterization of neuronal circuits that influence motivated behaviors, including aversion and reward-related behaviors. The ventral pallidum (VP) is a corticolimbic-motor interface that has been previously linked to conditioned aversion (Itoga et al 2015) and reward-seeking (Tachibana and Hikosaka 2012). Whereas most neurons in VP are GABAergic, a subpopulation of VP projection neurons is glutamatergic (Root et al 2015). VP neurons project to the lateral habenula (LHb), a key structure mediating reward and aversion encoding and, therefore, these projections likely gate aversion and reward encoding. Here, we aim to characterize whether VP neurons mediate motivation related to reward approach and aversion avoidance. First, we tested whether optogenetic activation or silencing of VP somata or projections to LHb generate real-time place preference in a two-chamber box. Optogenetic activation of VP GABAergic somata or their projections to LHb induced strong place preference for the chamber paired with laser stimulation (p < 0.001). Conversely, optogenetic activation of VP glutamatergic somata or their projections to LHb induced strong place aversion for the chamber paired with laser stimulation (p < 0.001). Silencing VP GABAergic or glutamatergic somata, or their projections to LHb, had no effect on real-time place preference (p > 0.1). We next tested whether VP neurons are necessary for reward approach. We developed a task in which five auditory tones predict different levels of reward (water) in head-fixed, water-deprived mice. To obtain the water reward, mice needed to lick the water spout within a time limit after the cue presentation. We found that silencing of VP GABAergic, but not glutamatergic (p > 0.1), somata or projections to LHb reduced reward attainment by diminishing licking responses (p < 0.05). Conversely, activation of VP GABAergic somata or projections to LHb increased licking responses (p < 0.05). Activation of VP glutamatergic somata decreased licking responses (p < 0.05). Together, these results suggest that VP GABAergic neurons are sufficient and necessary for reward approach, whereas VP glutamatergic neurons antagonize reward approach and generate aversion. Characterization of this VP circuit will broaden our understanding of how motivation is encoded in the basal ganglia.

Disclosures: C. Bravo-Rivera: None. M. Stephenson-Jones: None. C. Fernandes-Henriques: None. B. Li: None.

327. Appetitive and Incentive Learning and Memory II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 327.04/OO3

Topic: G.01. Appetitive and Aversive Learning

Title: A direct comparison of neuronal firing in passive aversive and passive appetitive conditioning and extinction in the prelimbic and infralimbic cortices of the mPFC

Authors: *B. KAMINSKA¹, D. E. MOORMAN²

¹Neurosci. and Behavior, Univ. of Massachusetts, Amherst, MA; ²Psychological and Brain Sci., Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Neurons of the rat medial prefrontal cortex (mPFC), specifically those in prelimbic (PL) cortex, are modulated by positive and negative cues and outcomes. Neurons in the infralimbic (IL) mPFC are modulated by previously-informative cues that no longer predict an outcome (i.e., after extinction learning). These dichotomous roles are not observed in all cases of conditioning, indicating that more work is required to understand PL vs. IL function during learning. To address this issue, we recorded from PL and IL during learning and extinction of Pavlovian cue and outcome pairings in both appetitive and aversive conditions. Female and male Wistar rats were surgically implanted with intraoral catheters which allowed a brief delivery of either an aversive or appetitive stimulus (quinine or sucrose). Electromyography wires were implanted into the anterior digastric which allowed the measurement of taste reactivity to the stimulus based on hedonic reaction (licks for appetitive stimuli and gapes for aversive). Drivable tetrode arrays were implanted with 4 tetrodes implanted in PL and 4 tetrodes implanted in IL, allowing simultaneous neuronal recordings. Electrodes were driven down ~500um to access a different population of neurons after every session for a total of 5 sessions. Each recording session consisted of 2 days, and the first day was split into 3 sections. During the first section, 3 auditory stimuli were presented in a random order (habituation). During the second section, each tone presentation was followed by sucrose, quinine, or no liquid delivery (conditioning). During the third section, no outcome was delivered after tone presentation (extinction). On the second day of recording the three tones were played with no outcome following them (extinction recall). Currently we have recorded from 2 rats with two sessions each, totaling 54 neurons. Of these, 32 neurons were located in PL and 22 in IL. In the subset of significantly event-modulated neurons (15 PL and 6 IL), there was no consistent pattern of neuronal activation or inhibition to cue or stimulus deliveries during habituation, conditioning, extinction, and extinction recall. These preliminary results suggest a highly heterogeneous coding structure by mPFC neurons during Pavlovian conditioning and extinction. More recordings and analysis, currently underway, will augment these preliminary data and better define the parameters of mPFC neuronal encoding of learning.

Disclosures: B. Kaminska: None. D.E. Moorman: None.

Poster

327. Appetitive and Incentive Learning and Memory II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 327.05/OO4

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant 5R01NS094667-02

Pew Biomedical Scholar

Klingenstein Fellowship in the Neurosciences

Title: Songbirds implement multiobjective reinforcement learning with action-specific cost functions

Authors: *R. CHEN, D. MURDOCH, J. H. GOLDBERG Neurobio. and Behavior, Cornell Univ., Ithaca, NY

Abstract: Standard reinforcement learning (RL) algorithms enable an agent to optimize a single cost function such as reward, but it remains poorly understood how an animal might pursue multiple objectives at once. Multi-objective RL can be implemented in machines by endowing a single agent with multiple sub-agents each tasked with optimizing objective-specific reinforcement. Here we use songbirds to test if two behaviors with distinct objectives - vocalization and navigation - are shaped by objective-specific reinforcers. We demonstrate that strobe light reinforces place preference but not song syllables. Playback of song-like sound reinforces song syllables but not place preference. This double dissociation demonstrates that a single animal can possess distinct action-generating systems shaped by correspondingly distinct cost functions.

Disclosures: R. Chen: None. D. Murdoch: None. J.H. Goldberg: None.

Poster

327. Appetitive and Incentive Learning and Memory II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 327.06/OO5

Topic: G.01. Appetitive and Aversive Learning

Title: Adaptive thermogenesis is increased by long term co-adimistration of central GLP-1 and GIP

Authors: *Y. LEE^{1,2}, D. CHUN^{1,2}, C. NAMKOONG^{1,3}, M. KIM^{1,2}, H. LEE¹, H. CHOI^{1,2,3,4} ¹Seoul Natl. Univ., Seoul, Korea, Republic of; ²BK21Plus Biomed. Sci. Project Team, Seoul, Korea, Republic of; ³Neurosci. Res. Inst., Seoul, Korea, Republic of; ⁴Wide River Inst. of Immunol., Hongcheon, Korea, Republic of

Abstract: The brain network that regulates adaptive thermogenesis receives inputs from temperature- and energy-sensing neurons through hypothalamic and brain stem areas. Glucagonlike peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are both wellknown gut hormones that acts on brain to regulate body weight and food intake. In this study, we investigated the effect of long term co-administration of central GLP-1 and GIP on energy expenditure. We co-administered GLP-1 and/or GIP intracerebroventricularly (ICV) in mice with osmotic mini pumps for 17 days. GLP-1 (3nmol/day), GIP (6nmol/day) or both GLP-1 and GIP were continuously administered during 17 days. Comprehensive Laboratory Animal Monitoring System was used to measure O₂ consumption, CO₂ production, RER, locomotion and energy expenditure. Thermogenic marker, UCP-1 was measured in white adipose tissue and brown adipose tissue. Body weight and food intake were significantly decreased by coadministration of GLP-1 and GIP during the first week. After two weeks, co-administration of GLP-1 and GIP significantly increased energy expenditure without changing locomotion. GLP-1 and GIP co-administrated mice showed significantly higher expression of UCP-1 in white adipose tissue and brown adipose tissue. Therefore, the present results suggest that coadministration of GLP-1 and GIP increases thermogenesis via direct modulation of adipocyte metabolism. Our findings suggest that co-administration of GLP-1 and GIP may have synergistic anti-obesity effect on both food intake and energy expenditure.

Disclosures: Y. Lee: None. D. Chun: None. C. NamKoong: None. M. Kim: None. H. Lee: None. H. Choi: None.

Poster

327. Appetitive and Incentive Learning and Memory II

Location: Halls A-C

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Program#/Poster#: 327.07/OO6

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant MH108924

NIH Grant MH101214

NIH Grant MH113316

NARSAD Independent Investigator Grant

Title: Genetically distinct ventral pallidal neurons encode the motivation for reward approach and punishment avoidance

Authors: *M. STEPHENSON-JONES, C. C. BRAVO-RIVERA, C. FERNANDES-HENRIQUES, B. LI

Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Motivated behaviours can be driven by two opposing processes, the desire to obtain a reward or the drive to escape a punishment. The ventral pallidum is critical for attributing motivation salience to cues that predict reward and for invigorating reward seeking behaviour. However, sparse evidence suggests that it may also play a role in motivating avoidance behavior. Here we show that two genetically distinct ventral pallidal populations encode the motivation to approach or avoid. Single-unit recording in the ventral pallidum of mice undergoing conditioned reward and punishment tasks revealed three functional distinct clusters, which encoded 1) incentive salience, 2) aversive salience and 3) general vigor. Optogenetic inhibitory tagging demonstrated that neurons that encoded incentive salience were GABAergic, as were the neurons that encoded general vigor. In a Pavlovian reward task, these incentive salience neurons encode the expected reward value through a phasic increase in neuronal firing. As with dopamine neurons, the response to expected reward diminishes with training; but unlike dopamine neurons, the response to reward cues inverts and is signaled with a decrease in neuronal firing when mice are sated. This suggests that GABAergic ventral pallidal neurons encode a state dependent prediction error signal. These neurons also signal state value on a longer timescale as their baseline firing was modulated by the state depending on block type (reward vs punishment). Taken together GABAergic VP neurons signal the positive motivational state and incentive value to invigorate reward seeking on a short and long timescale. In contrast the Glutamatergic ventral pallidal neurons were phasically excited by the expectation or delivery of punishment. As with GABAergic neurons, the baseline firing was modulated depending on the block condition, indicating that these neurons encode aversive salience on both long and short time scales. Together our data demonstrates that there are two genetically distinct types of ventral pallidal neurons that encode either incentive or aversive salience and are critical for driving reward approach or punishment avoidance.

Disclosures: M. Stephenson-Jones: None. C.C. Bravo-Rivera: None. C. Fernandes-Henriques: None. B. Li: None.

Poster

327. Appetitive and Incentive Learning and Memory II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 327.08/OO7

Topic: G.01. Appetitive and Aversive Learning

Title: Effect of paternal obesity on the central nervous system reward circuitry in offspring

Authors: *G. SINDI

Biol. Sci., Ohio Univ., Athens, OH

Abstract: Paternal and maternal diet-induced obesity strongly impact subsequent generations. The endocannabinoid system (ECS) is responsible for appetite, and modulating reward processing and includes cannabinoid receptor type-1 (CB1-R). CB1-R expression is altered due to overeating. Previous studies have focused on the direct effect of CB1-R in mice eating high fat diet (HFD) and not their offspring. Our team studied the effect of paternal obesity on offspring and demonstrated that voluntary physical activity occurred more in male offspring of HFD-fed fathers (Gr 2) at 1.5 months of age than offspring of low fat diet (LFD)-fed fathers (Gr 1). Female offspring ran more than males in three groups: HFD-fed father offspring at 6 and 12 months, and LFD-fed father offspring at 1.5 months. Female offspring from Gr2 showed higher food consumption at 1.5 months than Gr1 females. We hypothesized that alterations within the CNS reward circuitry are related to different levels of physical activity and food preference in offspring of obese HFD-fed fathers versus non-obese LFD-fed fathers. Brain samples from offspring at 1.5, 6, and 12 months of age were used for immunohistochemistry. Sections were incubated in primary polyclonal rabbit anti-CB1-R antibody against the C-terminus of the receptor, and secondary conjugated Horseradish Peroxidase (HRP) goat anti-rabbit secondary antibody. CB1-R were detected using DAB substrate HRP, which generates a brown to black product when cleaved by DAB and quantified using ImagePro. Our results demonstrate the effects of paternal obesity on the CNS reward circuitry in offspring mice. We will discuss the expression of CB1-R depending on age, sex, and paternal diet. This study further clarifies the relationship between paternal obesity, voluntary exercise and food intake and CB1-R expression alterations in the brain and may lead to discovery of new agents for prevention of obesity in offspring of obese parents.

Disclosures: G. Sindi: None.

Poster

327. Appetitive and Incentive Learning and Memory II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 327.09/OO8

Topic: G.01. Appetitive and Aversive Learning

Title: Effects of age, sex, and alcohol access on sign-tracking/goal-tracking and omissioncontingency learning in rats
Authors: A. COOK¹, B. GAEDDERT¹, H. FISHER², *C. L. PICKENS¹ ²Psychological Sci., ¹Kansas State Univ., Manhattan, KS

Abstract: Previous research from our laboratory has found that 6 weeks of chronic intermittent access of alcohol during adolescence and early adulthood (PND 26-66) in male rats leads to long-term behavioral changes that include faster omission contingency learning in a lever-based autoshaping task. Subsequent examinations did not find this pattern if the chronic intermittent access occurred in adult males or adolescent females. However, as the adolescent males consumed more alcohol than the adult males or adolescent females, it is unclear whether this differential effect is due to a heightened sensitivity to the effects of alcohol in adolescent males or merely related to the higher level of alcohol consumed in this group. In order to examine this, we gave adolescent male, adolescent female or adult male rats injections with fixed doses of saline or alcohol to ensure that all rats received the same dose of alcohol. Rats received 0, 0.875 or 1.75 g/kg alcohol injections twice per day on Monday, Wednesday and Friday for 6 weeks. Thus, rats received 0, 1.75, or 3.5 g/kg of alcohol during each injection day. Rats began behavioral training 18 days later. During autoshaping training, extension of two levers signaled food delivery regardless of responding. In the subsequent omission contingency phase, presentation of one lever signaled food delivery regardless of responding and presentation of the other lever predicted reward if the rat did not press the lever, but pressing this lever canceled the reward delivery. When compared with saline treatment, alcohol injections had no effect on behavior during the autoshaping phase. However, when we examined terminal levels of signtracking and goal-tracking behaviors (responses/trial) in the autoshaping phase, we found that rats in the adult male groups made significantly more sign-tracking responses than the adolescent female groups (but not the adolescent male groups), but there were no differences between the groups in goal-tracking behaviors. We will also present our research on the effects of alcohol on behavior in the omission contingency task.

Disclosures: A. Cook: None. B. Gaeddert: None. H. Fisher: None. C.L. Pickens: None.

Poster

327. Appetitive and Incentive Learning and Memory II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 327.10/OO9

Topic: G.01. Appetitive and Aversive Learning

Support: R01 DA019473

R01 DA038412

R21 DA041725

Title: Studying temporal decision making in a free-operant foraging paradigm

Authors: *C. CROUSE, S. NICOLA

Dominick D. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: The delay-discounting paradigm is used to study how animals make decisions about how long to wait to receive reward and analyze the brain areas involved. However, results from this paradigm conflict with ecological observations about animal foraging where food is distributed in discrete patches, bringing these conclusions into doubt. We are developing an operant-conditioning paradigm modeled on animal choice in the wild to study foraging behavior. Food-restricted rats are trained to press a lever to receive sugar, but each press increases the wait time before the next lever presentation. Pressing a second lever returns the wait time to its original value after a reset period, modeling the time to travel between patches of food. We have found that increases in the reset time causes animals to wait longer for rewards, suggesting that rats are responsive to the task parameters and validating predictions from foraging theory. Future experiments will create a model of optimal choice and test the role of nucleus accumbens dopamine in this task.

Disclosures: C. Crouse: None. S. Nicola: None.

Poster

327. Appetitive and Incentive Learning and Memory II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 327.11/OO10

Topic: G.01. Appetitive and Aversive Learning

Support: NIH-1R01DK108643

NIH/NIDDK DK26687

Title: Bariatric surgery reduces effort to obtain a sweet drink

Authors: *H. R. KISSILEFF¹, G. M. PAYNE², J. D. HAMM², M. HERZOG³, J. JANG³, A. SHECHTER³, B. LAFERRERE³, F. X. PI-SUNYER³, J. ALBU⁴ ¹New York Obesity Nutr. Res. Ctr. - Med., ³Med. - Obesity Res., ²Columbia Univ. Med. Ctr., New York, NY; ⁴Med. Endocrinol., Mt. Sinai-St. Luke's Hosp., New York, NY

Abstract: Mechanisms for reduction of food intake after bariatric surgery are not thoroughly understood. Bariatric surgery induces unpleasant after-effects of eating such as plugging and dumping syndromes, which may lead to the commonly reported avoidance of sweets by food-avoidance conditioning. The mechanism underlying the avoidance of sweets is one that requires more attention. Post-surgical patients worked less for a sweet tasting solid snack food (M&Ms)

than before surgery (Am J Clin Nutr 2012;96:467–73.). To determine whether reduction of work for a sweet tasting snack extends to beverages, gastric-bypass and gastric-sleeve bariatric surgery candidates (N = 30, mean BMI = 45 kg/m^2) used a sipometer (Physiol. Behav. 2017: 171, 216– 227), that requires oral sipping pressure, to sham-sip (sip and spit out) non-caloric cherry flavored Kool Aid. The beverages were presented either sweetened with 10% sucrose-equivalent aspartame, or unsweetened, in separate counterbalanced sessions on the same trial day, on both continuous and 3-sec time increment progressive ratio reinforcement schedules. Tests were conducted within 2 to 4 weeks before surgery and again at 3 mo after surgery. Before surgery, cumulative pressure difference exerted between sweetened and unsweetened beverages during trials on the progressive ratio schedule increased significantly with differences in patients' beverage ratings of "liking", "enjoyment" sweetness intensity and "wanting" of the beverage, (all p's <.05). After surgery there were no significant correlations between any of the pressure-rating relationships. Pressure exerted in relation to valuation provides a novel measure of effort and propensity to consume. Reduced propensity to consume sweet beverages could be a contributor to reduced intake and, therefore, to weight loss after bariatric surgery. Further study of neural and hormonal mechanisms for reduction in effort to consume sweets could be used to develop non-surgical treatments for obesity.

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Poster

327. Appetitive and Incentive Learning and Memory II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 327.12/OO11

Topic: G.01. Appetitive and Aversive Learning

Title: Localization of npy/agrp and pome neurons in hypothalamus arcuate nucleus (arc) using ihe and tissue clearing technique: Comparative study of human and mouse

Authors: *K. MIN SUN^{1,2}, H. SONG¹, C. NAMKOONG^{1,3}, Y. LEE^{1,2}, J. OH¹, Y. CHUNG¹, H. CHOI^{1,2,3,4}

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Abstract: Neuropeptide Y (NPY) / Agouti-related peptide (AgRP) and Pro-opiomelanocortin (POMC) neurons are the major regulators of feeding behavior and body weight. Both populations have different roles on appetite. In a previous study with mouse hypothalamus, AgRP/NPY neurons are located in the medial side of ARC while POMC neurons are located in

the lateral side of ARC. However, only few studies investigated the distribution of AgRP/NPY and POMC neurons in human hypothalamus. We investigated the distribution of AgRP/NPY and POMC neurons throughout the mouse brain and human hypothalamus by immunohistochemistry (IHC) and tissue clearing technique. Tissue clearing technique is a novel technique which enables three-dimensional visualization of immunostained tissue. We found that AgRP/NPY and POMC neurons were distributed in a specific locations as previously reported. We were able to document three-dimensional distribution of POMC neurons using tissue clearing technique. Also in human hypothalamus, AgRP/NPY and POMC neurons were localized in a specific locations throughout the ARC. To elucidate the functional aspects of each neuron, co-localization of GLP-1R (which is the target of anti-obesity drug, GLP-1) was investigated. In mouse brain ARC, GLP-1R was not expressed in NPY neurons. However, about half of POMC neurons expressed GLP-1R (45.9%). The distribution of AgRP/NPY and POMC neurons in human and mouse brain ARC, may facilitate obesity research and anti-obesity drug development.

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Poster

327. Appetitive and Incentive Learning and Memory II

Location: Halls A-C

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Program#/Poster#: 327.13/OO12

Topic: G.01. Appetitive and Aversive Learning

Support: NIMH 1RO1MH85724-01

NIMH 5T32MH019113-22

US DEPT of Veteran's Affairs

Title: A novel role for acid sensing ion channels in pavlovian conditioning

Authors: ***A. GHOBBEH**¹, S. M. ALAM², R. FAN³, R. J. TAUGHER⁴, R. T. LALUMIERE⁵, J. A. WEMMIE³

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Abstract: Pavlovian conditioning is a fundamental form of learning and memory implicated in a variety of behaviors. Pavlovian conditioning involves learning the association between a neutral, conditional stimulus (CS) paired withand an unconditional stimulus (US), which can be either intrinsically aversive (e.g. foot shock) or rewarding (e.g. food). Our previous studies suggest that Pavlovian conditioning to an aversive US depends on synaptic plasticity in the amygdala via Acid Sensing Ion ChannelsIs (ASICs). Here, we asked whether ASIC1A, a critical ASIC subunit,

is also required for Pavlovian conditioning to a rewarding US. We tested mice expressing ASIC1A versus mice lacking ASIC1A using automated chambers to quantify behavioral responses to 20 pairings per day over multiple days of a CS (tone + light) with a rewarding US (Ensure). Three experiments were performed testing three different CS-US pairing strategies: 1) CS immediately preceded US, 2) CS and co-initiated with US co-initiated simultaneously, and 3) CS explicitly unpaired fromand US explicitly unpaired. In these experiments, multiple ASIC1A-dependent effects were observed. Surprisingly,Together, the results suggest that rather than causing impairment, loss of ASIC1A unexpectedly improved Pavlovian reward conditioning by increasing learning precision and efficiencyenhancing learning and memory. NonethelessTogether, these results suggest a fundamental difference in the effects of ASICs in two opposingtwo forms of Pavlovian learning and memory. They also offer a potential target for differentially manipulating reward and aversion in the context of mental illnesses such as drug

addiction and depression. More work is needed to better understand this unexpected improvement in reward conditioning. Nonetheless, these results suggest a fundamental difference in the effects of ASICs in two forms of Pavlovian learning and memory. They also offer a potential target for differentially manipulating reward and aversion in the context of mental illnesses such as drug addiction and depression.

Disclosures: A. Ghobbeh: None. S.M. Alam: None. R. Fan: None. R.J. Taugher: None. R.T. LaLumiere: None. J.A. Wemmie: None.

Poster

327. Appetitive and Incentive Learning and Memory II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 327.14/OO13

Topic: G.01. Appetitive and Aversive Learning

Title: Calorie information modulates reward and control activation in response to food images

Authors: *A. L. COURTNEY, K. M. RAPUANO, E. K. PECONGA, T. F. HEATHERTON, W. M. KELLEY

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Abstract: Obesity is a major public health problem in the United States, with more than two out of three of adults categorized as overweight or obese (NIDDK, 2016). In response, public health departments have moved to mandate that restaurants publish caloric data at the point of purchase, with the hope of biasing consumer purchasing decisions toward lower calorie options (Berman, 2008; Roberto, 2010). However, the efficacy of this approach has not been well understood at the level of value processing and decision-making. The present study sought to interrogate the influence of calorie information on brain responses to food cues. Specifically, we hypothesized

that activation in reward regions—including the nucleus accumbens (NAcc) and orbital frontal cortex (OFC)-would be reduced in the presence of calorie information, but that activation in control regions-including the frontoparietal control network-would increase in the presence of calories. Moreover, because dieters are known to consider calorie content when making food choices, we evaluated dieting status as a moderator of these effects. During an fMRI scan, participants (N= 50) viewed pictures of food in which caloric information was either absent or present in a within-subject design. In the presence of calorie information, reward activation decreased whereas frontoparietal control activation increased. Further, reward activation was sensitive to both caloric information and the dieting status of the participant. A whole-brain searchlight multivariate pattern analysis (MVPA) was performed to identify regions of the brain whose activation patterns were most similar across conditions (calories VS. no calories). A group t-test comparing dieters to non-dieters revealed that dieters produced more similar activation patterns in the OFC for food images presented with calories and food images presented without calories—suggesting that dieters may spontaneously consider caloric information when viewing food cues. Taken together, these findings suggest that calorie information alters brain responses to food cues by simultaneously increasing control system activity and reducing reward system activity.

Disclosures: A.L. Courtney: None. K.M. Rapuano: None. E.K. PeConga: None. T.F. Heatherton: None. W.M. Kelley: None.

Poster

327. Appetitive and Incentive Learning and Memory II

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Program#/Poster#: 327.15/0014

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant DA035468

Title: C. elegans as a model system to identify novel pharmacotherapies for nicotine addiction

Authors: *B. S. NEAL-BELIVEAU¹, K. E. BREDHOLD², K. B. STEAGALL, II², S. N. KATNER³, E. A. ENGLEMAN³ ¹Psychology, ²Neurosci. Program, IUPUI, Indianapolis, IN; ³Psychiatry, IU Sch. of Med., Indianapolis, IN

Abstract: Despite the decrease in tobacco use in the United States since 1973, nicotine continues to be one of the most widely used psychoactive drugs in our society. Although the negative consequences associated with nicotine use via smoking cigarettes are well known, people find it very difficult to successfully quit. In fact, current smoking cessation pharmacotherapies only result in long-term abstinence in about 20% of quit attempts. Thus, there is a need to identify

novel, effective treatments for combating nicotine addiction. The purpose of this study was to examine the appetitive properties of nicotine in an invertebrate model of drug preference. Our laboratory has previously shown that the nematode *Caenorhabditis elegans* (*C. elegans*) demonstrates movement toward and self-exposure to various psychoactive drugs (i.e. cocaine, methamphetamine, morphine, ethanol and caffeine). The eventual goal is to use this invertebrate model to identify potential novel cessation aids at a fraction of the time and cost of many other animal models. Preference tests were performed in 6-well test plates spotted with nicotine (5, 50, 100 or 500 mM) and vehicle (water) in the opposing target zones of each well. Adult, agesynchronized worms (N2 Bristol wild-type) were placed in the center of each well and the number of worms in each target zone was counted 30 min later. Untreated worms showed a concentration-dependent preference for nicotine that was significantly enhanced when the nicotine solutions were titrated to a pH of 7. The worms were not immobilized by self-exposure to nicotine as they were able to move away when the aversive compound nonanone was subsequently added to the target zone. Preference for nicotine was significantly decreased by pretreatment with the opioid receptor antagonist naltrexone. In contrast, pretreatment with naltrexone did not alter preference for food or the known attractant benzaldehyde, suggestive of a specific effect on nicotine preference. Overall, these data suggest that the rewarding properties of nicotine can be studied in this invertebrate model. Furthermore, the ability of naltrexone pretreatment to alter the preference for nicotine lends support for the use of this model to test potential pharmacotherapies for nicotine addiction.

Disclosures: B.S. Neal-Beliveau: None. **K.E. Bredhold:** None. **K.B. Steagall:** None. **S.N. Katner:** None. **E.A. Engleman:** None.

Poster

328. Fear and Aversive Learning and Memory: Neural Circuits I

Location: Halls A-C

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Title: Disinhibitory amygdala microcircuits for aversive learning

Authors: *E. PARADISO¹, S. KRABBE², C. XU², S. D'AQUIN², M. MARKOVIC², J. GRÜNDEMANN², F. FERRAGUTI¹, A. LÜTHI² ¹Med. Univ. Innsbruck, Innsbruck, Austria; ²Friedrich Miescher Inst. for Biomed. Res., Basel,

Switzerland

Abstract: The basolateral amygdala (BLA) is a cortical-like structure known to be involved in simple forms of emotional learning such as fear conditioning. It is the main entry site for sensory information to the amygdaloid complex and local plasticity in the BLA is crucial for fear memory formation. BLA principal neurons (PN) excitability is under tight control of different subclasses of inhibitory interneurons (IN). However, the contribution of each subtype of IN to sensory processing and fear learning is still poorly understood.

Using a deep brain calcium imaging approach in freely behaving mice, we aim to understand how different IN subtypes control fear learning. We further apply novel intersectional viral tools and optogenetic approaches, together with rabies-based retrograde trans-synaptic tracing to analyse how different IN subgroups interact with each other, and how this disinhibitory interplay could affect plastic changes of BLA PNs and thus gate memory formation.

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Poster

328. Fear and Aversive Learning and Memory: Neural Circuits I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 328.02/OO16

Topic: G.01. Appetitive and Aversive Learning

Support: JSPS Kakenhi Grant-in-Aid Wakate B 16K21620

Strategic Research Program Nou Pro 11041047

Title: Noradrenergic modulation of aversive memory formation - from circuits to molecules

Authors: *B. TAN, J. JOHANSEN RIKEN Brain Sci. Inst., Wakoshi, Japan

Abstract: Noradrenergic neurons in the locus coeruleus play an important role in mood, attention, cognitive processes and learning through their diverse projections throughout the brain. Previously, we showed that noradrenaline in the lateral amygdala (LA) modulates Hebbian

plasticity mechanisms via beta-adrenergic receptors (β -ARs) to facilitate the formation of auditory fear memories, possibly via downstream signaling events that regulate long term memory formation. But it is unclear if locus-coeruleus-noradrenaline (LC-NA) projections to the LA modulate learning, and the intracellular changes that are triggered by NA release and β -AR activation in the LA to facilitate this learning. To understand these questions, we used optogenetic and viral approaches in rats to dissect the functional role of LC-NA projections to the LA during fear learning, and the underlying molecular mechanisms through which these neuromodulatory signals regulate long term memory formation. We injected retrograde canine adenovirus expressing Cre recombinase (CAV2-Cre-GFP) into the LA and adeno-associated virus expressing Cre-dependent ArchaerhodospinT (ArchT-tdtomato) into the LC. This produced expression of ArchT in the cell bodies and nerve terminals of the noradrenergic neurons, making it possible to optogenetically inhibit the LC-NA projections in the LA during auditory fear conditioning. We found that inhibition of LC-NA projections in the LA, specifically during the aversive shock period of fear conditioning, significantly reduced fear memory formation. CREBregulated transcription coactivator-1 (CRTC1) integrates calcium and cAMP signaling pathways synergistically to regulate transcriptional processes and neural plasticity through its nuclear translocation. We next examined the role of CRTC1 activity in the LA neurons during fear conditioning and found that fear learning produced a significant increase in CRTC1 nuclear accumulation in the LA neurons. To examine the function of this CRTC1 nuclear translocation, we virally expressed a dominant-negative CRTC1 (DNCRTC1) or shRNA targeting CRTC1 to reduce CRTC1 activity or expression respectively. Both manipulations reduced long term fear memory formation. These results demonstrate a temporally precise role for the activity of LC-NA projections in the LA during fear memory formation, and implicate CRTC1 as a potential mechanism for transducing noradrenergic signaling in the LA neurons into long term fear memories.

Disclosures: B. Tan: None. J. Johansen: None.

Poster

328. Fear and Aversive Learning and Memory: Neural Circuits I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 328.03/OO17

Topic: G.01. Appetitive and Aversive Learning

Support: 16H05928

16H01291

Title: Distinct long-range noradrenaline microcircuits control opposing emotional and flexible learning states

Authors: *A. UEMATSU, B. TAN, J. P. JOHANSEN RIKEN Brain Sci. Inst., Wako, Japan

Abstract: Noradrenaline neurons in the locus coeruleus (LC) play a critical role in many functions including emotional learning and behavioral flexibility. This relatively small population of neurons is traditionally believed to be anatomically and functionally homogeneous with all neurons projecting widely throughout the brain and responding similarly to salient stimuli. However, noradrenaline is important for opposing functions such as fear or extinction learning in different brain regions and some studies have reported specificity in the efferent connectivity of specific LC cell populations. Thus, one mechanism for noradrenergic modulation of opposing behavioral learning states is through distinct LC subcircuits that regulate specific aspects of behavior. We found that LC neurons displayed context-dependent interrelationships, with moderate, discrete activation of distinct cell populations by fear/safety cues and robust, global recruitment of most cells by strong aversive stimuli. Retrograde tracing studies demonstrated that distinct subpopulations of LC-noradrenergic neurons project to amygdala- and medial prefrontal cortex (mPFC) and brain-wide efferent mapping revealed that each neuronal subpopulation maintained specific connectivity with amygdala or mPFC. In a series of optogenetics experiments, we discovered that the amygdala-projecting ensemble promoted fear learning, while the mPFC-projecting cells extinguished aversive responses to enable flexible behavior. Importantly, amygdala projecting cells were more activated during high fear states, while mPFC projecting cells were more engaged during extinction. These results revealed a modular organization of the LC-noradrenaline system assembled from a mosaic of projectionand behavior-specific modules coupled with combinatorial activation modes which enables the adaptive tuning of emotional responding and behavioral flexibility.

Disclosures: A. Uematsu: None. B. Tan: None. J.P. Johansen: None.

Poster

328. Fear and Aversive Learning and Memory: Neural Circuits I

Location: Halls A-C

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant R01MH065961

NIH Grant F31MH107113

Title: The bed nucleus of the stria terminals mediates fear expression to temporally unpredictable threats

Authors: *T. D. GOODE, G. M. ACCA, S. MAREN

Inst. for Neurosci. and Dept. of Psychology, Texas A&M Univ., College Station, TX

Abstract: The bed nucleus of the stria terminalis (BNST) is thought to mediate conditioned fear responses to sustained contextual stimuli but not phasic cues. However, contexts differ from discrete cues not only in terms of their duration, but also in the degree to which they temporally predict the occurrence of aversive outcomes. The current experiments sought to determine whether the temporal predictability of a CS (whether a cue or context), rather than CS duration, is a factor in the recruitment of the BNST. To test this hypothesis, adult male Long-Evans rats were implanted with guide cannulae aimed at the BNST. After a week of recovery, rats were conditioned in a single context with an unsignaled footshock (2 sec, 1 mA) that occurred either 1 min (short-delay; "temporally predictable") or 9 min (long-delay; "temporally unpredictable") after the animal was placed in the context. Each animal received 4 trials over 2 days (2 trials per day) and total exposure to the context was equated for all groups (i.e., all rats remained in the context for 10 min independent of when the shock was delivered); freezing behavior served as the measure of fear expression. After training, the BNST was reversibly inactivated using muscimol (a GABA receptor agonist) or NBQX (an AMPA receptor antagonist) immediately prior to a retrieval session in the conditioned context in the absence of shock. Interestingly, BNST inactivation only disrupted contextual freezing in rats in the long-delay condition, despite the fact that total shock and context exposure was equated among the groups. This reveals that BNST mediates fear expression in unpredictable, but not predictable contexts. Next, we examined whether the BNST mediates fear to discrete CSs that are poor predictors of US onset. We used forward (i.e., CS-then-US) and backward (i.e., US-then-CS) fear conditioning procedures to establish CSs that were either good or poor predictors, respectively, of US onset. Twenty-four hours after conditioning in context A, rats were infused with drug or vehicle into the BNST and tested for fear to the CS alone in context B. Similar to the previous experiment, BNST inactivation attenuated fear to the backward CS, without affecting freezing to the forwardtrained CS. Additional experiments demonstrated increases in Fos-positive neurons in the anteroventral BNST following exposure to a backward CS, but not to a forward CS. Collectively, these results suggest that fear evoked by temporally unpredictable CSs, whether contexts or cues, are mediated by the BNST.

Disclosures: T.D. Goode: None. G.M. Acca: None. S. Maren: None.

Poster

328. Fear and Aversive Learning and Memory: Neural Circuits I

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Program#/Poster#: 328.05/OO19

Topic: G.01. Appetitive and Aversive Learning

Support: R01MH065961

Title: Locus coeruleus modulation of extinction retrieval and fear renewal

Authors: *T. F. GIUSTINO^{1,2}, P. J. FITZGERALD², S. MAREN² ²Dept. of Psychology and Inst. for Neurosci., ¹Texas A&M Univ., College Station, TX

Abstract: After a traumatic incident individuals often undergo exposure therapy or psychological debriefing in an attempt to prevent the development of stress- and trauma-related disorders (e.g., posttraumatic stress disorder, PTSD). These "extinction-like" therapies aimed to reduce fear-related behaviors are context dependent. That is, fear tends to relapse in other settings, which limits the efficacy of such therapies. Past work has demonstrated that the stress hormone norepinephrine (NE), produced by and released from the locus coeruleus (LC), is elevated in individuals with PTSD. Extinction learning and fear relapse can be modeled in the laboratory using Pavlovian fear conditioning procedures in rodents. Here, we used LC-specific DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) to examine how LC-NE contributes to both extinction retrieval (low fear) and fear renewal (high fear). Male rats first received bilateral infusions of either AAV9-PRSx8-hM3Dq-HA or AAV9-PRSx8-hM4Di-HA into the LC. Two weeks after surgery, rats underwent standard auditory fear conditioning and extinction procedures. After extinction learning, rats underwent two dual retrieval-renewal sessions (i.e., each session consisted of consecutive retrieval and renewal procedures using a within-subjects design). Thirty minutes prior to each test session, rats received either vehicle or clozapine N-oxide (CNO; 3 mg/kg, i.p.) injections in a counterbalanced fashion; under these conditions, CNO reliably increased (hM3Dq) or decreased (hM4Di) LC firing rates. In animals expressing the excitatory DREADD, CNO greatly increased freezing to the extinguished CS and strongly attenuated extinction retrieval. In contrast, CNO reduced freezing to the extinguished CS in animals expressing the inhibitory DREADD in the extinction context, but paradoxically increased fear renewal outside the extinction context. This suggests that elevated fear can result from either increases or decreases in LC firing, and presumably NE release in forebrain targets such as the amygdala and prefrontal cortex. Further work is required to understand how bidirectional changes in LC firing differentially regulate the context-dependent expression of extinction.

Disclosures: T.F. Giustino: None. P.J. Fitzgerald: None. S. Maren: None.

Poster

328. Fear and Aversive Learning and Memory: Neural Circuits I

Location: Halls A-C

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Topic: G.01. Appetitive and Aversive Learning

Support: McKnight Memory and Cognitive Disorders Award to SM

NIH R01MH065961

Title: The nucleus reuniens gates prefrontal-hippocampal modulation of extinction retrieval

Authors: *J. JIN, K. R. RAMANATHAN, S. MAREN

Inst. for Neuroscience; Dept. of Psychology, Texas A&M Univ., College Station, TX

Abstract: Fear extinction is a critical component of behavioral therapies for anxiety- and stressrelated disorders. Considerable work has shown that the amygdala and medial prefrontal cortex (mPFC) are crucial for learning fear extinction, whereas the hippocampus (HPC) regulates the context-dependent expression of fear after extinction. Recent work indicates that interactions between the mPFC and HPC mediate the context-dependent expression of extinction memory. Although direct projections from the HPC to the mPFC have been implicated in fear renewal, it is not known whether indirect projections from the mPFC to the HPC via the thalamic nucleus reuniens (RE) are involved in this process. In the present experiments, we explored the role of the RE and mPFC-RE-HPC circuits in the context-dependent expression of extinction. Male rats underwent standard auditory fear conditioning and extinction procedures followed by retrieval tests in either the extinction context or in an alternate context. Pre-testing pharmacological inactivation of the RE using the GABAA receptor agonist muscimol attenuated extinction retrieval, without affecting fear renewal. Consistent with this, RE inactivation was found to reduce the numbers of Fos-positive neurons in the mPFC and HPC after extinction retrieval testing. Lastly, silencing mPFC neurons projecting to RE produced deficits in extinction retrieval similar to those produced by RE muscimol; ongoing studies are exploring the role of RE projections to the HPC in these effects. Overall, our results indicate that the RE may be a novel hub gating information flow between the mPFC and HPC that is critical for the inhibition of extinguished fear.

Disclosures: J. Jin: None. K.R. Ramanathan: None. S. Maren: None.

Poster

328. Fear and Aversive Learning and Memory: Neural Circuits I

Location: Halls A-C

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Program#/Poster#: 328.07/OO21

Topic: G.01. Appetitive and Aversive Learning

Support: McKnight Memory and Cognitive Disorders

NIH R01 MH065961

Title: Nucleus reuniens mediates the acquisition of fear extinction

Authors: *K. R. RAMANATHAN¹, J. JIN², S. MAREN²

²Inst. for Neurosci. and dept of psychology, ¹Texas A&M Univ., College Station, TX

Abstract: The hippocampus (HPC) and medial prefrontal cortex (mPFC) are two brain areas that have an important role in the extinction of fear memories. Inactivation of the dorsal (Corcoran et al., 2005) or ventral (Sierra-Mercado et al., 2011) hippocampus or the infralimbic cortex (Sierra-Mercado et al., 2011) impairs the acquisition of fear extinction. The anatomy of this circuit suggests that unidirectional, monosynaptic projections from the HPC to the mPFC supports extinction learning. However, the mPFC is positioned to influence information processing in the HPC via indirect projections through the thalamic nucleus reuniens (RE); it is not known how the RE or mPFC->RE->HPC projections contribute to extinction learning. To address these questions, we first examined whether muscimol inactivation of RE would affect extinction learning. Animals were implanted with a single cannula targeting RE. After recover from surgery, rats were subjected to 5 CS (tone) -US (footshock) pairings in context A. Twenty-four hours later, they were infused with either muscimol (GABAA agonist) or saline in RE 10 mins before they received an extinction session wherein they received CS-alone trials in context B. This was then followed a day later by retrieval tests in the extinction context (B) and the conditioning context (A). RE inactivation attenuated both within-session extinction, as well as fear suppression in the extinction context the following day; the renewal of fear in the conditioning context was not affected by RE inactivation. To determine if extinction deficits subsequent to RE inactivation were due to blocking PFC input to the RE (and presumably to the HPC), we performed circuit manipulations using Cre-dependent DREADD's (designer receptor exclusively activated by designer drugs) to silence mPFC->RE and RE->HPC projections. Four weeks after surgery, the rats received the same behavioral protocol described above. We found that silencing RE projectors in the mPFC reproduced the extinction deficits we observed after muscimol inactivation of RE; studies exploring RE projections to the HPC are ongoing. Overall, these results indicate that the RE may be critical for gating information flow between the mPFC and HPC during the acquisition of extinction learning.

Disclosures: K.R. Ramanathan: None. J. Jin: None. S. Maren: None.

Poster

328. Fear and Aversive Learning and Memory: Neural Circuits I

Location: Halls A-C

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant MHO58883

NIMH Conte Grant P50 MH086400

Title: Avoidance over-conditioning impairs extinction of fear, induces persistent avoidance, and increases use of safety cues: Implications for OCD

Authors: *F. J. MARTINEZ, M. J. SANCHEZ-NAVARRO, C. D. VELAZQUEZ-DIAZ, G. J. QUIRK

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Abstract: Obsessive-compulsive disorder (OCD) is characterized by compulsive urges that can resemble avoidance of perceived danger. It is treated with exposure-with-response-prevention (ERP) therapy, in which patients are prevented from carrying out compulsions in response to triggers. Due to their repetitive nature, OCD compulsions are thought to resemble habit formation (Gillan et al., 2015), but little is known about the effects of repetitive avoidance on subsequent extinction. Using a platform-mediated avoidance task (Bravo-Rivera et al., 2014), we recently reported that a minority of rats persist in their avoidance following several days of extinction with a barrier that prevents access to the platform (extinction with response prevention, Ext-RP) (Rodríguez-Romaguera et al., 2016). A possible factor contributing to persistent avoidance after extinction is the development of habits over extended periods of training. Therefore, we trained two groups of rats with either 8 days (8d) or 20 days (20d) of avoidance conditioning, followed by 4 days of Ext-RP and a subsequent test with the barrier removed. Both groups showed similar avoidance conditioning. During Ext-RP, however, the 20d group showed impaired extinction of freezing (RM-ANOVA; $F_{(1,28)} = 57.37$, p<0.001), ending with elevated freezing levels. The following day, 20d rats showed increased avoidance at test compared to the 8d group (t-test; $t_{76} = 4.03$, p<0.001), but did not display differences in freezing. Thus, the ability to re-access the platform eliminated the excessive fear in this group. To assess whether 20d group interpreted the barrier as a safety signal, an additional test session was run with the barrier placed opposite to the platform. Under these conditions, avoidance was reduced in the 20d group (t-test; $t_{29} = 4.05$, p<0.001), suggesting the barrier signaled safety to these rats. We are currently using cFos to compare activity in prefrontal-striatal-amygdala circuits in the 8d and 20d groups. Our results suggest that repeated expression of avoidance-like compulsions may reduce the effectiveness of extinction-based therapies, and increase subjects' reliance on apparent safety cues.

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Poster

328. Fear and Aversive Learning and Memory: Neural Circuits I

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Topic: G.01. Appetitive and Aversive Learning

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Title: Time-dependent changes in conditioned responses of prelimbic neurons projecting to amygdala or thalamus

Authors: *K. QUINONES-LARACUENTE¹, A. VEGA-MEDINA³, E. M. MEDINA-COLÓN², F. H. DO MONTE⁴, G. J. QUIRK⁵

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Abstract: Memories are not static in the brain, but shift with the passage of time (Frankland and Bontempi 2005). Previously, we showed that the prelimbic - amygdalar projection (PL-BLA) is necessary to retrieve a 6-hour fear memory, whereas the prelimbic - thalamic projection (PL-PVT) is necessary to retrieve a 7-day fear memory (Do-Monte, et al., 2015). The somas of PL-BLA and PL-PVT neurons are in different layers of PL. What characterizes the activity of both neuronal populations in early vs. late fear retrieval? To address this, we infused separate retrograde tracers into BLA and PVT, exposed rats to auditory fear conditioning, and then assessed early (2 h) fear retrieval. One hour after test, rats were sacrificed and brains were processed for fluorescent labeling of the retrograde tracers, as well as the activity marker c-Fos. Compared to unconditioned controls, early fear retrieval increased activity in PL-BLA neurons (21% vs 5% of PL-BLA neurons expressing c-Fos, p=0.03) but not in PL-PVT neurons (4% vs 5% of PL-PVT neurons expressing c-Fos, p=0.47). To characterize the timing of PL neuronal responses during early and late timepoint, we recorded from 216 individual neurons in different PL layers, using 32 channel silicon probes (NeuroNexus). Neurons in layers 2-5 of PL, which project to BLA but not to PVT, showed conditioned excitatory tone responses during early retrieval (2, 24 h), but not during late retrieval (4 d). In contrast, neurons in layer 6, which project to PVT but not to BLA, showed no excitatory conditioned responses in early or late retrieval, but showed pronounced and long-lasting inhibitory responses during late retrieval. Our findings suggest that, following fear conditioning, the conditioned output of PL shifts with time from increased activity in projections to BLA to decreased activity in projections to PVT. We previously showed that PVT neurons develop excitatory tone responses with the passage of time after conditioning, and optogenetic silencing of PL-PVT fibers induces both excitation and inhibition of PVT neurons (Do-Monte et al., 2015). Thus, PL-PVT inhibition could indirectly increase PVT activity, through disinhibitory circuits.

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328. Fear and Aversive Learning and Memory: Neural Circuits I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 328.10/OO24

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant MH106332

NIH Grant MH058883

Title: Hippocampal-prefrontal BDNF in the extinction of active avoidance

Authors: *L. E. ROSAS-VIDAL¹, V. LOZADA-MIRANDA¹, Y. CANTRES-ROSARIO², L. MELÉNDEZ², G. J. QUIRK³

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Abstract: The infralimbic prefrontal cortex (IL) is necessary for both extinction of conditioned fear (Do-Monte et al., 2015) and extinction of platform-mediated active avoidance (Bravo-Rivera et al., 2014). Brain-derived neurotrophic factor (BDNF) is critical for learning and memory processes. Blocking extracellular BDNF in IL, but not PL, during extinction of conditioned fear impairs its acquisition and recall (Rosas-Vidal et al., 2014). Here we examined the role of BDNF in extinction of active avoidance. We found that blocking extracellular BDNF in either IL (p<0.05) or PL (p<0.01) during extinction of avoidance impaired its recall but not its acquisition. To determine the possible source of extinction-related BDNF, we combined retrograde tracers in IL or PL with immunohistochemistry. Interestingly, we found that extinction of avoidance increased BDNF expression in ventral-hippocampal (vHPC) neurons projecting to IL or PL (p=0.004 and p=0.010 respectively), but not amygdala or mediodorsal thalamic neurons projecting to IL or PL. To determine whether BDNF produced in vHPC is necessary for extinction of avoidance, we injected lentiviri expressing the CRISPR/Cas9 system targeting the *bdnf* gene. CRISPR-Cas9 was sufficient to impair BDNF expression in vHPC (p= 0.014). Furthermore, BDNF knock-down in vHPC neurons impaired recall of extinction of avoidance (p=0.047 and p=0.019 respectively). Post-traumatic stress disorder is associated with reduced hippocampal volumes (Bremner et al., 2002, Bremner et al., 1995, Chao et al., 2013) and with decreased hippocampal-prefrontal connectivity Admon et al., 2012). Our results suggest that reduced BDNF release by an impaired hippocampal-prefrontal pathway may be associated with increased avoidance behaviors seen in post-traumatic stress disorder.

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328. Fear and Aversive Learning and Memory: Neural Circuits I

Location: Halls A-C

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Topic: G.01. Appetitive and Aversive Learning

Support: NIMH Grant K01MH105731

NARSAD Young Investigator Award, BBRF

NIMH Grant P50MH096891

NIMHD Grant MD007599

Title: Chronic social defeat stress impairs the ability to discriminate between threat and safety

Authors: *I. S. GRUNFELD^{1,2}, G. E. SCHAFE^{3,1}, E. LIKHTIK^{2,1}, N. S. BURGHARDT^{3,1} ¹Neurosci. Collaborative, The Grad. Center, City Univ. of New York, New York, NY; ²Dept. of Biol., ³Dept. of Psychology, Hunter Col., New York, NY

Abstract: Numerous psychiatric disorders, including post-traumatic stress disorder (PTSD), are associated with a deficit in the ability to successfully discriminate between conditions of fear and safety. Chronic exposure to adverse stressful experiences may confer susceptibility to PTSD by altering the brain's fear memory system in a way that increases generalization of responses usually reserved for threat to innocuous stimuli that are unrelated to the trauma. To test this hypothesis, we exposed 129Sv/Ev mice (N=10) to social defeat stress or to no stress (N=10) for 10 days and then tested their ability to discriminate threat from safety using a threat discrimination learning paradigm. During discrimination learning, mice were exposed to two different auditory stimuli: one stimulus (CS+) was paired with a mild foot shock (0.5 mA), and the other (CS-) was never paired with a foot shock. Training involved exposing mice to 6 presentations of each CS a day for 3 consecutive days (Likhtik et al., 2014). We then placed the mice in a testing chamber that was distinct from the training context and tested them to both tones in the absence of foot shock. During the test session, we found that the non-stressed control group froze significantly more to the CS+ than the CS-, while our socially defeated group exhibited similar freezing behavior to both tones. We categorized mice as 'generalizers' if they froze similarly to both tones (<10% difference in freezing to CS+ vs. CS-) and 'discriminators' if they showed greater freezing to the CS+ than the CS-. We found that a greater proportion of mice exposed to chronic social defeat stress fit the criteria for generalizers (50%) than those in our non-stressed control group (20%). Our data suggest that chronic stress may increase susceptibility to PTSD by impairing the ability to discriminate threat from safety.

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Poster

328. Fear and Aversive Learning and Memory: Neural Circuits I

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Topic: G.01. Appetitive and Aversive Learning

Support: Undergraduate Research Fellowship

BP- Endure Fellowship

NIMH Grant K01MH105731

NARSAD Young Investigator Award, BBRF

NIMH Grant P50MH096891

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Title: Differential activation of GABAergic and cholinergic basal forebrain neurons during threat and safety

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Abstract: The basolateral amygdala (BLA) is an integral structure for rapid signaling about potential threats. Cholinergic afferents from the basal forebrain (BF) to the BLA make an important contribution to its role as a threat detector by potentiating cortical input onto BLA principal neurons and increasing the signal to noise ratio of BLA firing (Jiang et al., 2016; Unal et. al., 2014). Less is known, however, about the role of GABAergic projections from the BF to the BLA, which may serve to inhibit the acetylcholine-mediated signal amplification of threat-related learning in the amygdala. To investigate this question, we trained mice to either associate a conditioned stimulus (CS+, 2 kHz tone) with a shock US (0.5mA) or with a period of safety via explicitly unpairing the US and CS. Neural activity in the BF was analyzed via expression of the immediate early gene cfos in parvalbumin expressing GABAergic cells (PV+) and cholinergic cells expressing choline acetyltransferase (ChAT+). As suggested by previous work, paired threat conditioning led to higher cfos activation of cholinergic cells than unpaired conditioning (cfos:ChAT+ paired 23% vs unpaired 6%, p=0.014 in the substantia innominata (SI), and in the

horizontal limb of the diagonal band (HDB cfos:ChAT+ paired 21% vs unpaired 3%, p=0.0023). However, unpaired safety training led to increased cfos activation in PV+ cells of the HDB relative to the paired conditioning (cfos:PV+ paired, 15% vs. cfos:PV+ unpaired, 41% in p=0.015), as well as suppression of ChAT+ cellular activity across all sampled regions of the BF (ventral pallidum [VP], SI, HDB). In light of these findings, we are currently investigating how PV+ GABAergic inputs from the BF to the BLA impact threat and safety learning. These findings indicate that the BF may constitute an important gate for amygdala output at the time of predictive threat and safety.

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Poster

328. Fear and Aversive Learning and Memory: Neural Circuits I

Location: Halls A-C

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Topic: G.01. Appetitive and Aversive Learning

Support: Undergraduate Research Fellowship

BP-Endure Fellowship

NIMH Grant K01MH105731

NARSAD Young Investigator Award, BBRF

NIMH Grant P50MH096891

NIMHD Grant MD007599

Title: Infralimbic and prelimbic inputs to BLA-projecting cell groups in the basal forebrain: An anatomical and functional investigation

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¹Dept. of Biol., ²Dept. of Chem., Hunter Col., New York, NY; ³Neurosci. Collaborative, The Grad. Center, CUNY, New York, NY

Abstract: The medial prefrontal cortex (mPFC) and its projection targets play an important role in learning to discriminate threat from safety. Previous studies have shown that the prelimbic (PL) and infralimbic (IL) sub-regions of the mPFC are critically involved in fear expression and fear suppression, respectively. Investigation into how these two contiguous areas govern opposing behavioral responses has focused on their direct, reciprocal connectivity with the

basolateral amygdala (BLA). This work has shown that the mPFC drives both excitation and inhibition in the BLA, with a prevalence toward inhibition after safety or extinction training. However, the mechanism of mPFC-mediated inhibition in the BLA is not well understood. To address this question, we are investigating indirect connectivity between the mPFC and BLA via the basal forebrain (BF). Collectively, the BF (substantia innominata - SI, ventral pallidum - VP, horizontal limb of the diagonal band - HDB) send GABAergic and cholinergic inputs to the BLA, which are thought to increase the signal to noise strength of BLA firing and enhance fear expression. Thus, we reasoned that mPFC inputs to BLA-projecting cell groups in the BF could provide an indirect mechanism for control over BLA activity and fear expression. To investigate this possibility, we injected retrograde fluorescent beads (RB) in the BLA and an anterograde tracer (AAV8.2-hEF1a-DIO-synaptophysin-mCherry with AAVa - hSyn-Cre-WPRE-hGH) in either the IL (N=4) or PL (N=4) of C57BL6J mice. After a 3 week period for viral and RB expression, the BF region was stained for parvalbumin (PV+) and choline acetyltransferase (ChAT+) to identify GABAergic PV+ and cholinergic cell groups, respectively. Analyses show that the majority of projections from the VP region of the BF (VP-to-BLA) are ChAT+ (13.47%), whereas the majority of projections from the HDB are PV+ (5.72%). Surprisingly, despite the comparatively smaller overall number of PV+ cells in the BF overall, they were the principal target of IL synaptic vesicles in all three examined BF regions. We are investigating whether the PL-BF-BLA network shows similar connectivity patterns and the functional significance of this circuit for BLA activity and fear expression.

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Poster

328. Fear and Aversive Learning and Memory: Neural Circuits I

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Program#/Poster#: 328.14/OO28

Topic: G.01. Appetitive and Aversive Learning

Support: NIMH R01 MH069558

Title: Thalamic inputs onto the amygdala regulate fear memory retrieval

Authors: *N. FERRARA, P. K. CULLEN, S. E. PULLINS, F. J. HELMSTETTER Psychology, Univ. of Wisconsin--Milwaukee, Milwaukee, WI

Abstract: Pavlovian fear conditioning provides a way to investigate memory formation and retrieval. During fear conditioning, a conditional stimulus (CS) is paired with an aversive outcome and the CS acquires aversive value over several pairings. The CS may then be presented during a retrieval session where fear responding is measured as an indicator of memory strength.

Retrieval sessions allow for the incorporation of new information into the original memory trace by destabilizing amygdala synapses, which is accompanied by changes in synaptic expression of AMPA receptors. Inhibition of protein synthesis-dependent plasticity in the amygdala immediately following a retrieval experience impairs long-term memory retention and disrupts AMPA receptor synaptic expression. Previous work shows that terminals of auditory thalamic (MgN) neurons onto cells in the lateral amygdala are immediately presynaptic to AMPA receptor subunits, suggesting that MgN input may play a role in amygdala-dependent memory through regulation of AMPA receptor dynamics. However, very little is known about the sensory inputs to the amygdala that regulate fear memory at retrieval. Local inhibition of the MgN activity using pharmacological agents prior to fear conditioning, but not prior to retrieval, can disrupt memory, but pathway specific optogenetic manipulation provides more precise tool that potentially allows for isolation of MgN-LA connections. Recent work has shown that optogenetic terminal stimulation of thalamic and cortical inputs onto the amygdala during conditioning can serve as a CS to simulate auditory fear conditioning when paired with a UCS and that this is dependent on glutamatergic synaptic transmission. Importantly, groups conditioned with optogenetic stimulation as a CS show that terminal stimulation closely resembles auditory fear memory retrieval, suggesting a role for thalamic and cortical terminals in the amygdala during fear memory reactivation. Here groups undergo auditory fear conditioning followed by a brief retrieval session to characterize the role of MgN terminals in the amygdala during recall. We found that silencing MgN terminal activity during CS presentations at retrieval impaired fear responding, suggesting that MgN inputs to the amygdala are necessary for the retrieval of an auditory fear memory. Other experiments address if MgN terminal silencing alters anisomycin-induced amnesia following memory retrieval and synaptic destabilization evidenced by changes in AMPA receptor trafficking in the amygdala.

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Poster

328. Fear and Aversive Learning and Memory: Neural Circuits I

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Topic: G.01. Appetitive and Aversive Learning

Support: NIA R21AG053854

Title: The effects of methylene blue on trace fear memory and brain proteolytic activity in young and aged rats

Authors: *W. CRUZ¹, S. E. PULLINS¹, J. R. MOYER, JR.¹, F. J. HELMSTETTER² ¹Psychology, ²Deptartment of Psychology, Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Both humans and rats experience an age-related decline in episodic/explicit memory. Along with protein synthesis, protein degradation mediated by the ubiquitin proteasome system is critical for the synaptic plasticity underlying memory formation and retrieval. While a decrease in basal proteasome activity with increasing age is evident across various species, agerelated changes in plasticity dependent proteolysis have not been explored and may underlie the age-related deficit in trace fear conditioning (TFC) previously documented in aged rats. Using TFC to model episodic memory in rats, we attempted to rescue age-related memory decline by upregulating proteasome activity via dietary administration of the compound methylene blue (methylthioninium chloride, MB), since prior work showed that MB increases chymotrypsin-and trypsin-like proteasome activity in 3xTg-AD mice. Two experiments tested the effects of MB on TFC and proteolytic activity in male F344 young (3 months) and aged (18 months) rats. In Experiment 1, 3-month-old rats were administered 50mg of MB per 100g of powdered food for 4 months. While this treatment did not significantly enhance memory, it significantly increased proteasome activity in the retrosplenial cortex (RSC). Since MB treatment is dose dependent, we lowered the dose in Experiment 2, and 3 month and 18-month-old F344 rats were administered 25mg of MB per 100g of powdered food. MB treatment enhanced TFC in young rats, while not affecting TFC in aged rats. However, there was a significant increase in proteasome activity in the RSC for both young and aged rats, as well as a significant increase in proteasome activity in the dorsal hippocampus for young rats. These results suggest a lower dose of MB significantly enhances TFC in young but not aged rats. Therefore, additional research is needed to determine the optimal dose at which MB rescues TFC in aged rats.

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Poster

328. Fear and Aversive Learning and Memory: Neural Circuits I

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH MH069558

NIH MH112141

Title: Contributions of the retrosplenial cortex to event-related and contextual fear memory formation in trace fear conditioning

Authors: *S. E. PULLINS, P. K. CULLEN, N. C. FERRARA, F. J. HELMSTETTER Psychology, Univ. of Wisconsin - Milwaukee, Milwaukee, WI

Abstract: The ability to form memories for aversive events, as well as for where those events occur, is critical to survival. Recent research suggests a critical role for the retrosplenial cortex (RSC) in the encoding and storage of events and their associated contexts. However, the nature of information processing in the RSC during learning remains unclear due to previous manipulations of the RSC lacking spatial and temporal precision. Our initial immunohistochemical analysis of the RSC following auditory trace fear conditioning, a paradigm used to model declarative memory in rodents, showed increased neuronal activity throughout its longitudinal extent. While these results suggest that the RSC may be functionally homogeneous throughout its rostral-to-caudal expanse, previous lesion and immunohistochemical work implicates differential function between the anterior and posterior segments. We then used optogenetics to examine specific functions of RSC segments during trace fear conditioning. Rats were transfected with ArchT, under control of the neuron-specific promoter CAG, while control rats received a similar viral cassette that lacked the light-sensitive pump. By injecting these viruses into the anterior RSC (aRSC) or the posterior RSC (pRSC), and then mounting LEDs over a thinned skull window immediately above the site of transfection, we can achieve spatially and temporally precise reductions in neuronal excitability. All rats received light delivery during each learning trial of conditioning. The following days, rats were tested for memory to the tone and to the training context. Silencing of aRSC during each tone-shock pairing impairs memory formation for the training tone, whereas pRSC silencing selectively impairs contextual memory formation. These results suggest that the two poles of the RSC may differentially contribute to event-related and contextual memory formation, and that the RSC may normally aid in the final association of these two distinct aspects of the fear memory.

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Poster

328. Fear and Aversive Learning and Memory: Neural Circuits I

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH MH069558

NIH MH112141

Title: Neural activity in the ventrolateral periaqueductal gray provides a feedback mechanism to modulate fear network activity

Authors: *P. K. CULLEN, N. C. FERRARA, S. E. PULLINS, F. J. HELMSTETTER Psychology, Univ. of Wisconsin, Milwaukee, Milwaukee, WI

Abstract: Decades of research on defensive behaviors supports a model in which the amygdala initially processes emotional responses to threat and coordinates defensive responses via "downstream" structures including the midbrain periaqueductal gray (PAG). While the PAG was initially thought to be the final common pathway for fear expression, recent evidence suggests that the PAG plays a much more integrated role in coordinating defensive responding. Inactivation of the ventrolateral PAG (vlPAG) during fear conditioning or extinction training impairs neural plasticity in both the amygdala and medial prefrontal cortex (mPFC), suggesting that the PAG modulates plasticity in these structures. However, our present understanding of PAG function comes primarily from pharmacological manipulations or lesions that impair PAG activity for an imprecise amount of time following drug administration. To gain a better understanding of the role of the PAG in modulating network activity within the fear/extinction circuit, activity within the vlPAG 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 either a single 30-second white noise CS/laser presentation and were sacrificed 5-minutes later for Arc fluorescent in situ hybridization, or five 30-sec CS/laser presentations followed by two subsequent CS tests in either the same or shifted context. We found that 1) inactivation of vIPAG during one CS presentation significantly reduced retrievalrelated Arc expression in the amygdala and prelimbic (PL) region of the mPFC; 2) inactivation of vIPAG during 5 CS presentations reduced CS freezing during the retrieval session; 3) the reduction in CS freezing persisted during a laser-free test 24-hours following vIPAG inhibition and was renewed in a shifted context. These results indicate that the PAG modulates fear network activity during fear expression. Furthermore, inhibiting vlPAG activity during CS presentations resulted in a stable reduction in fear responding in the absence of optogenetic inhibition and the renewal of fear suggests that inhibiting vIPAG activity during fear expression may have triggered extinction learning. The current study provides new insight into the role of vlPAG during fear memory retrieval/expression and suggests that the vlPAG may participate in a feedback mechanism to modulate network activity, potentially to signal extinction learning.

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Poster

328. Fear and Aversive Learning and Memory: Neural Circuits I

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Title: BAF53b, a neuron-specific nucleosome remodeling factor, is induced after learning and facilitates long-term memory consolidation

Authors: *M. YOO¹, K.-Y. CHOI², J. KIM¹, M. KIM¹, J. SHIM³, J.-H. CHOI³, H.-Y. CHO¹, J. OH¹, H.-S. KIM¹, B.-K. KAANG³, J.-H. HAN¹

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Abstract: Although epigenetic mechanisms of gene expression regulation have recently been implicated in memory consolidation and persistence, the role of nucleosome-remodeling is largely unexplored. Recent studies show that the functional loss of BAF53b, a postmitotic neuron-specific subunit of the BAF nucleosome-remodeling complex, results in the deficit of consolidation of hippocampus-dependent memory and cocaine-associated memory in the rodent brain. However, it is unclear whether BAF53b expression is regulated during memory formation and how BAF53b regulates fear memory in the amygdala, a key brain site for fear memory encoding and storage. To address these questions, we used viral vector approaches to either decrease or increase BAF53b function specifically in the lateral amygdala of adult mice in auditory fear conditioning paradigm. Knockdown of *Baf53b* before training disrupted long-term memory formation with no effect on short-term memory, basal synaptic transmission, and spine structures. We observed in our qPCR analysis that BAF53b was induced in the lateral amygdala neurons at the late consolidation phase after fear conditioning. Moreover, transient BAF53b overexpression led to persistently enhanced memory formation, which was accompanied by increase in thin-type spine density. Together, our results provide the evidence that BAF53b is induced after learning, and show that such increase of BAF53b level facilitates memory consolidation likely by regulating learning-related spine structural plasticity.

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328. Fear and Aversive Learning and Memory: Neural Circuits I

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Topic: G.01. Appetitive and Aversive Learning

Support: MH113053

DA034010

Title: Ventrolateral periaqueductal gray neurons signal threat probability

Authors: *K. M. WRIGHT¹, M. A. MCDANNALD²

¹Psychology, ²Dept. of Psychology, Boston Col., Chestnut Hill, MA

Abstract: The ventrolateral periaqueductal gray (vlPAG) has long been implicated in Pavlovian fear conditioning. The canonical view is that the vIPAG is critical to fear expression. However, recent studies have failed to find adequate neural correlates in this region linked to fear expression. Using a combination of Pavlovian fear conditioning and single-unit recording, we tested the hypothesis that vIPAG neurons signal threat probability, a signal more consistent with threat prediction, than fear expression. Six, Adult Male Long Evans rats, were presented with three auditory cues, each associated with a different probability of foot shock: Safety, p = 0.00; Uncertainty, p = 0.38; and Danger, p = 1.00. Rats received eight discrimination sessions, then were implanted with drivable, microelectrode bundles aimed at the caudal vlPAG. Following recovery, discrimination sessions resumed with single-unit recording. Across all sessions, rats demonstrated excellent fear discrimination, achieving high fear to the Danger cue, low fear to the Safety cue, and intermediate fear to the Uncertainty cue. Our analysis of vlPAG activity during discrimination revealed a population (29/267) of neurons that showed rapid, phasic increases in firing at cue onset. Interestingly, this population showed graded firing to the three cues which appeared to follow a pattern similar to fear expression: high firing to the Danger cue, low firing to the Safety cue, and intermediate firing to the Uncertainty cue. However, using either population or single-unit analyses, we found that differential firing to each of the three cues was best explained by the probability of foot shock associated with a given cue, not the level of fear expressed. Our analyses also found a similar, but largely separate population of neurons (30/267) that showed a ramping of activity from cue onset to offset. This ramping also scaled with the probability of foot shock: Danger > Uncertainty > Safety. Together, these results suggest that vlPAG neurons signal threat probability, or *threat prediction*, and generate signals which may be critical to organizing an appropriate fear response that are independent of the response itself.

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328. Fear and Aversive Learning and Memory: Neural Circuits I

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BK21 PLUS BioKAIST Initiative, Biological Sciences, KAIST

Title: Reactivation-mediated organization of associative memory engram in systems consolidation

Authors: *J.-P. OH, J.-T. KWON, Y. LEE, M. KANG, H.-S. KIM, Y. JEONG, H.-Y. CHO, J.-H. HAN

Biol. Sci., Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: In rodent studies, it has been shown that memory retrieval circuit changes as memory is consolidated over time, suggesting dynamic regulation of memory engram organization during systems level consolidation. However, how memory is organized within neural circuits over time is poorly understood. Previously, we showed optogenetic stimulation of presynaptic auditory input in lateral amygdala (LA) can serve as a conditioned stimulus (CS) to form associative fear memory. In this study, we observed that the retrieval of such memory, driven by optogenetic CS input activation in the LA, was gradually decreased over time after learning. However, when the CS input was reactivated by optogenetic stimulation 1 day after learning, the memory retrieval through this CS input pathway was maintained. Importantly, the reactivation effect was highly specific such that reactivation of auditory input that was not activated during learning had no effect. Consistent with behavior results, we also found that LTP at CS input and c-Fos induction in the LA neurons during memory retrieval was maintained with the reactivation manipulation. Furthermore, in natural auditory fear memory, we found that prolonged inhibition of auditory brain regions activity for one week, starting from a day after conditioning, impaired memory retrieval at remote time, suggesting the possibility of reactivation-mediated maintenance of CS input as a critical part of retrieval circuit for associative memory.

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328. Fear and Aversive Learning and Memory: Neural Circuits I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 328.21/OO35

Topic: G.01. Appetitive and Aversive Learning

Title: Dissecting the contribution of auditory cortex to acquisition and expression of auditory fear memory

Authors: *T. DALMAY, E. ABS, R. B. POORTHUIS, J. J. LETZKUS Max Planck Inst. For Brain Res., Frankfurt Am Main, Germany

Abstract: Classical auditory fear conditioning is a powerful model to elucidate many key features of adaptive and maladaptive forms of plasticity underlying learning and memory. In particular, a large body of work demonstrated that neurons in the lateral amygdala (LA) that receive thalamic and cortical afferents conveying auditory and foot-shock information undergo plasticity during learning. The LA has therefore been regarded as the primary site of stimulus convergence and fear memory formation. More recent evidence indicates, however, that auditory cortex (ACx) also exhibits foot-shock-evoked activity, and undergoes plasticity as a result of fear conditioning. How the parallel processing of auditory and foot-shock information via the thalamo-amygdala and thalamo-cortico-amygdala circuits interacts to enable memory formation and expression is therefore a key open question. Furthermore, the role of ACx as a whole has been controversial, with lesion experiments providing evidence both for and against a critical involvement in fear memory acquisition and retrieval. Here, we use acute optogenetic silencing of pyramidal neurons with ArchT in freely-behaving mice during different forms of auditory fear conditioning to determine the conditions under which the ACx (areas A1 and AuV) is strongly required for both fear memory acquisition and expression. We find that optogenetically inhibiting ACx during conditioning leads to a robust reduction in fear responses (freezing) in the subsequent recall session. Similarly, our data indicate a strong and reversible reduction in freezing responses when ACx is inhibited during recall of both recent and remote memory. Control experiments demonstrate that the decrease in auditory stimulus-evoked freezing is not caused by potential off-target effects of ACx inhibition or other side effects of light stimulation. As a next step we are investigating how cortical afferents to the LA influence memory formation and expression, and the extent to which tone-evoked activity in the LA depends upon cortical processing. Together, these experiments enhance our understanding of the brain-wide interactions of distinct areas that enable behavioral functions such as learning and memory.

Disclosures: T. Dalmay: None. E. Abs: None. R.B. Poorthuis: None. J.J. Letzkus: None.

328. Fear and Aversive Learning and Memory: Neural Circuits I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 328.22/OO36

Topic: G.01. Appetitive and Aversive Learning

Support: DA034010

MH113053

Title: Ventrolateral periaqueductal gray neurons signal positive aversive prediction errors

Authors: *M. A. MCDANNALD, R. A. ZACHARIAS, K. M. WRIGHT Dept. of Psychology, Boston Col., Chestnut Hill, MA

Abstract: Fear is adaptive when confined to situations of danger, but is maladaptive when the level of fear expressed is inconsistent with a present threat. Generating appropriate fear in situations that threaten harm with uncertainty is particularly challenging. Prediction errors (PEs), discrepancies between predicted and actual outcomes, provide an updating mechanism by which appropriate fear in uncertainty may be achieved. Disrupting ventrolateral periaqueductal gray (vlPAG) activity can alter PE-dependent fear learning, and neural signatures consistent with PEs have been identified in rats (single-unit recording) and humans (fMRI). However, strong evidence for vlPAG single-unit activity as a *de novo* source of PEs is lacking. We implanted six male Long Evans rats with drivable microelectrode bundles aimed at the caudal vIPAG. Following recovery, single-unit activity was recorded during Pavlovian fear discrimination in which three auditory cues predicted different probabilities of foot shock: Danger (p = 1.00), Uncertainty (p = 0.38), or Safety (p = 0.00). Theoretically, positive PEs (+PEs) would be weak or absent to 'predicted' foot shock, on Danger trials, when prediction (1.00) is consistent with actuality (1.00). In contrast, +PEs would be generated to 'surprising' shock delivery on Uncertainty trials, due to the discrepancy between the predicted (0.38) and the actual shock (1.00). Across all trials, rats expressed high fear to Danger and low fear to Safety. Critically, rats expressed intermediate fear to Uncertainty, indicative of appropriate prediction error signaling. We identified a population of shock-responsive neurons (30/267) with phasic increases in firing to surprising and/or predicted foot shock. Despite not selecting for this criterion, population and single-unit activity was biased towards greater firing to the surprising foot shock - consistent with positive +PE signaling. In support of a mechanism for updating fear, single trials in which robust +PE responses were observed, resulted in increased fear to the Uncertainty cue on subsequent trials. In contrast, single trials with weak or anti-PE signaling resulted in decreased fear to the Uncertainty cue on subsequent trials. Combined with optogenetic findings from our lab, the present results suggest that +PE signaling is an intrinsic function of vlPAG neurons, and perhaps a neural source of dysfunction in disorders characterized by excessive fear.

Disclosures: M.A. McDannald: None. R.A. Zacharias: None. K.M. Wright: None. Poster

328. Fear and Aversive Learning and Memory: Neural Circuits I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 328.23/PP1

Topic: G.01. Appetitive and Aversive Learning

Support: CRC Tier 2 Behavioural Neuroscience

NSERC Discovery Grant RGPIN-2015-03658

CFI John R. Evans Leaders Fund

Title: VTA dopamine transients reduce prediction error about aversive outcomes

Authors: *A. MAHMUD¹, M.-P. COSSETTE¹, B. P. LAY², M. D. IORDANOVA¹ ¹Psychology, Concordia Univ., Montreal, QC, Canada; ²Univ. of New South Wales, Sydney, Australia

Abstract: Prediction-error, the discrepancy between real and expected outcomes, drives associative learning as exemplified in the blocking paradigm. In blocking, a cue established as a good predictor of an outcome generates a small error in prediction. Therefore little is learned about the relationship between another, novel cue (e.g., a tone) and the same outcome in the presence of the good predictor. When prediction error is large, however, as in the case when the novel cue is trained in the presence of a poor predictor of the outcome, learning proceeds normally. Dopamine in the ventral tegmental area (VTA) has been implicated in *reward* prediction-error. Whether a similar role can be attributed to VTA dopamine in learning about fear remains unknown. We used the fear blocking paradigm to show that optogenetic activation of tyrosine hydroxylase positive neurons in the VTA at time of expected footshock further hinder learning about the blocked cue, therefore, augmenting the blocking effect in the aversive setting. Our results also indicate that optogenetic activation of dopamine does not modulate learning in a series of behavioural and neural control conditions. Overall, our findings suggest that dopamine transients in the ventral tegmental area reduce prediction error about aversive outcomes and thus modulate associative learning via a valance-specific prediction-error mechanism.

Disclosures: A. Mahmud: None. M. Cossette: None. B.P. Lay: None. M.D. Iordanova: None.

328. Fear and Aversive Learning and Memory: Neural Circuits I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 328.24/PP2

Topic: G.01. Appetitive and Aversive Learning

Support: KTIA_NAP_13-2-2015-0010

Title: Pathway-specific thalamic modulation of amygdalar circuits

Authors: *K. KOCSIS^{1,2}, B. BARSY¹, A. MAGYAR¹, Á. BABICZKY¹, V. KANTI^{1,3}, M. HORVÁTH¹, K. VARGA¹, T. A. FÖLDES¹, F. MÁTYÁS¹

¹MTA TTK NAP-B Neuronal Network and Behaviour Res. Group, Res. Ctr. for Natural Sciences, HAS, Budapest, Hungary; ²Roska Tamás Doctoral Sch. of Sci. and Technol., Pázmány Péter Catholic Univ., Budapest, Hungary; ³János Szentágothai Doctoral Sch. of Neurosciences, Semmelweis Univ., Budapest, Hungary

Abstract: Affective memory formation is determined by arousal level as well as by the sensory information flow related to a certain memorable experience. Although the amygdala is considered the main scene for integrating these kinds of information, the cell-type-specific subcortical origins of these inputs are yet to be elucidated.

Hereby we study the calretinin(CR)-positive cell aggregates of the thalamus: the midline and the posterior intralaminar/suprageniculate (PIL/SG) nuclei which have been shown to project to the amygdala nuclei. The question thus arises about their specific role in affective learning.

We investigate the temporal dynamics of CR+ neuronal firing in innate and learned behaviour of CR-Cre mice applying optogenetic techniques along with chronic multichannel electrode recording. Spontaneous and stimulus-evoked electrophysiological characteristics of CR+ cells along the thalamo-amygdala pathways are presented.

Frequency-dependent optogenetic excitation of midline thalamic cells in CR-Cre mice leads to a heightened level of arousal and waking with short latency (see also poster of Komlósi et al.). Concerning the PIL/SG CR+ neurons, bidirectional optogenetic manipulation of their axon terminals in the amygdala shows that they contribute to the transmission of both cue- and context-dependent information in auditory fear learning. Optogenetically tagged single units exhibit diverse and plastic response patterns along the stages of threat conditioning, which are supposed to be shaped by distinctive brainstem input characteristics depicted here. Our results suggest that CR+ thalamic cells are good candidates to provide the amygdala with arousal- and sensory-related information in affective learning.

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328. Fear and Aversive Learning and Memory: Neural Circuits I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 328.25/PP3

Topic: G.01. Appetitive and Aversive Learning

Support: NIMH R01 MH052619

Title: Activating glutamatergic projections from the perifornical hypothalamus to the basolateral amygdala enhances conditioned fear learning and persistence

Authors: *E. T. DUSTRUDE¹, J. L. LUKKES¹, A. R. R. ABREU¹, A. I. MOLOSH^{1,2}, A. SHEKHAR^{1,2,3}

¹Dept. of Psychiatry, ²Stark Neurosciences Res. Inst., ³Indiana Clin. and Translational Sci. Inst., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Panic disorder is characterized by recurrent and unexpected panic attacks involving a surge of catastrophic fear accompanied by marked cardiorespiratory responses and is often associated with phobias. Disinhibition of the perifornical hypothalamic region (PeF), a panic generating site, by GABA antagonism produces panic associated responses including escape and anxiety-like behavior and enhancement of fear memory in rat models. Chronic infusion of glutamic acid decarboxylase inhibitor L-Allylglycine (L-AG), to inhibit GABA synthesis, generates rats that are vulnerable to interoceptive stimuli, like sodium lactate. Our laboratory has previously shown that L-AG, panic vulnerable, rats can be protected against panic responses by metabotropic glutamate receptor II agonism that reduces glutamate-mediated excitation. Furthermore, pyramidal neurons in the basolateral amygdala (BLA) of panic vulnerable rats undergo a shift in their membrane properties towards increased excitation. As a key region for fear and anxiety behaviors, the BLA may be a major glutamatergic target for the PeF in fear learning during panic; however, the excitatory PeF-BLA circuit has not been selectively activated to determine its role in panic or fear models. Here, we investigate circuit functions by utilizing Sprague-Dawley male rats and adeno-associated virus to express EYFP or EYFP and channelrhodopsin (ChR2) under calmodulin-kinase II promoter within the PeF. BLA-projecting glutamatergic PeF neurons were activated by optogenetic stimulation of fibers within the BLA region in behavioral and brain slice electrophysiology experiments. Blue light stimulation at 10Hz was bilaterally targeted to the BLA of freely moving animals to excite the PeF-BLA circuit during each of the five minutes preceding acquisition, consolidation, and extinction phases of conditioned fear behavior testing. ChR2 expressing animals exhibited enhanced freezing during acquisition as well as delayed extinction suggesting glutamatergic PeF-BLA circuit modulation of fear learning. Three weeks after stimulation, ChR2 expressing animals exhibited increased spontaneous recovery of fear memory and delayed extinction during reinstatement of fear

conditioning. Whole-cell recordings of BLA neurons were utilized to confirm that blue-light evoked excitatory responses were glutamatergic and inhibited by AMPA/NMDA receptor antagonists. Together, our data suggest that enhanced glutamate release from BLA-projecting PeF neurons is important in phobia development.

Disclosures: E.T. Dustrude: None. J.L. Lukkes: None. A.R.R. Abreu: None. A.I. Molosh: None. A. Shekhar: None.

Poster

328. Fear and Aversive Learning and Memory: Neural Circuits I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 328.26/DP11/PP4 (Dynamic Poster)

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant MH107238

Title: Region-specific rearrangement of synapses as a consequence of fear conditioning in zebrafish

Authors: *W. DEMPSEY¹, Z. DU¹, T. V. TRUONG², K. CZAJKOWSKI³, A. ANDREEV², G. G. GROSS¹, C. KESSELMAN³, S. E. FRASER², D. B. ARNOLD¹ ¹Mol. and Computat. Biol., ²Translational Imaging Ctr., ³Informatics Div., USC, Los Angeles, CA

Abstract: Information storage within the brain is thought to be facilitated by changes in the number and relative strength of synaptic connections between neurons. Visualizing these changes over time in vivo would help reveal the rules that govern this process. However, longterm, synapse-scale optical imaging in vivo - especially in structures that are found deep in the mammalian brain - continues to be a significant challenge. To circumvent this problem, we have used larval zebrafish, whose essentially transparent brains are organized such that virtually all areas are located close to the surface, within reach of in vivo imaging techniques. We have established an extensive labeling, behavioral training, imaging, and synapse analysis paradigm to track synaptic changes over time in living zebrafish larvae. FingRs (Fibronectin Intrabodies Generated with mRNA display) fused to fluorescent reporters label either PSD95 or Gephyrin in a mosaic subset of neurons within living fish. Before and after rounds of fear conditioning, we visualize labeled synapses at sub-micron resolution (~0.3x0.3x1.0µm voxel size) using selective plane illumination microscopy (SPIM). We focus mainly on the habenulae and pallia regions of the zebrafish brain, which have been suggested to be integral to fear memory formation. Synapses are classified using a custom analytical software package which allows a 3dimensional map of their positions and strengths to be produced. By comparing the differences between synapse maps obtained from the same fish before and after learning, we can map the

changes that occur in distributions of synapses. We have found that dramatic synaptic rearrangements occur in the brains of fish that have learned, but not in those that have not, suggesting that these rearrangements correlate specifically with learning.

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Poster

329. Motivation: Neural Circuits II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 329.01/PP5

Topic: G.02. Motivation

Title: Use of autoshaping measures to predict motivation for sucrose rewards

Authors: Z. E. BOND, M. MCWATERS, E. M. ANDERSON, *L. MATUSZEWICH Dept. of Psychology, Northern Illinois Univ., DeKalb, IL

Abstract: Dysregulation of motivational systems has been implicated in several disorders, including addiction to natural and stimulant rewards (for reviews see DiLeone, Taylor, & Picciotto, 2012; Kalivas & Volkow, 2005). Salient cues are thought to increase the likelihood of the development of addiction and reinstatement after extinction (Flagel, Akil, & Robinson, 2009). In order to better understand the contribution of cue saliency in motivational behavior, individual differences in rats have been characterized using operant procedures (for review, see Flagel & Robinson, 2017). Rats can be categorized as those that exhibit salience toward the reward dispenser ("goal-trackers"), the cue (lever) for reward ("sign-trackers"), or both the reward dispenser and cue equally ("intermediates"; Yagers & Robinson, 2013). These categorizations have been assessed using an autoshaping procedure in rats participating in laboratory experiments to examine individual variance in motivational responding for a sucrose reward and stress-induced reinstatement of responding. A total of 211 adult Sprague-Dawley rats, 101 males and 110 females, were exposed to 25 sucrose pellets in their home cages for one day, received one day of pretraining in the operant box in which 25 sucrose pellets were administered on a variable interval schedule, and then received five days of the autoshaping procedure. During autoshaping, the lever extends into the operant box for 8 seconds, retracts, and the reward is immediately dispensed into the magazine, irrespective of responding. The autoshaping paradigm tracks several behaviors during the session, including lever presses and magazine entries while the lever is either in or out; therefore, assessing each rat's individual tendency to approach either the conditioned stimulus (lever) or the reward dispenser. Across 3 laboratory studies, more subjects attributed salience toward the cue for reward and were therefore categorized as sign-trackers (approximately 77%) than either other category ($\chi^2(2) =$

191.2, p < .01). However, the distribution of goal, intermediate, and sign-trackers was similar between males and females (χ^2 (2) = .71, p = .70) and between studies (χ^2 (4) = 8.73, p = .07). Further analyses will investigate individual differences in approach behaviors within each category (e.g. does one sign tracker differ from another?) that may contribute to results found in measures of motivation and reinstatement behaviors for sucrose rewards.

Disclosures: Z.E. Bond: None. **M. McWaters:** None. **E.M. Anderson:** None. **L. Matuszewich:** None.

Poster

329. Motivation: Neural Circuits II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 329.02/PP6

Topic: G.02. Motivation

Title: Influence of juvenile methylphenidate on motivational behavior in adulthood

Authors: *M. MCWATERS, Z. BOND, L. MATUSZEWICH Northern Illinois Univ., Dekalb, IL

Abstract: Methylphenidate (MPH) is a commonly prescribed psychostimulant for attention disorders, particularly in children. There is concern that MPH increases the potential for developing other addictions in adulthood because MPH enhances motivation (Chelonis et al., 2011; Crawford et al., 2007). Importantly, motivation can also be influenced by several individual characteristics, such as sex and the salience toward reward-associated cues. A greater degree of salience toward cues may contribute to drug addiction (Christiansen et al., 2012) and can be quantified in rats using the Pavlovian Conditioned Approach (PCA) Index (Meyer et al., 2012). Although research has investigated the role of juvenile MPH treatment on the rewarding value of substances in rats (Andersen et al., 2002; Carlezon, Mague, & Andersen, 2003; Crawford et al., 2011), the influence of juvenile MPH on reward-associated cues in operant conditioning has not been assessed. The objective of the current study was to investigate whether juvenile MPH exposure altered the salience of reward cues and motivation in adulthood, comparing male and females. Therefore, the current study exposed 40 male and female rats to 0 or 2.0 mg/kg MPH on a cookie from postnatal day (PD) 21-35. At ~ PD 80, rats began the following training and testing procedures in operant chambers: pretraining, 5 days of autoshaping, 5 days of increasing fixed ratio, progressive ratio (PR), 10 days of fixed ratio 1 (lever with light cue), extinction (lever with no light cue), and 1 day of cue-induced reinstatement (lever with light cue). Sex and drug condition did not influence PCA index, although 70% of these animals had heightened salience toward the cue as measured by a high PCA index. During the PR assessment of motivation, there was an interaction between sex and drug condition on active lever pressing [F(1,36)=5.22, p=.03]. Juvenile MPH exposure,
compared to vehicle, increased females' motivation, but decreased males' motivation for sucrose. Males tended to have more inactive lever presses than females overall [F(1,36)=4.02, p=.05]. Surprisingly, active lever pressing in PR did not vary by PCA index (p>.05). Preliminary findings suggest that although all rats exhibited cue-induced reinstatement, the number of active level presses were similar across males and females and drug conditions. Overall, early exposure to MPH appears to differentially affect motivation in adulthood for a sucrose reward in males and female rats, which may have clinical implications for sex differences in motivational disorders (drug/food addiction), regardless of inherent individual differences in the salience of reward-associated cues.

Disclosures: M. McWaters: None. Z. Bond: None. L. Matuszewich: None.

Poster

329. Motivation: Neural Circuits II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 329.03/PP7

Topic: G.02. Motivation

Support: the Research Pool of the University of Fribourg, Grant 578

Title: Reward under stress: Striatum involvement in reward processing from early detection to reward delivery

Authors: *C. GAILLARD¹, M. GUILLOD¹, A. FEDERSPIEL², D. SCHOEBI¹, R. RECABARREN¹, X. OUYANG¹, C. MUELLER-PFEIFFER³, A. HORSCH⁴, G. HASLER², C. MARTIN-SOELCH¹

¹Dept. of Psychology, Univ. of Fribourg, Fribourg, Switzerland; ²Univ. Hosp. for Psychiatry and Psychotherapy, Univ. of Bern, Bern, Switzerland; ³Dept. of Psychiatry and Psychotherapy, Univ. Hosp. of Zurich, Zurich, Switzerland; ⁴Dept. of Woman-Mother-Child, Univ. Hosp. of Lausanne, Lausanne, Switzerland

Abstract: Introduction: Stress and reward are two important determinants of motivated behavior (Haber & Knutson, 2009; Ossewaarde et al., 2011). Experimentally induced acute stress affects reward-related neural responses to monetary reward, especially in the dorsal striatum (Porcelli et al., 2012). Striatal regions play a crucial role in reward processing (Hare et al., 2008). However, it remains unclear how the striatum is involved in the different phases of reward processing. Here, we aimed to investigate (1) the effect of acute stress on neural activation elicited by monetary reward and (2) what role striatal regions play regarding early reward detection to reward delivery.

Method: We recorded changes in BOLD signal using a 3 Tesla MRI scanner (EPI pulse sequence, TE: 30ms, TR: 2s, slice thickness: 3 mm) in 23 healthy subjects (age: 23.5 ± 4.3 years;

13 women), while they performed a spatial working memory reward task with two difficulty levels (low and high) and two reinforcement conditions (non-reinforced and reinforced). All functional images were acquired within two scanning sessions, one without stress induction and one with stress induction through the administration of unpredictable mild electric shocks. Data were analyzed using the general linear model for event-related designs in SPM12. Statistical parametric maps of voxel t-values were assessed for the comparison between reinforced and non-reinforced trials during the different phases of reward processing (trial cue, stimulus presentation, decision making, reward expectation, reward delivery).

Results: Results revealed significant activation in two striatal regions, the putamen and the caudate nucleus when comparing reinforced and non-reinforced trials in the absence of stress induction (p<0.05, FDR-corrected). These regions were activated exclusively in the condition without experimental stress induction. The putamen showed a specific reward reactivity during trial cue, whereas caudate nucleus was involved later in reward processing starting at stimulus presentation to reward delivery.

Conclusion: These findings indicate a stress-induced reduction in striatal reactivity to monetary reward and differential reward reactivity in dorsal striatum throughout the different phases of reward processing in healthy subjects. Thus, putamen is important at early detection of reward, whereas caudate nucleus is implicated in later phases of reward processing.

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Poster

329. Motivation: Neural Circuits II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 329.04/PP8

Topic: G.02. Motivation

Title: Reward preference induced by optogenetic CeA stimulation persists despite competitive physiological motivation

Authors: *O. M. LOFARO, M. J. F. ROBINSON Wesleyan Univ., Middletown, CT

Abstract: Drug addiction involves compulsive pursuit of a reward that persists even in the face of adverse consequences. The DSM-V draws a distinction between external and internal adverse consequences in its criteria for Substance Use Disorder, where external adverse consequences consist of legal or social problems. In contrast, internal adverse consequences consist of continued drug-seeking and drug consumption despite health problems, aversive drug effects, and unpleasant side effects of withdrawal. Previous research has established that optogenetic

stimulation of the central amygdala (CeA) generates an addiction-like preference for laser-paired reward that persists in the face of external punishment. The present study however aims to examine whether CeA stimulation-induced reward preference is maintained despite internal physiological consequences using sodium depletion and conditioned taste aversion (CTA). To this end, rats infused with light-activated Channelrhodopsin (ChR2) or control virus (EYFP) were trained to press levers for either a sucrose reward paired with CeA laser stimulation, or an unpaired salt pellet. During the sodium depletion experiment, rats developed a strong preference for the sucrose pellet, and were then repeatedly sodium-depleted in order to shift preference towards the salt reward. Here, EYFP rats shifted their behavior while ChR2 rats did not, maintaining a focused preference for the laser-paired reward despite their growing sodium appetite. In the CTA experiment, rats were presented with two novel sugar pellet flavors, one of which was repeatedly paired with the aversive agent lithium chloride. ChR2 and EYFP rats were then given the option to lever press for the aversive reward paired with laser stimulation, or the unpaired alternative flavored reward. Here, EYFP rats demonstrated a stronger avoidance of the aversive pellet and reduction in laser preference compared to ChR2 rats. These results suggest that CeA stimulation produces a powerful compulsion that can overcome intense physiological motivation, which could provide insight into why addicts forego their own health and biological needs to pursue their preferred reward.

Disclosures: O.M. Lofaro: None. M.J.F. Robinson: None.

Poster

329. Motivation: Neural Circuits II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 329.05/PP9

Topic: G.02. Motivation

Title: The impact of junk-food on 'liking' responses to sucrose, saccharin and salt in obesity-prone and obesity-resistant rats

Authors: ***A. BEN-EZRA**¹, E. HALTER¹, C. FREELAND¹, C. L. POISSON¹, A. WANG¹, C. R. FERRARIO², M. J. F. ROBINSON¹ ¹Psychology, Wesleyan Univ., Middletown, CT; ²Pharmacol., Univ. of Michigan, Ann Arbor, MI

Abstract: Diet-induced obesity is a growing problem influenced by the widespread availability of "junk-foods". However, consumption of these high-fat and high-sugar foods can cause varied weight gain due to genetic predisposition. This is reflected in rodent models, where a junk-food diet causes a weight increase in some Sprague Dawley rats (gainers), but not others (non-gainers). We have recently shown that male gainers experience a blunting of pleasure or 'liking' following prolonged junk-food exposure (Robinson et al, 2015). However, it is unclear whether these effects are the cause or consequence of excessive junk-food consumption. We can explore

this question using selectively-bred obesity-prone (OP) and obesity-resistant (OR) rats to examine hedonic 'liking' and 'disliking' responses both before and after consumption of junkfood. We used male and female OP (n = 20) and OR (n = 20) rats to study hedonic taste reactivity responses to sucrose (1%, 3%, 9%), salt (1%), and saccharin (0.15%) solutions. We found that OP rats showed fewer positive hedonic responses to sucrose and saccharin, both before and after junk-food exposure, as compared to OR rats. We also found that after junk-food exposure OP rats demonstrated reduced hedonic sensitivity towards the different sucrose concentrations, but no overall significant sex differences. Together, these data show that hedonic responses to sweet tastes are lower in obesity-prone rats, suggesting that pleasurable responses to sweetness do not drive over-consumption and that these responses are only mildly reduced by junk-food consumption.Furthermore, after junk-food, we observed that OP, but not OR rats, found salt less aversive, possibly suggesting the development of tolerance for salt overconsumption. Previous research suggests that mu-opioid receptor (MOR) transmission in the ventral and dorsal striatum modulates food intake and the preference for palatable foods, and we have previously shown that male gainers show decreased expression of MOR mRNA in these areas. To explore this in OP and OR rats and its possible relationship to changes in taste reactivity, we tested mu opioid receptors sensitivity via a morphine conditioned place preference paradigm before and after junk-food. Ultimately, our results suggest that animals predisposed to obesity have blunted hedonic responses to sweet solutions, even prior to consumption of a junkfood diet. Coupled with previous research suggesting increased attraction to food cues in obesityprone animals, these findings suggest basal differences and interactions between responses to food, highlighting a possible pre-existing dissociation between 'liking' and 'wanting'.

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Poster

329. Motivation: Neural Circuits II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 329.06/PP10

Topic: G.02. Motivation

Title: Effects of nicotine exposure and anxiety on motivation for gambling-like cues

Authors: *T. I. RUSSELL¹, M. J. ROBINSON² ²Psychology, ¹Wesleyan Univ., Middletown, CT

Abstract: More than half of disordered gamblers (60.4%) report comorbid tobacco dependence. Furthermore, 41.3% of disordered gamblers report the diagnosis of an anxiety disorder. The cooccurrence of gambling disorder and tobacco dependence might suggest a common mechanism for their pathology, and anxiety might promote the maintenance of these maladaptive behaviors.

One of the most important characteristics common to all gambling games is the uncertainty associated with the probability of reward delivery and the magnitude of the reward. Evidence suggests that uncertainty may powerfully enhance attraction to cues, such as the flashing lights and celebratory sounds of casino slot machines. However, it is unknown how nicotine or anxiety might contribute to cue-attraction. In the present study, we investigate the effects of nicotine (0.3 mg/kg, s.c.) on the desire for cues associated with uncertain rewards in male and female Sprague-Dawley rats. In an autoshaping task, rats learned to associate lever and tone cues (CS) with the delivery of sucrose pellet rewards (UCS) on either a certain or uncertain reward contingency in the presence of nicotine or saline. In the certain condition, each cue presentation was followed by the delivery of one sucrose pellet (100%-1). In the uncertain condition, only half of the trials were reinforced with either 1, 2, or 3 sucrose pellets (with equal probability) (50%-1-2-3). Subsequently, we tested the ability of gambling-like cues to serve as a conditioned reinforcer, and to promote motivation for the sucrose reward during a progressive ratio task. Finally, the Elevated Plus Maze was used to measure the effects of anxiety and its interaction with nicotine and uncertainty. During the autoshaping task, nicotine enhanced attraction to CS cues under certain conditions, but not for uncertain ones. Conversely, in the progressive ratio task, nicotine enhanced motivation to obtain the reward in uncertain conditions, but not for certain conditions, and this effect was largely driven by females. Lastly, we examined the role of anxiety in moderating cue-directed behaviors. In all, nicotine appears to promote motivated behaviors by increasing incentive salience to cues associated with certain rewards and increasing motivation for rewards associated with uncertainty. Since motivation to obtain rewards appears to be greater in females injected with nicotine, comorbidity rates may be sex specific rather than uniform across both sexes. Future studies should consider nicotine comorbidity and these sexdependent effects when developing successful intervention programs for disordered gambling.

Disclosures: T.I. Russell: None. M.J. Robinson: None.

Poster

329. Motivation: Neural Circuits II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 329.07/PP11

Topic: G.02. Motivation

Title: Distinguishing between predictive and incentive value of uncertain gambling-like cues in a Pavlovian autoshaping task

Authors: *A. S. KNES, T. I. RUSSELL, C. CLIBANOFF, J. R. COTE, M. J. F. ROBINSON Psychology, Wesleyan Univ., Middletown, CT

Abstract: Gambling Disorder is a behavioral addiction that affects nearly 1% to 2% of the United States adult population each year. In gambling games, cues such as flashing lights and

celebratory sounds are present during reward delivery, and can attract and motivate an individual to continue playing. Cues that reliably predict reward often become attractive and imbued with incentive properties. However, gambling-like cues are typically associated with uncertainty in the form of reward probability and magnitude. Although the predictive value of uncertain cues is degraded, previous research has shown that these cues become more attractive. Currently, it remains unclear how uncertainty affects the attribution of incentive salience to the predictive versus the incentive property of these cues. Here, we used a novel pavlovian autoshaping approach to investigate the effects of reward uncertainty on the predictive and incentive value of CS cues in Sprague Dawley rats. In this task, we presented a CS1 followed by a CS2, which resulted in the delivery of sucrose pellets. The first CS gains predictive value as it is presented immediately before the second CS and predicts the possibility of sucrose reward delivery. The second CS provides no additional predictive information, and instead primarily holds incentive value as its presentation most closely precedes reward delivery. Rats learned to associate the CS1 and CS2 lever presentations with the delivery of sucrose pellets on either a certain or uncertain reward contingency. In the certain reward condition, one sucrose pellet is delivered immediately after the CS period. In the uncertain reward condition, half of the CS presentations are not reinforced, while the other half are reinforced with either 1, 2, or 3 sucrose pellets. Here, we demonstrate the separation of incentive and predictive value for cues that are associated with the uncertain reward contingency. Our results suggest that the presentation of a CS2, which is more proximal to reward, leads to increased attraction primarily for animals assigned to the uncertain reward contingency. In contrast, under certain reward conditions, rats demonstrate increased attraction to solely the first, predictive CS1. These findings suggests that uncertainty might alter attraction and promote the attribution of greater salience specifically to incentive cues, while leaving the attraction to predictive cues unchanged. Therefore, in gambling games with a high degree of uncertainty, attraction to cues such as lights and sounds might be greater due to the increased incentive value that is attributed to these cues.

Disclosures: A.S. Knes: None. **T.I. Russell:** None. **C. Clibanoff:** None. **J.R. Cote:** None. **M.J.F. Robinson:** None.

Poster

329. Motivation: Neural Circuits II

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Program#/Poster#: 329.08/PP12

Topic: G.02. Motivation

Support: NIDA T32 DA007281 to KEY

NIDA DA039952 to JBB

NSF IOS-1353263 to JBB

Title: Regulation of food reward by female gonadal hormones

Authors: *K. E. YOEST¹, K. E. SHASHLO¹, J. A. CUMMINGS¹, J. B. BECKER^{1,2,3,4} ¹Dept of Psychology, ²Neurosci. Program, ³Mol. and Behavioral Neurosci. Inst., ⁴Dept of Psychiatry, Univ. of Michigan, Ann Arbor, MI

Abstract: In female rodents, motivated behaviors are influenced by cyclic changes in levels of gonadal hormones. Induction of sexual receptivity by estradiol and progesterone modulates motivated behavior in response to both natural and drug rewards, and estradiol alone is sufficient to enhance motivation for cocaine (Becker & Hu, 2008, Front. Neurosci. 29(1): 36-47). Here we sought to parse the pharmacological and behavioral mechanism by which changes in circulating gonadal hormones levels over the course of the estrous cycle influences motivation for palatable food reward.

We have previously demonstrated that hormone priming with estradiol and progesterone in a regimen that induces sexual receptivity reduces both food consumption and motivation for food when animals are tested on fixed interval (FI) or progressive ratio (PR) schedules of reinforcement. However, we had not yet verified whether administration of estradiol alone is sufficient to induce changes in motivation for food. Therefore, we tested whether repeated administration of estradiol benzoate could induce the same changes in motivation that were seen after giving estradiol benzoate followed by progesterone. This priming regimen also induces sexual receptivity but may not activate progesterone receptors outside of the hypothalamus. We found that administration of estradiol alone reduced both the total number of rewards received and the average number of responses made on the FI schedule. Estradiol did not reduce breakpoint on the PR, however, suggesting that progesterone is required for the decreased motivation for food on the PR schedule. Interestingly, on the FI schedule there was an increase in the number of intervals initiated but not completed when females were treated with estradiol and progesterone, and not estradiol alone.

These behavioral effects led us to hypothesize that estradiol and progesterone may be acting on different neural circuits to modulate different aspects of food reward during periods of sexual receptivity. While estradiol alone can decrease consumption of rewards and overall responding on a well-trained task, supplementary progesterone is required to reduce perseveration toward a food reward. To investigate if this effect of progesterone on perseverative responding is dependent on the ventral striatum, we implanted ovariectomized female rats with bilateral cannula aimed at the nucleus accumbens. Animals were then hormone primed with and without chronic intracranial administration of the progesterone antagonist, RU486. These findings will further elucidate the mechanism by which induction of sexual receptivity induces changes in feeding behavior and food reward.

Disclosures: K.E. Yoest: None. K.E. Shashlo: None. J.A. Cummings: None. J.B. Becker: None.

Poster

329. Motivation: Neural Circuits II

Location: Halls A-C

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Program#/Poster#: 329.09/PP13

Topic: G.02. Motivation

Support: NSERC

Title: Chronic stress impairs reward responsiveness in a rat test of anhedonia

Authors: *S. J. LAMONTAGNE¹, M. C. OLMSTEAD² ¹Psychology, ²Queen's Univ., Kingston, ON, Canada

Abstract: Anhedonia, a severe deficit in reward processing, is an endophenotype of several neuropsychiatric disorders (APA, 2000) and increases susceptibility to depression (Atherton et al., 2015), substance abuse (Hatzigiakoumis et al., 2011), and suicidality (Winer et al., 2016). Recent literature suggests that anhedonia reflects a maladaptive interaction between reward circuitry and stress, the former being mediated by the mesolimbic dopamine (DA) system and the latter by the glucocorticoid system (Vrieze et al., 2013). The role of the DA system in the development and maintenance of anhedonia has been studied extensively, but little is known about the contribution of the glucocorticoid system in this process. The goal of the current study was to investigate the effect of stress on reward processing using a validated rat test of anhedonia, the probabilistic reward task (PRT). In this task, rats learned to discriminate between two ambiguous tones for a sucrose reward. During testing, one lever was programmed to produce three times more reward than the other lever. Similar to healthy humans, rats develop a response bias toward the more frequently reinforced stimulus, regardless of which tone is presented. Consistent with prior literature (Der-Avakian et al., 2013), the response bias was enhanced following administration of a DA agonist, amphetamine, and reduced following administration of a DA antagonist, pramipexole. Additionally, a 28-day chronic mild stress (CMS) regimen attenuated the increase in response bias that normally develops over the test session. Finally, the glucocorticoid antagonist, mifepristone, had no effect on the response bias relative to saline; however, the glucocorticoid agonist, dexamethasone, attenuated the response bias relative to both saline and mifepristone. These results confirm a role of DA in reward processing, and highlight an additional contribution of the glucocorticoid system in the mediation of this effect. Thus, it is plausible that stress directly interferes with normal mesolimbic DA functioning, which leads to an overall impairment in reward responsiveness. Future studies on the etiology of anhedonia should carefully investigate the interaction of these systems in reward processing.

Disclosures: S.J. Lamontagne: None. M.C. Olmstead: None.

Poster

329. Motivation: Neural Circuits II

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Program#/Poster#: 329.10/PP14

Topic: G.02. Motivation

Support: NICHD, NIH intramural research program project Z01-HD-000642-04

Title: Deletion of Prkar2a, with its exclusive habenular brain expression, confers obesity resistance through decreased hedonic behavior and increased drive for voluntary exercise

Authors: *E. LONDON¹, J. C. WESTER², C. A. STRATAKIS¹ ¹NICHD, Bethesda, MD; ²NIH, Bethesda, MD

Abstract: The cAMP-dependent protein kinase (PKA) system is crucial in regulating energy balance and many other cellular functions as it mediates the effects of numerous hormones, neurotransmitters and catecholamines that bind G-protein coupled receptors. Prkar2a codes for PKA regulatory subunit RIIa, that unlike other PKA subunits, has minimal expression throughout the brain except in the habenula where it is highly expressed. The habenula has been implicated in regulating hedonic state, voluntary exercise, and primary reinforcement. Here we show that RIIa knockout (KO) mice resist diet-induced obesity, and this phenomenon cannot be explained by enhanced metabolic rate. Instead, RIIa KO consistently consumed less palatable high fat (HF) chow than wild type (WT) mice, despite their comparable preference for HF over regular chow. RIIa KO mice also consistently consumed less 10% sucrose solution during a twoweek sucrose preference test. Alternatively, when provided home cage running wheels, RIIa KO mice ran two to three times more than WT littermates. These differences were more profound in females. Pkrar2a colocalizes with Chrna3 and Chrnb3 in the medial habenula, and with Mor1 in the lateral habenula, yet it is not known how it might regulate nicotinic or mu opioid receptor pathways. Enzymatic activity assays showed decreased habenular PKA activity in RIIa KO compared to WT mice; PKA activity was unchanged in striatum, a region that provides input to the habenula. Others have shown that mice with dorsal medial habenula lesions have blunted sucrose preference as well as poor performance in voluntary wheel running. These data combined suggest that Prkar2a has diverse roles in modulating behaviors related to reward reinforcement and motivation for voluntary exercise, likely by affecting downstream targets such as ventral tegmental area or substantia nigra. Ongoing studies involve virus-mediated reexpression of Prkar2a in the habenula and habenula-specific KO of Prkar2a. The study of Prkar2a in brain may serve as a useful model for better understanding the role of the habenula in regulating behaviors involved in obesity, addiction, and depression.

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Poster

329. Motivation: Neural Circuits II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 329.11/PP15

Topic: G.02. Motivation

Title: An examination of the effects of D1, D2, and mu-opioid receptors of the nucleus accumbens on appetitive and consummatory motivation in a modified effort-related choice paradigm

Authors: *H. N. CARLSON, C. MURPHY, W. E. PRATT Psychology Wake Forest Univ. Winston Salem NC

Psychology, Wake Forest Univ., Winston Salem, NC

Abstract: The nucleus accumbens (NAcc) is critical for regulating appetitive and consummatory phases of motivated behavior. It is innervated by dopaminergic pathways which promote incentive and effort-inducing properties of stimuli. Additionally, opioid receptor activation of the NAcc increases food consumption, particularly on palatable diets. In these experiments, we compared the impact of dopamine or µ-opioid receptor manipulations of the NAcc core on a modified effort-related choice paradigm in which rats could choose between working for a preferred sugar pellet or consuming freely available rat chow. Male Sprague-Dawley rats (n = 12/group) were food restricted to 90% ad libitum weight and trained on a progressive ratio 2 (PR2) schedule to earn sugar pellets. Following training, food was returned, and rats were surgically implanted with bilateral guide cannulas targeting the NAcc core. Rats were then retrained on the PR-2 task, with rat chow also freely available in the operant chamber. Access to the sugar-associated lever and the food was continuous throughout each 1-hr session. Once behavior had stabilized, separate groups were tested following NAcc D1 receptor stimulation or blockade (with 0, 0.25, and 1.0 µg SKF 38393 or 0, 0.5, and 2.0 µg SCH 23390/side, respectively), D2 receptor stimulation or blockade (with 0, 1.0, and 5.0 µg raclopride or 0, 0.2, and 1.0 μ g quinpirole/side, respectively), or μ -opioid receptor stimulation (with 0, 0.025, and 0.25 µg DAMGO/side). Individual rats in each drug group were tested on all doses of a single drug, and then again in separate sessions following 24-hr food restriction. NAcc blockade of the D1 receptor or stimulation of the D2 receptor dose-dependently reduced the break point for earning sugar pellets, but had no impact on consumption of the freely-available rat chow, regardless of the deprivation condition of the animals. Neither NAcc D1 receptor stimulation nor D2 receptor antagonism impacted progressive ratio performance or chow intake. NAcc µ-opioid receptor stimulation increased intake of the freely-available chow and also reduced break point for the sugar pellets. These data support a role for NAcc dopamine for regulating incentive and effort-related processes of appetitive motivation but not consummatory processes. In contrast, stimulation of µ-opioid receptors shifted rats' behavior away from sugar-seeking and towards food consumption, even when additional effort would yield a preferred food. Future use of this

task may be beneficial in teasing apart the motivational functions of other neurotransmitter systems involved in food-directed motivation.

Disclosures: H.N. Carlson: None. C. Murphy: None. W.E. Pratt: None.

Poster

329. Motivation: Neural Circuits II

Location: Halls A-C

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Program#/Poster#: 329.12/PP16

Topic: G.02. Motivation

Title: Fenfluramine and lorcaserin inhibit the binge-like feeding induced by mu-opioid receptor stimulation of the nucleus accumbens in the rat

Authors: *W. E. PRATT, S. BLUMENTHAL

Dept. Psychology, Wake Forest Univ., Winston Salem, NC

Abstract: One cause of the current obesity epidemic is the increased availability of highly palatable diets that engage brain reward circuitry and promote overconsumption of food. Prior experiments have shown that the stimulation of µ-opioid receptors in the nucleus accumbens (NAcc) powerfully increases intake of palatable and high-fat diets. In contrast, serotonin agonists reduce feeding by advancing satiety processes, and several serotonin agonists have been prescribed to promote weight loss. Despite this knowledge, it is unclear if serotonin signaling can modulate the food intake elicited by activation of µ-opioid signaling within reward circuits. These experiments assessed the effects of systemic treatments with the serotonin agonists dfenfluramine and lorcaserin on the binge-like feeding induced by mu-opioid receptor stimulation of the NAcc. Male Sprague-Dawley rats (n = 8/group) were surgically implanted with bilateral guide cannulas targeting the NAcc core. Upon recovery, they were acclimated to 2-hr daily sessions, during which they were given free access to Crisco® and water. On subsequent drug testing days, rats received systemic injections of d-fenfluramine (Experiment 1; at 0, 0.6, or 3.0 mg/kg) or lorcaserin (Experiment 2; at 0, 0.3, or 1.0 mg/kg) prior to injections of vehicle or the mu-opioid receptor agonist DAMGO bilaterally into the NAcc (at 0.025 µg/0.5 µl/side). Each rat randomly received all possible combinations of systemic and intracranial injections across 6 treatment days. Food and water intake, rearing, and ambulatory behavior were monitored throughout each session. Consistent with previous reports, stimulation of NAcc µ-opioid receptors significantly increased consumption of the high-fat vegetable shortening, and systemic treatment with d-fenfluramine and lorcaserin dose-dependently decreased intake. Interestingly, both d-fenfluramine and lorcaserin reversed the binge-like feeding observed following stimulation of NAcc µ-opioid receptors. At 3.0 mg/kg, fenfluramine completely blocked feeding on the vegetable shortening, whereas 1.0 mg/kg of lorcaserin reduced DAMGO-induced feeding to baseline at the conclusion of the 2-hr session. Both serotonergic drugs also reversed the

increases of ambulation observed following DAMGO injections into the NAcc; rearing was inhibited at the highest doses of d-fenfluramine and lorcaserin given. There was no impact of any treatment on water intake. These data demonstrate for the first time that serotonergic systems, in addition to impacting homeostatic regulation of feeding, also can inhibit food intake elicited by activation of mu-opioid receptors within the ventral striatum.

Disclosures: W.E. Pratt: None. S. Blumenthal: None.

Poster

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Topic: G.02. Motivation

Support: NIH Grant R21AA021445

NIH Grant RO1 AA024109

Title: Anterior insula-Locus Coeruleus area inputs and α 1-adrenergic receptors promote compulsion-like alcohol intake

Authors: *K. LEI¹, S. A. WEGNER¹, D. DAREVSKY¹, F. W. HOPF² ¹Neurol., Univ. of California, San Francisco, San Francisco, CA; ²Dept Neurol, UCSF, San Francisco, CA

Abstract: Compulsive alcohol intake, where drinking continues despite negative social, legal and physical consequences, is a major obstacle to treating alcohol use disorders (AUDs) in humans. Our previous work identified an anterior insular (aINS) circuit that is specifically required for promoting compulsive alcohol intake, but with no role in the regular, unpunished alcohol drinking. This was observed when inhibiting aINS-NAcCore or mPFC-NAcCore inputs with optogenetics, and inhibiting non-canonical NMDARs in NAcCore with pharmacology and shRNA. Interestingly, the idea that compulsive drinking would be driven by cortical-subcortical circuits that process conflict has been proposed based on clinical findings (Tiffany and Conklin, 2000; Bechara and Naqvi, 2010). In particular, it is the conflict, where intake persists despite negative consequences that requires the use of specific cortical circuits to successfully maintain compulsive behavior such as drinking despite punishment. For regular drinking, there is no conflict and no need for any of this circuitry, and habit-related circuits are likely involved. In fact, ours is the first and only evidence for this model. In addition, we found that both aINS-NAcCore and mPFC-NAcCore projections are critical for driving compulsive intake regardless of whether the aversive consequence is shock or bad tasting quinine. This is important because the quinine-resistant compulsive drinking model is widely used and technically simple, but the

insula is also related to primary taste, but our results implicate these circuits for compulsive drinking independent from the actual type of punishment. Since compulsion-like drinking also involves aversion, we examined here whether the adaptive stress system, especially the Locus Coeruleus (LC) and its noradrenergic projections, also play a specific and potent role in compulsion-like intake. Inhibition of the α 1-adrenergic receptor with prazosin systemically or in the mPFC significantly and selectively reduced compulsive alcohol intake. Prazosin inhibition of compulsive drinking was not related to basal intake levels, suggesting compulsion and self-administration are separate factors, similar to compulsive drinking, with no role in regular alcohol intake. Thus, adaptive stress responding may be a central part of the mechanism that allows the anterior insula to drive compulsive responding for alcohol, in particular through a feed-forward mechanism whereby aINS drives the LC to activated the mPFC, which is essential for allowing compulsive drinking patterns.

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Poster

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Topic: G.02. Motivation

Support: NIH Grant R21AA021445

NIH Grant RO1 AA024109

Title: Compulsion-like alcohol drinking involves more automatic licking: Evidence for the head down and push model of compulsive intake

Authors: *F. W. HOPF, D. DAREVSKY, S. WEGNER, K. LEI Dept Neurol, UCSF, San Francisco, CA

Abstract: Alcohol addiction remains a major social, economic and emotional cost. Compulsive drives, where drinking continues even in the face of negative consequences (loss of job, family or freedom), represent a particular and potent obstacle to successful treatment. Our lab's recent findings suggest that compulsive drinking involves conflict between desired intake and feared consequences, which recruits cortical circuits and specific NMDAR subtypes, providing a specific intervention for compulsive drives for alcohol. Thus, we used a lickometer system to examine whether rats exhibited concentrated patterns of intake, and perhaps increased motivation for intoxicating effects, during what has been considered binge-like drinking or compulsion-like. Adult male rats drank alcohol under an intermittent schedule (Monday, Wednesday and Friday

overnights) which is known to strongly escalate drinking, a sign of addictive behavior. After ~ 3 months, rats drank 20 min/day, 5 days/week for ~1 month, and then intake patterns were assessed using lickometers. For each rat (n=14) we analyzed 4 alcohol-only sessions (binge intake) and 4 sessions of alcohol adulterated with bitter quinine (compulsion-like drinking). Different parameters of licking were analyzed using multiple linear regression and other methods. Using the widely used 1 sec cutoff, our results support that both binge and compulsive alcohol drinking (and saccharin intake) are based around a particular response pattern, which may represent an optimum for the central pattern generator for licking. Drinking of alcohol with higher levels of quinine, where intake is reduced, also shows evidence of disrupted bout generation, including slower and shorter bouts, suggesting that the stereotyped aspects of licking do not reflect a floor/ceiling effect in terms of licking. Also, different measures of licking also suggest that both forms of alcohol drinking exhibit high motivation. In addition, analyses suggest that compulsive like drinking is more focused and less variable than binge-like licking, perhaps indicating a process where aversion is acutely experienced and the individual tightens their focus to just get through the punished drinking session, our newly described Head Down and Push model. Thus, concentrated patterns of intake during binge-like or compulsion-like alcohol consumption could imply high motivation for, with a greater focus on lick fidelity that may be something akin to just gritting one's teeth and pushing through to get the desired intake done.

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Poster

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Location: Halls A-C

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Topic: G.02. Motivation

Support: AG045380

Title: Age-related changes in licking microstructure and motivation for palatable rewards

Authors: *I. A. MENDEZ¹, N. P. MURPHY¹, S. B. OSTLUND², N. T. MAIDMENT¹ ¹Psychiatry and Biobehavioral Sci., UCLA, Los Angeles, CA; ²Dept. of Anesthesiol. and Perioperative Care, UCI Sch. of Med., Irvine, CA

Abstract: While food reward processing in adolescents has been well studied, how it changes as individuals age beyond adolescence is less understood. Analysis of licking microstructures may be used to discern the incentive properties from the hedonic impact of rewards; therefore, licking microstructure for various concentrations of sweetened condensed milk (SCM) was assessed in adult (20 weeks old) and aged (21 months old) rats. Following training in a lickometer, within and between subjects SCM concentration-response curves were established to assess licking

microstructures. Motivation to work for either a low or high concentration of SCM was also examined by studying instrumental responding for SCM under various reinforcement schedules over several days. We found that aged rats showed more bouts of licking compared to adults, but only at lower concentrations of SCM. Additionally, differences between age groups on lever pressing for SCM under high demand schedules also depended on concentration. Our results suggest that age-related changes in licking microstructure and motivation go well beyond adolescence and may depend on the palatability of the orosensory reward being consumed. These findings may help understand healthy and pathological age-related changes in food reward processing and associated diseases.

Disclosures: I.A. Mendez: None. N.P. Murphy: None. S.B. Ostlund: None. N.T. Maidment: None.

Poster

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Topic: G.02. Motivation

Support: NIH Grant AG045380

Title: Response allocation in Pavlovian-to-instrumental transfer

Authors: *A. T. MARSHALL¹, C. N. MUNSON¹, S. B. OSTLUND²

¹Univ. of California - Irvine, Irvine, CA; ²Dept. of Anesthesiol. and Perioperative Care, UCI Sch. of Med., Irvine, CA

Abstract: Individual's actions are influenced by external reward cues, which may promote suboptimal reward-seeking behavior. For example, drug-associated cues appear to motivate drug seeking even when individuals are engaged in other, more adaptive behaviors. The presumed mechanism of this phenomenon is that cues induce a state of heightened incentive motivation for reward (increased desire/wanting). The Pavlovian-to-instrumental transfer (PIT) paradigm is a powerful behavioral tool for studying motivated behavior, and a selective assay of the incentive motivational properties of reward cues. In tests of PIT, Pavlovian reward-predictive cues, presented while an instrumental reward-seeking response (e.g., lever pressing) is available, typically result in an invigoration of reward seeking, even though reward-seeking responses were never reinforced during the cues. Unsurprisingly, cues also elicit conditioned approach behavior directed towards the reward delivery receptacle (goal approach), which can compete with cues' abilities to promote instrumental reward seeking. This Pavlovian-instrumental tradeoff is a fundamental component of various behaviors, but much remains unknown about the conditioning factors that govern how individuals allocate behavior between these two reward-related activities

(i.e., instrumental reward seeking vs. Pavlovian goal approach). Thorough analyses of the neural and behavioral mechanisms of this interaction may provide insight into adaptive decision making and aberrations in behavior control (e.g., compulsive drug seeking, gambling). Here, hungry rats pressed a lever for food and then learned to associate auditory cues with food delivery. Reward delivery frequency during the cues was manipulated. In PIT tests, rats could press the lever in extinction while the cues were intermittently presented. We predicted that cues would invigorate lever pressing, and that the cue associated with random reward delivery during the cue would elicit the greatest increase in lever pressing. Microstructural analyses of response allocation showed that rats' lever pressing was augmented during the cue, and that their distribution of lever pressing in the cues was sensitive to cue-specific predictions of reward, elucidating Pavlovian-instrumental interactions in cue-motivated behaviors. Further analyses investigated reward-magnitude effects when the timing of reward was constant. Planned manipulations of dopamine signaling will characterize the involvement of this modulator in cue-triggered response allocation. Overall, these results shed light on the neural substrates and microstructure of cue-motivated behavior.

Disclosures: A.T. Marshall: None. C.N. Munson: None. S.B. Ostlund: None.

Poster

329. Motivation: Neural Circuits II

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Topic: G.02. Motivation

Support: NIH SC1DA 034995

CUNY Five-Year Graduate Fellowship

Title: Habit formation does not depend on the correlation between response rates and reward rates

Authors: *E. GARR¹, A. R. DELAMATER² ¹Psychology, City Univ. of New York, Brooklyn, NY; ²Brooklyn Col. CUNY, Brooklyn, NY

Abstract: It is known that an animal's behavior can become insensitive to the value of its goal (i.e. habitual). One important variable in determining this phenomenon is the schedule of reinforcement. Rodents trained to press a lever on a random interval (RI) schedule are more likely to develop habits than rodents trained on a random ratio (RR) schedule (Dickinson et al., 1983; Gremel & Costa, 2013; O'Hare et al., 2016). One long-standing hypothesis is that habits do not form as readily under RR schedules because the animal is able to experience a correlation between the rate of responding and the rate of reward—a correlation which is degraded under an

RI schedule (Dickinson, 1985). We tested this theory by allowing rats to press a lever for pellets under an RI schedule whose mean reinforcement rate either remained constant or became increasingly dense over 10 training sessions. The purpose of the latter manipulation was to effectively induce a positive correlation between response rate and reward rate over sessions because we expected responding to increase with training. In Experiment 1 we found that training with a positive or flat response rate-reward rate correlation resulted in equivalent goaldirected lever pressing (F(1,14) = 5.23, p < .05). In Experiment 2, we adjusted the schedules to make the overall reward rate lower. In that case, rats were habitual regardless of whether they were trained with a positive or flat response rate-reward rate correlation (F(1,16) = 0.46, p > .05). Rather than pointing to a role for the correlation between response rates and reward rates, our findings suggests that schedule density or temporal uncertainty of rewards may play more influential roles in habit formation. In fact, switching habitual rats to a denser and less temporally uncertain schedule resulted in goal-directed lever pressing (F(9,135) = 1.87, p < .05). We are currently in the process of independently examining the role that each of these variables plays in habit formation. Given that the transition to habits is mediated by changes in the striatum and medial prefrontal cortex, these findings will inform theories of cortico-basal ganglia function by implying a more precise role played by these structures in habit formation.

Disclosures: E. Garr: None. A.R. Delamater: None.

Poster

329. Motivation: Neural Circuits II

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Topic: G.02. Motivation

Support: NSERC

Title: High-fructose corn syrup in alcoholic beverages? A study in laboratory rats

Authors: *S. AYOUB, M. MINHAS, F. LERI Psychology: Neurosci. and Applied Cognitive Sci., Univ. of Guelph, Guelph, ON, Canada

Abstract: Heavy alcohol use is the third leading risk factor contributing to the global burden of disease (The World Health Organization, 2009) and therefore it is important to identify factors that contribute to excessive consumption. It has been observed that the addition of sweet substances (i.e. sucrose, saccharin) to alcohol solutions drastically increases self-administration of alcohol in rats. High-fructose corn syrup (HFCS) is a calorically-dense corn-based sweetener that is frequently found in alcoholic beverages. In the current study, we used an intraoral alcohol self-administration procedure to determine if the addition of HFCS (25%) to a 5% alcohol solution would increase alcohol intake in male Sprague Dawley rats. In the first experiment, it

was found that the addition of 25% HFCS robustly increased the amount of alcohol selfadministered. A second experiment explored whether this effect could be attributable to the sweet taste or to the caloric value of HFCS. Previously, we established that 0.1% saccharin (a non-caloric sweetener) and 25% HFCS are equally palatable to rats (Levy, 2015). Thus, animals were trained to self-administer 5% alcohol mixed in a 0.1% saccharin solution. It was found that saccharin increases alcohol self-administration, but this increase was much smaller than the increase induced by the addition of HFCS. These results in rats suggest that efforts aimed at reducing the harmful consumption of alcohol may include removal of HFCS from the list of ingredients.

Disclosures: S. Ayoub: None. M. Minhas: None. F. Leri: None.

Poster

329. Motivation: Neural Circuits II

Location: Halls A-C

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Program#/Poster#: 329.19/PP23

Topic: G.02. Motivation

Support: NIDA022582

Dartmouth Brain Imaging Center (DBIC) pilot award

Title: Neural representations of observed appetitive actions encode information about reward

Authors: *K. M. RAPUANO, R. H. HYON, S. A. NASTASE, W. M. KELLEY Psychological and Brain Sci. Dept., Dartmouth Col., Hanover, NH

Abstract: Recent neuroimaging studies have shown that viewing dynamic appetitive cues (e.g., smoking, fast food commercials) engage regions of the action observation network (AON), and that this activity is meaningful for appetitive behavior. However, it is unclear what factors influence the spontaneous simulation of appetitive actions. We hypothesized that the simulation of appetitive actions encodes information about the motivational content of the stimuli. To test this hypothesis, we scanned participants using fMRI during passive viewing of naturalistic video clips depicting appetitive actions (e.g., eating food, drinking alcohol) and non-appetitive actions (e.g., applying chapstick). Appetitive actions included a range of appetizing items in a condition-rich design, and each action was presented from both the first- and third-person perspectives. Outside of the scanner, participants indicated their preferences for each item. A univariate parametric modulation analysis was performed in order to identify brain regions where activity correlated with subjective ratings of food preferences. In addition to reward-related regions (e.g., nucleus accumbens [NAcc]; orbitofrontal cortex [OFC]), activity in somatomotor cortex was greater for items that were more preferred. A similar relationship was observed for univariate

comparisons between high- and low-appetitive items (e.g., alcoholic versus nonalcoholic beverages). Additionally, we examined whether distributed patterns of activity encode motivational content using representational similarity analysis (RSA). In order to investigate whether there was shared information between reward and action observation regions, we examined the similarity of representational geometry between NAcc and somatomotor cortex and observed significant correlations between these regions bilaterally. Further, we performed an exploratory searchlight regression analysis to identify regions where local representational geometry was predicted by a weighted sum of three RDMs: perspective (i.e., first, third), categories (i.e., food, beverages), and preference ratings. Perspective and category were encoded in posterior visual areas, while category was additionally represented in ventral somatomotor cortex. Preferences were represented in lateral occipitotemporal cortex, postcentral gyrus, and ventral premotor cortex—canonical members of the action observation network. Taken together, these results suggest that reward-related information shapes representational geometry in the AON, which may influence the motivation to engage in potentially risky behaviors.

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Poster

329. Motivation: Neural Circuits II

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Program#/Poster#: 329.20/PP24

Topic: G.02. Motivation

Support: Conacyt 220772

Title: Endocannabinoids but not dopamine mediate the sexual behavior inhibition and drug hypersensitivity induced by copulation to satiety in male rats

Authors: *E. GONZÁLEZ-MORALES, G. RODRIGUEZ-MANZO Pharmacobiology, Cinvestav-Sede Sur, Ciudad de Mexico, Mexico

Abstract: The mesolimbic dopaminergic system is involved in the regulation of the motivational component of male rat sexual behavior and it is activated by copulation. When a male rat is allowed to copulate without restriction with a same sexually receptive female, it will ejaculate repeatedly until becoming sexually exhausted. Copulation to satiety induces the instatement of a long lasting (72h) sexual behavior inhibitory period and, at the same time, these rats exhibit a generalized hypersensitivity to drug actions. Both phenomena suggest the occurrence of copulation-induced brain plasticity. Besides, ventral tegmental area (VTA) dopamine (DA) neurons synthesize and release endocannabinoids (eCN) in response to its continued activation. During copulation to satiety, these neurons are continuously activated, inducing a prolonged

increase in DA release in the nucleus accumbens (NAcc). Previous data showed that eCNs are involved in the induction of the long-term sexual behavior inhibition of satiated rats, but it is not known if they are also involved in the induction of drug hypersensitivity in these animals. Due to the increased levels in the NAcc, DA might also play a role in the induction of both phenomena. To test these hypotheses we administered the CB1 receptor antagonist, AM251, or the DA receptor antagonist, haloperidol, immediately before subjecting male rats to copulation to satiety and tested them 24 or 48h later for sexual behavior and drug hypersensitivity. The latter was measured by the appearance of signs of the 5-HT syndrome in response to low doses of 8-OH-DPAT or by the loss of the sexual facilitative effects of yohimbine, at low doses. Results showed that antagonism of CB1 receptors during copulation to satiety interfered with the establishment of the long-term sexual behavior inhibition and of the hypersensitivity to 8-OH-DPAT and yohimbine in sexually exhausted rats. On the other hand, dopaminergic receptor antagonism did not prevent the occurrence of both phenomena. These results show that eCNs, but not DA participate in the induction of both the long-lasting sexual behavior inhibition and the drug hypersensitivity that characterize sexually satiated rats.

Disclosures: E. González-Morales: None. G. Rodriguez-Manzo: None.

Poster

329. Motivation: Neural Circuits II

Location: Halls A-C

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Program#/Poster#: 329.21/PP25

Topic: G.02. Motivation

Support: Childhood Obesity Prevention Training Grant #2011670013011

Title: Does reward type matter? Examining differences in reward types in healthy weight vs. overweight adolescents

Authors: *N. ROBERTS, S. ADISE, V. BRITTAIN, K. L. KELLER, C. F. GEIER Human Develop. and Family Studies, The Pennsylvania State Univ., State College, PA

Abstract: Adolescence is a period of development associated with a heightened response to rewards and limitations in cognitive control. The majority of research studying reward response has utilized monetary incentives, and only a limited number of studies have used addition types of incentives (e.g. food). While many of these studies compare incentives to a neutral condition, few studies have examined different types of rewards in the same study paradigm. Furthermore, how the brain responds to rewards (anticipation and receipt) has been linked to weight gain and obesity in adults. However, this relationship has not been examined in adolescence, a period where increases in autonomy, including food choice decisions (e.g. types, amounts), occur. In the present study, we used behavioral and imaging methods to examine differences in reward types

(food, money, no reward). We also examine differences by weight status (healthy weight vs. overweight or obese). Seventy participants (35 healthy weight) completed 8 blocks of an incentivized Go/NoGo task. Prior to each randomized block, participants were instructed if they would win money (3 blocks), food (3 blocks), or no reward (2 blocks) based on performance on that block. Our preliminary results of the Go/NoGo task do not show a significant difference by reward type, however, the data suggest a speed accuracy tradeoff. On average, when participant's reaction times were similar in the rewarded conditions relative to the neutral conditions, participants made more errors in the neutral condition relative to the money and food conditions. This suggests that participants took additional time in the rewarded blocks in order to make less errors. A subset of participants (N=30) performed a card-guessing task, again with 3 reward anticipation conditions (food, money, neutral). We measured BOLD response with fMRI to assess brain activation in a priori defined reward and cognitive control regions. Our preliminary imaging results indicate a differential response to the anticipation of food versus neutral in the striatum and food versus money in healthy weight versus overweight or obese adolescents. In healthy weight adolescents, there was greater activation in anticipation of money vs. neutral in the right medial frontal gyrus (BA10/11). Additional analyses in both tasks will be presented. Understanding the neurobiological risk factors for obesity are crucial for obesity prevention.

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Poster

329. Motivation: Neural Circuits II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 329.22/PP26

Topic: G.02. Motivation

Title: The effects of depletion and brain stimulation on motivation

Authors: *S.-B. BELL¹, M. M. YEE²

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Abstract: Transcranial direct current brain stimulation (tDCS) is a novel method of neuromodulation that can be used in humans and non-human animals. Electricity is applied to a targeted area of the brain, making neurons in that part of the brain more likely to fire. In this study, we stimulated a self-control area of the brain called the right ventrolateral prefrontal cortex. 224 college students participated in this study. First, participants completed personality questionnaires. Next, the tDCS was attached to the head and turned on. Half of the participants received real brain stimulation. The other half of the participants received sham brain stimulation, which involved turning on the device but covertly turning it back off before it had

time to start working. While the tDCS was attached, participants completed depletion exercises meant to induce a state of mental fatigue. We hypothesized that the participants who got real brain stimulation would be less fatigued in a subsequent motivation task. After the brain stimulation, the tDCS was removed. Participants were asked how hard they planned to work on the next task on a scale of 1 to 7. Then, they completed a progressive ratio motivation task where they had to press the computer mouse hundreds of times to earn points. When participants only got the sham brain stimulation, how hard they planned to work on the progressive ratio task did not affect performance; everyone clicked the mouse about the same amount of time. This was different for the people who got brain stimulation. When they did not plan to work very hard on the progressive ratio task, they performed about the same as the people who only got the sham brain stimulation. However, when they did plan to work hard on the task, they were actually able to press the mouse significantly more on the task. These findings suggest that brain stimulation can help increase work output, but only in people who are actually motivated to work. Future directions for understanding tDCS will require work in non-human animals to better understand mechanisms of action with this device.

Disclosures: S. Bell: None. M.M. Yee: None.

Poster

329. Motivation: Neural Circuits II

Location: Halls A-C

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Topic: G.02. Motivation

Support: R15DA015351

Title: Pde-4 inhibitor, rolipram, partially reverses scopolamine-induces behavioral deficits

Authors: *I. M. WHITE¹, B. K. WARD², S. L. CASE², W. WHITE²

¹Psychology, ²Neurosci. Program, Psychology, Morehead State Univ., Morehead, KY

Abstract: Phosphodiesterase-4 (PDE-4) inhibitors have been implicated in enhancing memory and cognitive function, possibly by regulating amyloid protein and other cellular functions. Previously, we reported that scopolamine given in combination with prefrontal lesions severely impaired simple memory, and that stress augmented the behavioral deficits, outcomes resembling those seen in the aged and in Alzheimer's patients. This study examined the capacity of rolipram, a class of PDE-4 inhibitors, and of nicotine to reverse scopolamine-induced deficits in learning and motivation. Male Wistar rats were shaped to press a lever for a food reward, then were trained on a fixed ratio 20 (FR20), which required of the rats moderate motivation and sustained attention to procure food. Scopolamine (0.25 and 0.5mg) reliably impaired performance in a dose-dependent manner, without affecting consummatory behavior. Rolipram

(0.05mg) and nicotine (0.25mg) partially reversed the scopolamine-induced impairment by decreasing the first response latencies, but failed to affect the time taken to complete the task or to retrieve rewards. Results were similar following a combination of nicotine and scopolamine. Our findings suggest that the muscarinic receptor modulates learning and motivation and that a partial reversal by rolipram and nicotine may reflect common target sites of drug action.

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Poster

329. Motivation: Neural Circuits II

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Topic: G.02. Motivation

Support: Michigan State University Foundation

Title: Individual differences in palatable food consumption following GABA-mediated inhibition of the medial prefrontal cortex in female rats

Authors: *E. B. SINCLAIR¹, K. L. KLUMP², C. L. SISK³

¹Neurosci. Program, ²Dept. of Psychology, ³Psychology & Neurosci Prgm, Michigan State Univ., East Lansing, MI

Abstract: Binge eating (BE) is the core, maladaptive symptom that cuts across most sub-types of eating disorders in women, and is defined as the uncontrollable consumption of a large amount of highly palatable food (PF, high-fat/sugar) within a short period of time. To study the neural underpinnings of BE, we use an individual differences model of binge eating that identifies binge eating prone (BEP) and binge eating resistant (BER) rats based on consistently high and low intake of intermittently-presented PF (vanilla frosting), respectively. We previously found that Fos expression during PF consumption was higher in the medial prefrontal cortex (mPFC) of BEP vs. BER female rats, suggesting that mPFC regulation of PF consumption differs between the two behavioral phenotypes. Here we sought to determine the functional significance of the mPFC in binge eating proneness by examining the effect of GABA-mediated inhibition of mPFC neural activity on PF consumption. BEP and BER rats were identified from a group of 50 young adult female Sprague-Dawley rats following two weeks of feeding tests consisting of ad libitum chow and intermittent (4h/day, 3d/week) PF access. Thereafter, bilateral guide cannulae were implanted into the ventral mPFC, and after recovery, BEP (N=9) and BER (N=10) rats underwent additional feeding tests immediately following intra-mPFC infusions of saline, 15ng, or 30ng of the GABA-A receptor agonist muscimol. PF and chow intake after 1 and 4hrs of access were measured and rats were video-recorded for the first hour of access to quantify feeding behavior. One hr PF intake in BEPs increased significantly in response to 30ng of

muscimol vs. saline and 15ng muscimol, yet BERs showed only a small non-significant increase in 1hr PF intake after 15ng and 30ng muscimol vs. saline. Moreover, BEPs showed a substantial increase in 4hr PF intake in response to 30ng muscimol vs. saline (Cohen's d = 0.75), while 4hr intake in BERs was unaffected by muscimol. Quantification of feeding behavior revealed that 30ng muscimol significantly lengthened the average duration of individual episodes of PF consumption in BEPs and BERs as compared to saline. These findings suggest that the mPFC normally functions as a "brake" on PF intake, given that pharmacological inhibition of mPFC activity increased (i.e., disinhibited) PF intake. However, the magnitude of the behavioral change induced by mPFC inhibition (i.e., the amount of PF consumed) was larger in BEPs than in BERs, indicating that normal mPFC inhibition of PF intake is weaker in rats prone to binge eating. Thus, aberrant prefrontal cortical control over PF consumption may contribute to heightened risk for binge eating PFs in women.

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Poster

329. Motivation: Neural Circuits II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 329.25/PP29

Topic: G.02. Motivation

Title: The neural mechanism of interaction between intrinsic motivation and external incentives

Authors: *L. QIU

Beijing Normal Univ., Beijing, China

Abstract: Minimum uncertainty and maximum rewards are the goal of human beings. We usually make decisions with uncertainty, and are intrinsically motivated to reduce the internal uncertainty through metacognition. Previous studies have found that dorsal anterior cingulate cortex (dACC) and lateral prefrontal cortex (lPFC) play important roles in metacognition. The activity of ventral striatum (VS) encoding the intrinsic motion modulated the PFC activities in metacognition. Alternatively, metacognition could be driven by external incentives. However, the neural mechanism of interaction between intrinsic motivation and external incentives remains unclear. We here devised a novel "decision-redecision" paradigm to allow participants to make two sequential decisions on the same Sudoku problem. Prior to the secondary decision, the cues were presented to indicate different external incentives (positive, neural and negative) for the second correct answers. Behaviorally, the performance accuracy was not significantly improved under the positive incentives, but was significantly reduced under the negative incentives, in comparison to the neural condition. However, we found that the fMRI activities in the metacognition network gradually increased as the magnitudes of both positive and negative incentives incentives increased, indicating the participants tried hardly to avoid punishments under negative

incentives. Although the metacognition network activities increased in both groups, performance accuracy did not. Physiology-psychological interaction (PPI) analyses showed that VS had significant modulations on the metacognition network, and in turn, the VS activities were modulated by the dopamine neural activities in the midbrain (VTA/SN), which were separately sensitive to the positive and negative incentives. Alternatively, the dopamine neural activities sensitive to the positive incentives also projected to ventromedial PFC (vmPFC). In the participants who were sensitive to external incentives, the vmPFC activities gradually increased as the positive incentives increased, impaired the proper function in metacognition. Alternatively, the vmPFC activities were not altered for insensitive participants. Taken together, our findings show external incentives have both effects to modulate the neural activities in the metacognition network and the default-mode network, the former was driven by intrinsic motivation to improve performance, while the latter was driven by external rewards. Although everyone is motivated to better solve the problems, too sensitivity to the external rewards would paradoxically impair the performance.

Disclosures: L. Qiu: None.

Poster

329. Motivation: Neural Circuits II

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 329.26/QQ1

Topic: G.02. Motivation

Support: NIGMS Grant 1FI2GM117583-01

Title: Compulsive addiction-like aggressive behavior in mice

Authors: *S. A. GOLDEN, C. HEINS, M. VENNIRO, D. CAPRIOLI, M. ZHANG, D. H. EPSTEIN, Y. SHAHAM Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Background: Some people are highly motivated to seek aggressive encounters, and among those who have been incarcerated for such behavior, recidivism rates are high. These observations echo two core features of drug addiction: high motivation to seek addictive substances, despite adverse consequences, and high relapse rates. Here we used established rodent models of drug addiction to determine whether they would be sensitive to "addiction-like" features of aggression in CD-1 mice.

Methods: In Exp. 1-2, we trained older CD-1 mice to lever-press for opportunities to attack younger C57BL6/J mice. We then tested them for relapse to aggression seeking after forced abstinence or punishment-induced suppression of aggression self-administration. In Exp. 3, we trained a large cohort of CD-1 mice, and tested them for choice-based voluntary suppression of

aggression seeking, relapse to aggression seeking, progressive ratio responding, and punishmentinduced suppression of aggression self-administration. We then used cluster analysis to identify patterns of individual differences in compulsive "addiction-like" aggressive behavior.

Results: In Exp. 1-2, we observed strong motivation to acquire operant self-administration of opportunities to aggress, and relapse vulnerability during abstinence. In Exp. 3, cluster analysis of the aggression-related measures identified a subset of "addicted" mice (~19%) that exhibited intense operant-reinforced attack behavior, decreased likelihood to select an alternative reinforcer over aggression, heightened relapse vulnerability and progressive ratio responding, and resilience to punishment-induced suppression of aggressive behavior.

Conclusion: Using procedures established to model drug addiction, we showed that a subpopulation of CD-1 mice demonstrate "addiction-like" aggressive behavior, suggesting an evolutionary origin for pathological aggression.

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Poster

329. Motivation: Neural Circuits II

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Program#/Poster#: 329.27/QQ2

Topic: G.02. Motivation

Title: Between-person differences in sensation-seeking: Vulnerabilities for cigarette-smoking and opportunities for intervention

Authors: *D. M. LYDON¹, B. LUNA³, C. F. GEIER²

¹Human Develop. and Family Studies, The Pennsylvania State Univ., University Park, PA; ²The Pennsylvania State Univ., Unviersity Park, PA; ³Dept Psychiatry, Lab. Of Neurocognitive Develop., Pittsburgh, PA

Abstract: Brain-based models of adolescent risk-taking have highlighted the importance of sensation-seeking and its associated neural correlates in explaining vulnerabilities for engagement in risky behaviors, including cigarette smoking. In a series of three studies, we present findings providing insight into the association between sensation-seeking and risky behaviors. Using data from the National Longitudinal Study of Adolescence to Adult Health (Add Health), time-varying effect models (TVEM) were implemented to estimate how the associations between sensation-seeking and impulse control (predictors) and daily smoking (outcome) varied from adolescence into young adulthood (ages 12 to 34). Sensation-seeking was significantly associated with daily smoking during adolescence and in the late 20s and early 30s, although the association was strongest during adolescence. Using resting state data from 43

children, adolescents, and adults, we observed that sensation-seeking was negatively associated with between-network connectivity across salience and both the default mode and central executive networks - suggesting that sensation-seeking is associated with patterns of functional connectivity that have previously been associated with limitations in cognitive control. Finally, in the context of an incentivized, anti-saccade task, we show that, although sensation-seekers are vulnerable for engaging in risky behaviors (study 1) and show evidence for limitations in cognitive control (study 2), they exhibit more accurate anti-saccade performance and greater engagement of key ocolumotor control regions (e.g., frontal eye fields, supplementary eye fields) when motivated with monetary incentives relative to low sensation-seekers.

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Poster

329. Motivation: Neural Circuits II

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Topic: G.02. Motivation

Support: DGAPA Grant IN307417

Title: Effects of binge eating behavior on incentive motivation evaluated with two types of progressive ratio schedule

Authors: *W. ZEPEDA-RUIZ¹, N. V. VÁZQUEZ-HERRERA², D. N. VELAZQUEZ-MARTINEZ²

¹Univ. Nacional Autonoma De Mexico, Mexico, Mexico; ²Univ. Nacional Autónoma de México, Mexico City, Mexico

Abstract: Binge eating behavior is characterized by overconsumption of palatable food without energy deficit and changes in motivation to obtain palatable food. In our laboratory, we trained subjects in an exponential progressive ratio schedule; after binge eating induction with sucrose we did not found the changes in motivation previously reported. The main difference of our study was that we used an exponential progressive ration while other studies used arithmetic progressive ratio. Therefore, the main objective of the present work was: first, evaluate the sensibility of the reinforcement schedule through a replication of the results with haloperidol administration, and second, evaluate if the use exponential or arithmetical progressive ratio schedule may influence the changes in incentive motivation. Thirty-six male Wistar rats were divided in three groups: A) haloperidol, B) exponential and C) arithmetic progressive ratio schedule. Subjects of group A were previously trained in the exponential schedule and three doses of haloperidol (0.01, 0.03 and 0.1 mg/k i.p.) were evaluated after subjects reach stability criteria. Subjects of group B and C were trained in an exponential or arithmetic progressive ratio

schedule, respectively. When all of the subjects reach the stability criteria, binge eating induction started. During binge eating induction, subjects in groups B and C, were subdivided in two groups: control and experimental. In all groups access to water and standard food was *ad libitum*; in the control group access to sucrose was also *ad libitum* while the experimental groups had access to sucrose on Tuesday, Thursday and Saturdays two hours per day. When haloperidol was administrated, there was a decrease in the breakpoint as reported previously; this result suggest that the schedule was sensitive enough. However, we did not found changes in the breakpoint either with the exponential or arithmetic progressive ratio after binge eating induction. It is important to note that other groups reported changes on incentive motivation using vegetable shortening; also, there are differences between strains in the susceptibility to binge eating induction: Wistar rats are less prone to binge and this could have influenced the motivational changes observed since the other group used Sprague-Dawley rats.

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Poster

329. Motivation: Neural Circuits II

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Topic: G.02. Motivation

Support: NIDA R01: R01-DA-038599 (SBF)

NIDA T32 Training Program in Neuroscience Grant T32 DA 7281-21 A1 (SAL)

Title: Assessment of the corticosterone profile and anxiety-related behaviors in sign-trackers and goal-trackers

Authors: *S. A. LOPEZ¹, P. CAMPUS⁴, M. KLUMPNER², S. B. FLAGEL³ ¹Neurosci., ³Mol. and Behavioral Neurosci. Inst., ²Univ. of Michigan, Ann Arbor, MI; ⁴Psychiatry, Univ. of Michigan Dept. of Psychiatry, Ann Arbor, MI

Abstract: Learning to associate cues in the environment with appetitive and/or aversive stimuli allows organisms to develop a behavioral repertoire fit to obtain valuable resources (e.g. finding food and avoiding predators). Cues, however, can also gain inordinate control and lead to maladaptive behaviors (e.g. relapse in addicts). Using an animal model, we have shown that there is individual variation in the ability of reward cues to control behavior. Rats that undergo Pavlovian conditioned approach (PCA) training, consisting of cue (e.g. lever) presentation followed by the delivery of a food reward, develop either a sign- or goal-tracking conditioned response. For both sign-trackers (ST) and goal-trackers (GT) the cue attains predictive value, but

only for ST does the cue also attain incentive value. The attribution of incentive value to the cue transforms it into a "motivational magnet", rendering it attractive and desirable. The ST/GT model can therefore be exploited to investigate individual variation in the propensity to attribute incentive salience to reward cues and the underlying neurobiological mechanisms. Using this model, we have shown that different neural circuits regulate predictive vs. incentive learning, with the latter dependent on dopamine and subcortical mechanisms. Additionally, relative to GT, ST show greater impulsive action as well as other addiction-like behaviors, indicating the coexistence of other behavioral traits that might contribute to the individual variation in cuereward learning. Interestingly, relative to GT, ST also show higher levels of corticosterone (CORT), the major hormone mediating stress response, after a single session of PCA training. It remains to be determined, however, if preexisting or emerging differences in "stressresponsivity" affect the propensity to attribute incentive salience to reward cues. Here, we assessed whether ST and GT differ in stress-responsivity, using both behavioral and neuroendocrine measures. Currently, we have examined CORT profiles in ST and GT under baseline conditions and following PCA training and anxiety-related behavioral paradigms. There does not appear to be a relationship between the propensity to attribute incentive salience to reward cues and anxiety-related behaviors, based on performance on the elevated plus maze or locomotor response to novelty. However, CORT may play an important role in mediating individual differences in cue-reward learning. These data contribute to our understanding of stress responsivity in sign- and goal-tracking behaviors and prompt future investigations examining the interaction between CORT and dopamine during cue-reward learning.

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Poster

329. Motivation: Neural Circuits II

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Program#/Poster#: 329.30/QQ5

Topic: G.02. Motivation

Support: DGAPA Grant IN307417

Title: Effect of different intensities of food deprivation over the preference of sucrose and corn oil intake

Authors: *S. ORTEGA-TINOCO¹, N. V. VÁZQUEZ-HERRERA², W. A. ZEPEDA-RUIZ², D. N. VELAZQUEZ-MARTINEZ² ¹Psicofisiología, UNAM, Ciudad de México, Mexico; ²UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO, MEXICO CITY, Mexico **Abstract:** Food intake is regulated by two systems: homeostatic and hedonic. Homeostatic system makes reference to hormones, peptides and neurotransmitters that are released when subjects are hungry or satiated. On the other hand, the hedonic system is related to the incentive value of food and/or the stimulus related to it. It has been suggested that the incentive value integrates two elements: wanting and liking; wanting is related to the approach to food, effort to obtain it and the consumption sustained by food, while liking is related to the sensorial characteristics of the stimulus and represents the hedonic impact of it. Also, it has been suggested that the physiological state of the subject influences incentive motivation. Therefore, the aim of the present work was to evaluate the effect of different magnitudes of food deprivation in the incentive value (measured as the amount of intake) of sucrose and corn oil. Twenty-four male Wistar rats were employed. Subjects were trained in a two bottle choice test with saline solution and water for 10 days. Then, subjects had access for 30 minutes to sucrose (10%) and corn oil (4.8%, diluted with mineral oil) after 2 h or 22 h of food deprivation, it is important to note that concentrations were isocaloric and the order of deprivation hours varied semirandomly. Body weight, intake of both substances and standard food were registered. In either magnitude of food deprivation, subjects preferred sucrose over corn oil. The intake of sucrose under 22 h of food deprivation was higher than intake of 2 h; however the difference did not reach statistical significance. Corn oil intake was similar under both conditions of food deprivation. Our results (intake during 6 days per condition of food deprivation) are at odds to those of Sclafani (Physiol Behav 1992, 53:1091), who observed that rats had a greater intake of fat when they were food deprived but prefer sucrose when sated. Differences in results may be related to the higher concentrations of sucrose and corn oil used in our study. Also, the differences in rat strain may be relevant: CD-IGS rats are more prone to obesity compared to Wistar rats.

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Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 330.01/QQ6

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: JSPS KAKENHI Grant Number JP16K10231

Title: A sucrose preference test modified for evaluation of depression-like behavior in macaque monkeys

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Abstract: Depression is a serious mood disorder with a high prevalence in all developed countries. Because of their close proximity to humans, macaque monkeys have a potential to advance our understanding of the etiology and pathophysiology of major depression. However, there are few experimental paradigms suitable for investigating a depression-like sign of the monkey characterized by anhedonia. The sucrose preference test (SPT) is generally used for analysis of depressive behaviors in rodents, but no reports are so far available on the application to the monkey. In the present study, we made an attempt to improve the SPT for macaque monkeys to delineate a transient or fluctuating sign of anhedonia. For determining the sensitivity to a sucrose solution, nine levels of sucrose concentrations ranging 0 to 3.0% were tested in six monkeys. The result obtained from a concentration-response curve showed a critical preference at higher than 0.25% of the sucrose concentration level. Interestingly, all six monkeys displayed a high value of sucrose preference that is more than 99% when 3.0% sucrose solution was presented. In addition, sucrose consumption increased up to 1.5% in a concentration-dependent manner, and then reached a plateau. The most important issue in disease model research is what concentration of a sucrose solution should be used. If the concentration of 5% is too high, animals might still prefer it even if becoming anhedonia-like condition. Therefore, to establish the procedure for evaluating the effect of three sucrose concentrations (0, 0.25 and 1.0%) within a day, we subsequently examined their test sequence and confirmed that a 0.25-1.0-0% sequence was suitable for anhedonia-targeted analysis. Lipopolysaccharide (LPS) is known to induce neuroinflammatory processes and depression-like behaviors in rodents and also humans. To detect an anhedonia-like sign in LPS-treated monkeys, we administered LPS in seven monkeys and analyzed the sucrose consumption and preference using our improved SPT. Administration of LPS (0.1 and 0.3 mg/kg) resulted in the decreased sucrose consumption in all monkeys, and the decreased sucrose preference in two monkeys. Thus, our improved SPT for macaque monkeys may be possible to detect anhedonia, although further investigations are needed, with special reference to the species-specific response pattern and the dosage of LPS.

Disclosures: H. Yamanaka: None. N. Hosaka: None. M. Takada: None. H. Onoe: None.

Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

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VCU Massey Cancer Center

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Title: Molecular and behavioral mechanisms mediating paclitaxel-induced changes in affect-like behavior in mice

Authors: *J. A. MEADE, W. TOMA, Y. ALKHLAIF, D. E. SELLEY, M. I. DAMAJ Pharmacol. & Toxicology, Virginia Commonwealth Univ. Hlth. Syst., Richmond, VA

Abstract: A common finding in longitudinal studies of cancer survivors is long-term changes in mood, such as dysphoria and emotional deficit, including anhedonia. However, there is no efficacious treatment for chemotherapy-induced depression in survivors who had received paclitaxel, an antineoplastic commonly used in the treatment of breast, pelvic, head and neck, and non-small cell lung cancers. Unfortunately, paclitaxel-induced changes in affect can persist for up to five years or longer following completion of treatment. With the Baby Boomer population approaching peak cancer age, it is dire that the mechanisms behind paclitaxel-induced changes in mood are uncovered so as to improve the quality of life of the projected 19 million cancer survivors in 2024. Therefore, in order to study paclitaxel-induced reward deficit over an extended period, cancer-free male C57BL/6J mice were treated with one cycle of four injections of vehicle or paclitaxel (32mg/kg cumulative) and periodically assessed for anhedonia-like behaviors. Paclitaxel caused significant time-dependent deficits in sucrose preference and morphine-induced conditioned place preference (CPP). These initial results suggested an aversive-like state induced by paclitaxel. We therefore investigated kappa opioid receptor (KOR) signaling as a putative mechanism of paclitaxel-induced anhedonia. The selective KOR antagonist norbinaltorphimine (norBNI) reversed paclitaxel-induced sucrose preference deficit. Because KOR signaling in the nucleus accumbens (NAc) can cause anhedonia via modulation of dopamine (DA) signaling, we used the $[^{35}S]GTP\gamma S$ assay to measure KOR and DA D₂ receptor (D₂R) function in the NAc from paclitaxel-treated mice. Surprisingly, a history of paclitaxel had a trend of reducing both U50,488H and quinelorane -stimulated binding, suggesting decreased function of KOR and D₂R, respectively. Our data suggest that paclitaxel-induced changes in affect-like behavior may be due to time-dependent dysregulation of KOR and D₂R signaling in the limbic system.

Disclosures: J.A. Meade: None. W. Toma: None. Y. Alkhlaif: None. D.E. Selley: None. M.I. Damaj: None.

Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NSERC DG RGPIN-2011-222912

NSERC DG RGPIN-2015-04276

Title: Repeated corticosterone alters membrane protein clustering in lymphocytes along the lines seen in patients with depression

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Abstract: Depression is a serious psychiatric disorder affecting about 16% of the population. In previous studies, we suggested that the pattern of serotonin transporter clustering on cell membranes from peripheral lymphocytes could be a biomarker for diagnosis and treatment responsivity in depression patients. Here we evaluated whether similar alterations are seen in lymphocytes collected from rats subjected to repeated corticosterone (CORT) injections - a well-characterized animal model of depression - and whether altered protein clustering in CORT-treated rats, correlates with measures of depression-like behavior.

Long-Evans male rats received a single daily CORT injection (40 mg/kg) for 21 consecutive days, whereas control rats received vehicle injections. On day 22, depression-like behavior was assessed using the forced swim test and blood samples were drawn by cardiac puncture. Lymphocytes were isolated using the Ficoll-Paque gradient. A panel of membrane proteins were analysed in collected lymphocytes using immunohistochemistry: the serotonin transporter (SERT), serotonin 2A receptor (5-HT2A), beta-2 adrenergic receptor (β -2AR), cellular prion protein (PrPc), N-Methyl-D-Aspartate receptor subunit 2B (NR2B), and dopamine transporter (DAT). After immunolabeling, we measured the size and number of membrane protein clusters in lymphocytes using a computerized image analysis system.

Our results indicated that CORT had significant effects on the size and number of membrane protein clusters for several of the proteins included in the experiment. For example, there was an increase of 13%, 6%, 12%, 11%, and 7% in the size of SERT, 5HT2A, PrPc, NR2B, and DAT protein clusters, respectively. However, we noted a 7% decrease in β -2AR cluster size with CORT treatment. No significant group differences were detected in the number of protein clusters, except for clusters of PrPc proteins, which decreased by 20% after CORT treatment. Importantly, CORT-induced alterations in membrane protein clustering for the SERT, PrPc, NR2B, and DAT were significantly correlated with increased immobility in the forced swim test. These results largely parallel previous findings in depression patients, particularly with respect to the SERT and 5HT2A clusters. In addition, we have identified changes in the clustering of additional proteins (β -2AR, PrPc, NR2B, and DAT) that were not previously studied in human patients. Our next step will be to determine whether these addition proteins are also altered in a patient population.

Disclosures: R. Romay-Tallon: None. **E.Y. Fenton:** None. **M.A. Mitchell:** None. **L.E. Kalynchuk:** None. **H.J. Caruncho:** None.

Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Italian Ministry of University and Research (PRIN – grant number 2015SKN9YT)

Fondazione CARIPLO (grant number 2012-0503)

Title: Long-lasting behavioral & molecular alterations following social isolation during adolescence in rats

Authors: V. BEGNI, L. LONGO, S. ZAMPAR, *M. A. RIVA Dept Pharmacol. and Biomolecular Sci., Univ. of Milan, Milano, Italy

Abstract: The exposure to adverse events early in life is associated with long-lasting neurochemical, structural and behavioural changes that may enhance the vulnerability to mental disorders, such as major depression. The pathologic outcome may depend upon the timing and duration of the adverse experience, possibly due to the differential impact on specific brain regions and circuits. In the present study we aimed to investigate the effects of stress exposure during adolescence, a critical developmental period characterized by intensive neural and behavioural changes and thus highly vulnerable to adverse events. We used social isolation, a well-established paradigm of stress for adolescent rats, in order to investigate the long lasting effects of this manipulation. 60 male and 60 female pups were weaned at PND21 and housed either in group or individually until PND49, followed by re-socialization in groups of 3 rats per cage until adulthood. Animals underwent behavioural tests at PND70-80 and they were killed two weeks later.

In the sucrose preference test, aimed to investigate the presence of a depressive like phenotype, isolated rats, both males and females, show a reduction of sucrose preference, as compared to their group-housed counterpart (p<0.05). However the cognitive performance of isolated rats was not different from control rats, as revealed in the novel object recognition test (p>0.05). The results of the molecular analyses performed in the prefrontal cortex and dorsal hippocampus revealed that social isolation produced a significant reduction of *Bdnf* mRNA levels in female, but not in male, rats (p<0.05). In order to investigate if the stressful experience during adolescence may alter the ability to cope under a challenging condition, a group of rats (control or isolated) were exposed to a 1h-session of immobilization stress immediately before killing. We found that while acute stress produced a significant up-regulation of the activity regulated

genes *Arc* and *Zif 268* (p<0.05) in the prefrontal cortex of group housed as well as of isolated rats, the stress-induced transcriptional profile of other genes was significantly affected as a consequence of isolation rearing during adolescence.

In summary, our results demonstrate that stress exposure during adolescence produce a depressive like phenotype, which is associated with a reduction in the expression of the neuroplastic marker BDNF. Future and on-going analyses will try to establish the pattern of transcriptional and translational changes that may sustain the behavioural impairment, with the aim to delineate sex and anatomical specificity as a consequence of the adverse experience early in life.

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Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

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R01NS088627

Rutgers BHI-RUN-NJIT Grant Program

Title: Cognitive, emotional and microglial assessments of mice bearing mutations of prepro orphanin FQ/nociceptin (OFQ/N) or its receptor, ORL-1, maintained on C57BL/6 and 129S6 mouse strains: Applicability to chronic stress paradigms of resilience

Authors: *A. W. KUSNECOV¹, J. E. PINTAR³, E. FRILING¹, E. B. UKPONG^{2,4}, L.-J. WU^{2,4}, B. PENG³, M. ANSONOFF³, R. GLASS¹ ¹Psychology, ²Cell Biol. and Neurosci., Rutgers Univ., Piscataway, NJ; ³Dept Neurosci & Cell Biol, Rutgers Robert Wood Johnson Med. Sch., Piscataway, NJ; ⁴Neurol., Mayo Clin., Rochester, MN

Abstract: The neuropeptide OFQ/N and its cognate receptor (NOP or ORL-1) have been linked to cognitive, emotional and motivational behaviors, as well as neuroinflammation. Human studies have linked the OFQ/N system to anxiety and depression, which is also associated with inflammatory cytokines. Further, animal models point to an anxiolytic role for OFQ/N, although full consensus has yet to be reached. Here we behaviorally compare prepro OFQ/N and ORL-1 mutations maintained on different background mouse strains (C57BL6/J or 129S6) to test the potential role of OFQ/N or ORL-1 as a resilience mechanism - and a possible anti-inflammatory influence - during chronic stress. Transgenic mice were compared to wild type C57/BL6 or 129S6 mice from the same breeding colonies. Male and female mice of at least 2-3 months of age (N was at least 5/group) were subjected to food-motivated T-maze learning and the elevated plus maze (EPM). For T-Maze learning, mice were trained to locate condensed milk in each of two opposing arms. Subsequent to habituation, alternation was tested for 5 days (4 trials/day). Percent correct choices (choosing the alternate arm, after visiting the baited opposite arm) were calculated for each day, and analyzed by ANOVA. Irrespective of genotype, learning was superior in mice of the C57BL/6 background (p <0.001), with the 129S6 mice displaying poor learning. Despite the superior C57 strain learning, ORL mutation delayed acquisition in male mice compared to the C57 WT mice (Day x Genotype, p = 0.054). Females and the ppOFQ/N KO mice are currently being tested. Among the 129S6 strain, while poor learners overall, the 129/ORL and 129/ppOFQ/N mutant mice were significantly worse (p < 0.05). These genotype effects were due mainly to ORL deletion. EPM: Mice were tested twice on days 1 and 4. Percent time in the open arms did not differ between strains (p<0.001), but ORL mutations in C57 mice did reduce percent open time (p < 0.001 compared to C57/wt), supporting an anxiolytic role for ORL-1. The ORL mutation in 129S6 mice is currently being tested for EPM. Microglial Activity: C57 WT or C57/ORL KO mice were crossed with CX3CR1GFP/+ mice to study microglial activity by 2-photon live imaging subsequent to acute or chronic stress exposure. Preliminary data suggest that basal microglial activity in CX3CR1GFP/+ ORL ko mice does not differ from heterozygous or wildtype CX3CR1GFP/+ mice. Conclusion: Further studies are ongoing to determine the potential protective benefits of ORL signaling on T-maze learning after chronic stress using the C57 background. Moreover, the role of ORL in modulating microglial activity during chronic stress is being investigated.

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Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

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Program#/Poster#: 330.06/QQ11

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders
Support: CNPq 159503/2013-2

FAPESP 2013/18897-7 FAPESP 2015/08098-5 FAPESP 2016/19824-1 FAPESP 2015/20789-3

Title: Mapping brain activity after CMS protocol through cytochrome c oxidase histochemistry

Authors: *K. S. HOMEM¹, A. DE TOLEDO RAMOS², C. M. DOMINGUEZ³, M. M. SOARES³, C. SCAVONE², L. R. P. TRONCONE³ ¹Univ. of São Paulo - IPUSP, Sao Paulo, Brazil; ²Pharmacol., Univ. of Sao Paulo - Inst. of Biomed. Sci., Sao Paulo, Brazil; ³Pharmacol., Inst. Butantan, São Paulo, Brazil

Abstract: Previous studies developed in this lab described relevant differences among types of stressors and HPA axis response. Stressors induce remarkable ACTH secretion, but vasopressin 1b antagonists can inhibit physical stressors like ether vapor inhalation while CRH1 antagonists inhibit psychological stressors such as restraint. Mixed stressor such as forced swimming only had its effects blocked by concomitant use of both antagonists. Continuous Mild Stress (CMS) is an important animal model of depression in which anhedonia is measured by sucrose preference. This model demands a long-term (2-3 weeks) exposure to a variety of stresses and sometimes the protocol results unsuccessful. We hypothesize that different stress modalities, physical or psychological, may be more effective in inducing anhedonia. Brain mapping of metabolic changes induced by stresses may offer clues to the understanding of the ongoing processes. Cytochrome c oxidase activity reflects long-term trends in energy demands allowing for an effective mapping of long-term changes in regional brain activity. We used 3 groups of 6 Wistar rats each that underwent CMS protocols: a control non stressed group (CON), a physical stress group (PHY) and a psychological stress group (PSY) for two weeks. All animals were then decapitated and brains were frozen at -80°C and later sliced at 30 µm in a cryostat. Slices were stained for cytochrome oxidase activity mapping by the DAB/cytochrome c standard method. Regions of interest were marked based on Watson&Paxinos rat brain atlas and measured by optical densitometry. Preliminary results are the following, always in comparison to CON: in PHY, areas that had statistically significant increase (1-way ANOVA) were lateral periaquedutal gray (LPAG), 6%; medial preoptic area (MPA), 9%; anterior paraventricular hypothalamic nucleus (PaAP), 8%; ventral paraventricular hypothalamic nucleus (PaV), 3%; ventral bed nucleus of stria terminalis (STLV), 10%; medial anterior bed nucleus of stria terminalis (STMA), 6,5%; medial ventral bed nucleus of stria terminalis (STMV), 11%. In PSY, the areas were LPAG (4%), MPA (5%), PaV (3%), STLV (4%), STMA (6%) and STMV (4%). There were no significant differences in cytochrome c oxidase activity on the other areas examined (anterior and posterior basolateral amygdaloid nuclei, anterior and posterior basomedial amygdaloid nuclei, central amygdaloid nucleus, dorsomedial periaquedutal gray, substancia nigra compacta dorsal, lateral and reticular). These results are in agreement with expected results for stressed animals. A full comparative approach is envisaged.

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Poster

330. Animal Models for Affective Disorders: Mechanisms I

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH Grant R01 MH112716

NARSAD YI 24877

Title: Cell-type specific SIRT1 signaling pathways in the nucleus accumbens modulate depression-like behaviors

Authors: *H.-D. KIM, T. CALL, S. CAROTENUTO, R. JOHNSON, M. TANG, D. FERGUSON Basic Med. Sci., The Univ. of Arizona Col. of Med., Phoenix, AZ

Abstract: Major depressive disorder (MDD) is a significant cause of disability and 20% of individuals will suffer from a major onset of clinical depression during their lifetime. Despite the prevalence and considerable impact of depression, its pathophysiology is still elusive. The nucleus accumbens (NAc) in the reward circuitry is a key region which integrates rewarding and aversive information from various brain regions, and thus thought to contribute to the pathophysiology and symptomatology of depression. GABAergic medium spiny neurons (MSNs) are major projection cells in the NAc and two different subtypes expressing D1- or D2type dopamine receptors seem to differentially modulate depression-like behaviors. Recently, we demonstrated that increase of Sirt1, a well-known deacetylase, in D1-MSNs, but not in D2, significantly promotes anxiety- and depression-like behaviors. To further elucidate downstream regulation mechanisms of SIRT1, we performed cell-type specific transcriptomics using MSNssubtype specific Sirt1 knock-out mice expressing RiboTag. Based on our unbiased analysis of transcriptome data, Sirt1 signaling pathways modulate various neuronal physiologies in the MSNs and seem to correspond with electrophysiological and morphological changes of MSNs subpopulations in depressed mice. These evidences demonstrate that Sirt1 signaling differentially regulates depression-like behaviors in a cell-type specific manner, implicating novel antidepressant targets.

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Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: The effects of medial prefrontal cortex inhibition on symptoms of depression in long evans rats

Authors: *J. J. CORTRIGHT, A. MILLER, B. PODGORSEK, A. BUTTERBRODT, A. WILLARD

Psychology, Univ. of Wisconsin River Falls, River Falls, WI

Abstract: Self-focus (i.e. the process by which one engages in self-referential processing) is a core issue in the psychopathology of major depression, which affects 350 million people worldwide. Previous studies have used functional neuroimaging to identify that the cortical midline structures, including the medial prefrontal cortex (mPFC), play a key role in selfreferential processing in depressed subjects. The current study investigates the hypothesized link between the mPFC and depression using an animal model of learned helplessness to measure self-referential processing. It is hypothesized that a decrease in symptoms of depression will be seen in animals that have undergone inhibition of the mPFC. This research holds significance in that it builds on previous studies, with conflicting results, that have aimed to link specific patterns of activity to the PFC as mediating symptoms of depression. Further examination of the mPFC is therefore warranted not only as a possible precursor to the implication of its involvement in mediating depression, but also in order to provide support for a theory of dominant pattern of brain activity (inhibition) which interacts with symptoms of depression. The current study utilized female Long Evans rats in order to more accurately generalize findings to the population of women, which make up the majority of depressed individuals in humans. Subjects were exposed to 28 days of randomized stressors during adulthood including forced swim, cage tilt, wet bedding, mild restraint and restriction of food and water. Control animals were housed in pairs, while animals exposed to randomized stress were housed in isolation. Following surgical placement of guide cannula animals were either infused with an inhibitory cocktail (0.3 nmol/0.5µl/side baclofen/0.3 nmol/0.5µl/side muscimol) or sham (artificial cerebrospinal fluid) before being tested for resiliency against learned helplessness. Subjects were tested for latency in a forced swim test and hot plate test, for motivation in a radial arm maze, for lethargy in an open field test, and for anhedonia using sugar pellets. To date, attenuation of learned helplessness symptoms have been found in stress-exposed animals which had undergone inhibition of the mPFC (having also had their self-referential processes inhibited) compared to controls. Collectively, these findings hold significance in that they build on recent research that has aimed to link areas of the PFC to symptoms of depression.

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Poster

330. Animal Models for Affective Disorders: Mechanisms I

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Assessing the antidepressant-like effects of the (R) and (S) isomers of the atypical antipsychotic amisulpride in C57BL/6 mice

Authors: *R. RICE¹, D. SMITH¹, H. NANGUNURI¹, S. RAMAN¹, C. FAIR¹, M. A. FRIAR², T. M. HILLHOUSE³, T. J. DONAHUE¹, J. H. PORTER¹ ¹Psychology, Virginia Commonwealth Univ., Richmond, VA; ²Psychology, American Univ., Washington, DC; ³Psychology & Neurosci., Weber State Univ., Ogden, UT

Abstract: The atypical antidepressant amisulpride is used at low doses for the treatment of mild, chronic depression (dysthymia). Unlike most atypical antipsychotics, it exhibits a selective binding profile displaying high binding affinity to dopamine D_{2/3} receptors and serotonin 5-HT₇ and 5-HT_{2B} receptors. Amisulpride is comprised of a mixture of two optical isomers: (S)amisulpride and (*R*)-amisulpride. The enantiomers bind at dopamine $D_{2/3}$ and α_2 -adrenoceptors in a stereoselective manner with marked differences in affinity: (S)-amisulpride displays high affinity binding at $D_{2/3}$ receptors and is approximately twice as potent as racemic amisulpride and 20-50 times more potent than (R)-amisulpride at these receptors. To assess the antidepressant-like activity of amisulpride and its enantiomers, adult C57BL/6 mice were tested in the differential-reinforcement-of low rate (DRL) 72 sec operant procedure and the Porsolt forced swim test (FST). Amisulpride produced an antidepressant-like profile in the DRL task producing significant increases in reinforcers at 1.78, 32, and 56 mg/kg doses and a significant decrease in responding at 56 mg/kg. In contrast, amisulpride did not produce a significant decrease in immobility in the FST at 0.1, 1.0, or 10 mg/kg. Testing with the (R)- and (S)amisulpride isomers in the DRL task revealed that (S)-amisulpride significantly increased reinforcers at 10 mg/kg without any changes in responses. The (R)-amisulpride isomer did not produce any significant changes in reinforcers or responses. The tricyclic antidepressant imipramine and the NMDA noncompetitive antagonist ketamine served as positive controls and produced antidepressant-like profiles in the DRL procedure and FST. Additional testing is being conducted with the amisulpride isomers in the FST and with ketamine isomers in both tasks. These results demonstrate the importance of using multiple behavioral tasks for assessing the antidepressant-like profile of drugs, as different results may be obtained.

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Poster

330. Animal Models for Affective Disorders: Mechanisms I

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Title: Exploring differences in gene expression and relative cell-type balance in the hippocampus of a selectively bred rat model for internalizing and externalizing psychiatric disorders

Authors: *I. BIRT¹, M. HAGENAUER¹, C. AYDIN¹, P. BLANDINO, Jr.¹, R. THOMPSON¹, S. M. CLINTON², J. STEAD³, H. AKIL¹, S. J. WATSON, Jr.¹ ¹Univ. of Michigan, Ann Arbor, MI; ²Neurosci., Virginia Tech. Univ., Blacksburg, VA; ³Dept. of Neurosci., Carleton Univ., Ottawa, ON, Canada

Abstract: For many years our laboratory has been exploring hippocampal gene expression differences between selectively-bred high responder (HR) and low responder (LR) rats in order to provide insight into the biology underlying mood and temperament. In total, as part of different experiments run across 53 generations of selective breeding, we have collected eight different microarray or RNA-Seq datasets from samples of the whole hippocampus of HR and LR rats. Recently, we ran a formal meta-analysis to determine which molecular candidates are indicated repeatedly across these different datasets. Using the statistical program R, we reanalyzed six separate HR/LR datasets, and also used the results from previous analyses of an additional two datasets. We included datasets from studies that observed gene expression across

several developmental age groups: P7, P14, P21, and adult. In addition, there was a special focus on observing differences in relative cell type expression across phenotype through the use of a cell type-based matrix deconvolution. For the comprehensive analysis, a meta-analysis of effect sizes using a random effects model was performed on both adult and developmental data. Results indicate major differences in the expression of genes with known relationships to various mood disorders, as well as potential differences in immune activation in the LR rats, supporting the theory of altered immune function in the pathophysiology of internalizing disorders.

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Poster

330. Animal Models for Affective Disorders: Mechanisms I

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIMH (MH072672)

Veterans Administration Merit Award 1I01BX003512

William and Ella Owens Medical Research

Title: Fear extinction as a model of exposure therapy in rats: Developing a sub-maximally effective fear extinction procedure to test adjunct pharmacotherapies

Authors: *D. PAREDES, *D. PAREDES, E. A. FUCICH, D. A. MORILAK Pharmacol. and Ctr. for Biomed. Neurosci., Univ. of Texas Hlth. Sci. Ctr. San Anto, San Antonio, TX

Abstract: Adjunct treatment combining psychotherapy and pharmacotherapy can improve symptoms of stress-related psychiatric disorders such as depression and PTSD, and can increase remission rates in patients. Exposure therapy, a form of Cognitive Behavioral Therapy, shares many similarities to fear extinction learning. Our lab has previously shown that fear extinction (i.e. learning that an innocuous cue previously associated with a fearful stimulus no longer predicts that stimulus) can be used to model the effects of exposure therapy on cognitive flexibility that has been compromised in chronically stressed rats. Thus, we will use this model of exposure therapy to investigate the mechanisms underlying adjunct treatment, with the goal of enhancing the efficacy of exposure therapy. The aim of these experiments is to investigate variations of vary the parameters of our standard extinction protocol to produce a sub-maximal therapeutic effect on cognitive flexibility and coping style after chronic stress. In these experiments, we varied two parameters, the strength of initial conditioning and the number of trials in the extinction session. Animals were first fear conditioned by 4 pairings of a tone (20 sec) coinciding with a mild footshock (0.5 sec), varying the shock intensity (0.6, 0.8 or 1.0 mA) to vary the strength of conditioning. They were then subject to 2 weeks Chronic Unpredictable Stress. For extinction treatment, in a different context they received either 8 or 16 tone exposures without shock. Cognitive flexibility, measured using the attentional set-shifting test, or coping style, measured on the shock probe defensive burying test, were assessed 24 hr after extinction. We hypothesized that exposing rats to only 8 tones may constitute sub-maximal extinction, and diminish the therapeutic effects of extinction on stress-compromised behavior. Reducing the shock intensity during fear conditioning may also weaken the fear memory, thereby reducing the magnitude of extinction learning and its subsequent therapeutic effect. Alternatively, increasing the shock intensity during conditioning may make it more difficult to achieve full extinction. Preliminary data suggest that decreasing tone exposure from 16 tones to 8 decreased the effect of fear extinction on coping behavior. Experiments are ongoing to determine how varying the shock intensity affects the therapeutic effect of fear extinction.

Disclosures: D. Paredes: None. **E.A. Fucich:** None. **D.A. Morilak:** A. Employment/Salary (full or part-time):; UT Health Science Center at San Antonio, Center for Biomedical Sciences, South Texas Veterans Health Care System, San Antonio TX.

Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 330.12/QQ17

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NRF Grant 2016R1A6A3A11932526, 2016R1A2B2009063, 2016M3A9D5A01952414

DGIST R&D Program of the Ministry of Science, ICT & Future Planning Grant 17-BD-06, 17-BT-02

Title: Psychiatric effect of MCFA via odorant receptor

Authors: D. KIM¹, J. KIM¹, N. KANG², N. LEE¹, Y. JAE¹, *J. KOO² ¹Dept. of Brain & Cognitive Sciences, DGIST, Daegu, Korea, Republic of; ²Dept. of New Biology, DGIST, Daegu, Korea, Republic of

Abstract: Psychiatric regulations through fatty acid receptors (FFARs) have been extensively studied in a variety of metabolic tissues, including gut and cranial nerve endings. Recent studies on the gut-brain axis support a close relationship between FFAR and the hypothalamic-pituitary-adrenal (HPA) axis, a neuroendocrine system that regulates stress response and mood. However,

the expression and functional studies of FFARs in the HPA axis are poorly understood. Here, we present a specific odorant receptor (OR) that is expressed in pituitary gland responding to medium-chain fatty acid (MCFA). The histological location and cell type were also identified by immunohistochemistry, and it was revealed that OR (+) cells were located in the anterior lobe of the pituitary gland, co-localizing with ACTH. The blood ACTH and corticosterone level were measured after intraperitoneal injection of medium-chain fatty acid (MCFA) followed by acute restraint stress. Interestingly, MCFA injection showed an inhibitory effect on both ACTH and corticosterone releases. Furthermore, anxiety and depression behaviors were assessed under chronic restraint stress condition through MCFA treatment, and decreased anxiety and depression behaviors. Finally, the generation of specific OR-KO mice exhibited abnormal HPA axis activity as well as depression- and anxiety-related behaviors. In conclusion, our study has demonstrated the potential role of OR response to stress through the HPA axis, suggesting that OR may be an important therapeutic target for mood disorders

Disclosures: D. Kim: None. J. Kim: None. N. Kang: None. N. Lee: None. Y. Jae: None. J. Koo: None.

Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NRF-2017R1A2B3011098

The Brain Korea 21+ Program

Title: Excitatory/inhibitory synaptic imbalance of hippcampus in mouse learned helplessness

Authors: *G.-E. CHANG, D. LEE, J. KIM, H. LEE, G. KIM, G. HA, E. CHEONG Yonsei Univ., Seoul, Korea, Republic of

Abstract: Major Depressive Disorder (MDD) is one of mental disorders characterized by disturbed symptoms in emotional states, cognitive functions and physical conditions. Alteration of excitatory and inhibitory (E/I) balance in diverse brain regions such as Nucleus Accumbens (NAc), Ventral Tegmental Area (VTA) and medial Prefrontal Cortex (mPFC) has been reported as a conserved pathophysiological brain structures in depressed patients. However, precise correlation between depressive-like behavior and E/I imbalance is still not well-understood. In present study, we used learned helplessness model from adult male C57BL/6J mice by exposing to a set of random electrical foot shock in inescapable chamber. Shocked-mice were tested to evaluate having negative coping strategies and depressive-like behavior using various behavior

tests. By using whole cell patch clamp and immunohistochemistry, we observed changes of GABAergic synaptic inputs and neuronal activity within hippocampus in learned helplessness mouse. These findings suggest that GABAergic synaptic dysfunction of hippocampus is strongly related to pathophysiology of major depressive disorder.

Disclosures: G. Chang: None. D. Lee: None. J. Kim: None. H. Lee: None. G. Kim: None. G. Ha: None. E. Cheong: None.

Poster

330. Animal Models for Affective Disorders: Mechanisms I

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Program#/Poster#: 330.14/QQ19

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Beca CONICYT Gastos Operacionales 21130549

Beca CONICYT Gastos Operacionales 21141115.

Title: Role of CaV1-2 calcium channel in hippocampal neurons of animals with depressive likebehaviors

Authors: *C. MORENO NARANJO^{1,2}, P. HARDY¹, T. HERMOSILLA², D. VARELA², P. ROJAS¹

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Abstract: Major depression (MD) is the most common mood disorder worldwide, and a serious public health problem in most countries. The biological study of MD has partly elucidated the development of this condition. Hippocampal neurons from patients with MD show a reduced dendritic arborization and dendritic spines, nevertheless, the molecular mechanisms responsible for these changes are still to be defined. Cav1.2 calcium channels are the principal pathway for calcium influx in neuronal soma, and have been associated to several cellular processes such as changes in neuronal morphology and excitation-transcription coupling. Our work is centered in studying the role of Cav1.2 in the changes in morphology and function at hippocampal neuron in animals subjected to chronic restraint stress (CRS), an animal model of depression. Behavioral parameters such as anhedonia, reduction in social interaction and behavioral despair, demonstrated that these animals share characteristics of MD as has been reported for this model previously. The expression level of Cav1.2 in whole hippocampal samples were found significantly increased in CRS animals. This correlates with a higher current density observed in CRS neurons compared to control, suggesting an alteration in intracellular calcium handling and maybe in excitation-transcription coupling. By using immunolocalization techniques we are

currently studying the changes in signaling cascades related to L-type calcium channels to test the hypothesis that they are key playerers in the development of depression.

Disclosures: C. Moreno Naranjo: None. P. Hardy: None. T. Hermosilla: None. D. Varela: None. P. Rojas: None.

Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

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Program#/Poster#: 330.15/QQ20

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Modulation of adult neural plasticity sets behavioural response in an animal model of recurrent depression

Authors: *N. D. ALVES, J. CORREIA, P. PATRÍCIO, A. MATEUS-PINHEIRO, A. R. MACHADO-SANTOS, E. LOUREIRO-CAMPOS, M. MORAIS, J. BESSA, N. SOUSA, L. PINTO

Life and Hlth. Sci. Res. Inst. (ICVS), Braga, Portugal

Abstract: Depression is a highly prevalent and recurrent neuropsychiatric disorder associated with alterations in emotional and cognitive domains. Neuroplastic phenomena are increasingly considered central to the etiopathogenesis of and recovery from depression. Nevertheless, a high number of remitted patients experience recurrent episodes of depression, remaining unclear how previous episodes impact on behavior and neuroplasticity and/or whether modulation of neuroplasticity is important to prevent recurrent depression. Through re-exposure to an unpredictable chronic mild stress protocol in rats, we observed the re-appearance of emotional and cognitive deficits. Furthermore, treatment with the antidepressants fluoxetine and imipramine were effective to promote sustained reversion of a depressive-like phenotype, however their differential impact on adult hippocampal neuroplasticity triggered a distinct response to stress re-exposure: while imipramine re-established hippocampal neurogenesis and neuronal dendritic arborization contributing to resilience to recurrent depressive-like behavior, stress re-exposure in fluoxetine-treated animals resulted in an overproduction of adult-born neurons along with neuronal atrophy, accounting for an increased susceptibility to recurrent behavioral changes typical of depression. Strikingly, cell proliferation arrest compromised the behavior resilience induced by imipramine and buffered the susceptibility to recurrent behavioral changes promoted by fluoxetine. This study shows that previous exposure to a depressive-like episode impacts on the behavioral and neuroanatomical changes triggered by subsequent reexposure to similar experimental conditions and reveals that the proper control of adult hippocampal neuroplasticity triggered by antidepressants is essential to counteract recurrent depressive-like episodes.

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Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 330.16/QQ21

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Wistar Kyoto (WKY) rats exhibit memory deficits in addition to exaggerated depressionlike behavior

Authors: *Y. LI, D. EYERMAN, C. SANCHEZ Biol., Alkermes, Inc., Waltham, MA

Abstract: Wistar Kyoto (WKY) rats exhibit increased depression-like behavior and anxiety-like behavior, as well as neurochemical changes in serotonin, glutamate, and gamma-aminobutyric acid (GABA) systems. As these neurotransmitters are involved in diverse cognitive processes, we assessed adult male WKY rats in different memory tasks and in a test for depression-like behavior. Adult male Sprague-Dawley (SD) rats were included in the study as comparators. In the object placement test, WKY rats exhibited an impaired visuospatial memory, while SD rats exhibited an intact memory. This deficit in WKY rats was not accompanied by any reduction in object exploration. In the Y-maze test for spatial working memory, WKY rats made significantly fewer spontaneous alternations than SD rats. However, the performance of WKY rats in the novel object recognition test was intact, similar to that of SD rats. Consistent with previous studies, immobility in the forced swim test was increased in WKY rats compared to SD rats. In summary, WKY rats exhibited deficits in hippocampal-dependent memories and an exaggerated depression-like behavior. Therefore, we hypothesize that WKY rats may be considered as a rodent model for depression comorbid with memory deficits.

Disclosures: Y. Li: A. Employment/Salary (full or part-time):; Alkermes, Inc. D. Eyerman: A. Employment/Salary (full or part-time):; Alkermes, Inc. C. Sanchez: A. Employment/Salary (full or part-time):; Alkermes, Inc..

Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 330.17/QQ22

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Harvard Seminal Grant

Title: Role of ankyrin 3 in bipolar disorder

Authors: *X. QI, T. PETRYSHEN

Ctr. for Genomic Med., Massachusetts Gen. Hosp., Boston, MA

Abstract: Background The Ankyrin 3 gene (ANK3) has been repeatedly implicated by genome-wide association studies as one of the strongest risk genes for bipolar disorder (BD). However, the functional genetic variants in ANK3 have not been identified, and its role in BD is unknown. Our recent RNA sequencing and proteomic studies indicate that Ank3 reduction induces neuronal cytoskeletal instability of microtubules, suggesting that Ank3 may contribute to BD via disturbing microtubule function. The present study therefore examined the role of Ank3 in microtubule functions in mouse, primary neuron, and cellular models.

Method Expression of the microtubule plus-end binding protein 3 (EB3), a marker of microtubule stability, was measured in hippocampus of Ank3 +/+ and Ank3 +/- mice with western blot. The dynamic polymerization and depolymerization of microtubules was directly monitored by tracking the GFP-tagged EB3 puncta movement in primary mouse neuronal axons from Ank3+/- and Ank3+/+ mice using time-lapse live-cell confocal imaging. Activity of the lithium target glycogen synthase kinase 3 (GSK3) and its microtubule-associated substrates collapsin response mediator protein 2 (CRMP-2) and Tau, were measured by western blot in lithium- or vehicle-treated Neuro 2A cells in which brain-specific Ank3 isoforms were suppressed by CRISPR/Cas9 transcriptional repression.

Results EB3 expression was increased in the hippocampus of Ank3+/- mice compared to Ank3+/+ littermates (p<0.05), indicating instability of microtubules. Time-lapse video analysis demonstrated that, compared to Ank3+/+ axons, Ank3+/- axons have fewer anterograde and more retrograde moving EB3 puncta (both p<0.01), suggesting the axonal polarity of microtubules is altered. In addition, EB3 puncta in Ank3+/- axons move a shorter distance at reduced speed compared to Ank3+/+ axons (both p<0.05), suggesting microtubule stability is reduced. CRISPR-Cas9-induced repression of Ank3 in Neuro 2A cells reduced inhibitory GSK3-Ser9 phosphorylation and increased CRMP-2-T514 phosphorylation, indicative of reduced microtubule stability, which was rescued by lithium (both p<0.001). In contrast, Tau-S396 phosphorylation was unchanged by Ank3 repression.

Conclusion Our results suggest that Ank3 suppression reduces microtubule stability through a

GSK3/CRMP-2 dependent pathway and this is related to the treatment mechanism of BD. Future studies will focus on microtubule dynamics using human induced pluripotent stem cells carrying Ankyrin 3 genetic variants associated with BD risk.

Disclosures: X. Qi: None. T. Petryshen: None.

Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH Grant R01-MH0104344

Veteran's Administration VISN 22 Mental Illness Research, Education, and Clinical Center

Title: 'Spring' births induce anxiety/risk averse behavior in normal female mice, but resilience is seen in mice with reduced dopamine transporter expression

Authors: *M. A. KWIATKOWSKI, Z. A. COPE, L. A. LAVADIA, M. A. GEYER, D. DULCIS, J. W. YOUNG

Dept. of Psychiatry, UCSD, San Diego, CA

Abstract: BACKGROUND: Gestation during short-active (SA) photoperiods in humans (late winter/early spring births) increases psychiatric diagnoses. Previously, we determined that SA photoperiods during gestation resulted in psychiatry-relevant behavioral changes in the forced swim and saccharin preference tests vs. normal-active (NA)-born mice when tested in adulthood. Not every spring birth results in a psychiatric condition, however, indicating an important role of genetic susceptibility. Given a dysfunctional dopamine (DA) system has been implicated in most psychiatric disorders - and genetic polymorphisms of the DA transporter (DAT) are associated with some conditions - we tested whether mice with ~50% reduced expression of DAT (DAT-HY) would exhibit exaggerated psychiatry-related behaviors when born in SA photoperiods. **METHODS:** C57/BL6 dams were placed into either SA (19:5 light:dark (L:D)) or NA (12:12 L:D) photoperiods for 2 weeks, then paired with DAT-HY sires. Resultant DAT-HY and wildtype (WT) littermates were maintained in these conditions until weaning (P21), then placed into a NA photoperiod until tested in the elevated plus maze (EPM, n=120) during the active (dark) phase of the L:D cycle.

RESULTS: A significant sex by genotype by perinatal photoperiod interaction was seen for time spent in EPM open arms ($F_{(1,112)} = 6.0$, p < 0.05). When analyzed separately by sex, females exhibited a trend toward a gene by perinatal photoperiod interaction ($F_{(1,51)} = 3.7$, p = 0.061). SA-

born female WT mice spent significantly less time in open arms vs. NA-born female WT mice $(t_{(29)} = 2.1, p < 0.05)$. Female DAT-HY mice spent equal time in open arms regardless of perinatal photoperiod exposure. SA-born female DAT-HY mice spent significantly more time in open arms vs. SA-born female WT mice $(t_{(26)} = -2.9, p < 0.01)$, whereas no gene effect was observed in NA-born female DAT-HY and WT mice. Males exhibited no main effects or interactions on EPM open arm duration.

DISCUSSION: SA gestation photoperiods induce risk-averse/anxiogenic behavior in female WT mice, an effect not seen in mice with reduced DAT function as evidenced by: 1) female WT mice born in SA photoperiods spend less time in open arms compared with WT mice born in NA photoperiods, and 2) no photoperiod effect was observed in DAT-HY mice, but they did spend significantly more time in open arms vs. WT mice. Separating anxiogenic vs. risk-averse behavioral effects of SA photoperiod gestation remains important. Hence, we will test these mice in the Iowa Gambling Task, specifically designed to assess risk-preference and directly translatable to human/psychiatric populations.

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Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 330.19/RR2

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

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Title: Sex-dependent effects of Cacna1c haploinsufficiency on juvenile social play and 50-kHz ultrasonic vocalizations in rats

Authors: T. M. KISKO¹, M. D. BRAUN¹, M. BARTZ¹, C. HOHMEYER², S. WITT², M. RIETSCHEL², R. K. SCHWARTING¹, *M. WÖHR¹ ¹Philipps-University of Marburg, Marburg, Germany; ²Central Inst. of Mental Hlth., Mannheim, Germany

Abstract: Social play behavior is the earliest form of mammalian social behavior directed at peers and not the mother. Vital to healthy development, play deprivation can result in social and cognitive deficits. Rats have complex behavioral repertoires that include both social play and ultrasonic vocalizations (USV). USV serve as situation-dependent affective signals and fulfill important communicative functions, e.g. positive 50-kHz USV ("rat laughter") as social contact calls. *CACNA1C* is a novel, yet well-established risk gene implicated in numerous

neuropsychiatric disorders, most notably affective disorders, i.e. major depression and bipolar disorder, but also schizophrenia and autism. The gene encodes for the pore-forming alpha1c subunit of the L-type voltage-gated calcium channel Ca_v1.2, mediating depolarization-dependent calcium influx into the cell. In this study, our aim was to explore the role of *Cacnalc* in social behavior, specifically during the critical period of early development. Using a newly developed genetic rat model, we investigated juvenile social play behavior and concomitant 50-kHz USV emission in male and female wildtype (+/+) and heterozygous (+/-) Cacnalc rats. In addition to 50-kHz USV in the sender, we assessed behavioral responses displayed by the recipient exposed to 50-kHz USV playback. Our results indicate that in males there are no genotype effects on social play behavior, but male Cacna1c+/- rats displayed less 50-kHz USV emission compared to Cacnalc+/+ controls across all three play sessions. In females, in contrast, evidence for prominent genotype effects was obtained, with female Cacnalc+/- rats spending more time playing and specifically pinning more than *Cacnalc+/+* controls during the second and third play session. However, increased play behavior did not result in elevated 50-kHz USV rates in female Cacnalc+/- rats and during the first play session, where play levels did not differ, 50kHz USV were reduced. Moreover, *Cacna1c* haploinsufficiency affected the behavioral response in recipient rats exposed to 50-kHz USV playback. Despite that Cacnalc+/+ and Cacnalc+/rats both displayed strong social approach behavior and presumably started to search for a conspecific in response to 50-kHz USV, strong place preference for the area in proximity to 50kHz USV was seen in Cacnalc + /+ but not Cacnalc + /- rats after the playback presentation ended, indicating that only Cacnalc + /+ rats kept searching for a conspecific. Together, our findings suggest that Cacnalc haploinsufficiency leads to behavioral changes in social and communication development with relevance to neuropsychiatric disorders, both in sender and receiver.

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Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH Grant R01MH104261

ONR Grant N00014-12-1-0366

Hope for Depression Research Foundation

Title: Examining the role of cell adhesion molecules in emotional reactivity using the bred high-responder/bred low-responder model

Authors: ***A. V. STEFANOV**¹, K. L. HILDE¹, I. BIRT¹, M. H. HAGENAUER¹, E. K. HEBDA-BAUER¹, S. CLINTON², C. AYDIN¹, P. BLANDINO, Jr.¹, J. STEAD³, R. C. THOMPSON¹, S. J. WATSON, Jr.¹, H. AKIL¹

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Abstract: In the United States, over a quarter of adults eighteen years or older are diagnosed with a psychiatric disorder over a twelve-month period, where the most common classes reported are anxiety and mood disorders. Developing treatments for individuals susceptible to depression and anxiety requires a better understanding of the underlying neural and molecular mechanisms. Cell adhesion molecules (CAMs) are essential for the organization and communication of neighboring cells in the nervous system of a developing organism, as they orchestrate growing neurons into neural networks and help develop and maintain the synapses between them. The structures, molecular pathways, and interactions of various CAMs have been extensively studied in neural systems, and individual CAMs have been implicated in psychiatric disease. However, we know very little about the role of this broad class of molecules in the development and fine-tuning of emotional brain circuitry.

To assess how CAMs affect the trajectory of neural circuits implicated in vulnerability to depression, we use a selectively-bred rat model of emotional reactivity: the bred high-responders (bHRs) and the bred low-responders (bLRs). bHRs exhibit higher locomotor activity, show greater sensation-seeking and impulsivity, have a propensity toward addiction, and demonstrate a greater tendency for social interaction. bLRs display lower locomotor activity, exhibit greater anxiety and depression behaviors, are more reactive to stress, and are less socially interactive. In this study, we have examined the developmental pattern of CAM gene expression in the hippocampus, an area critical for learning, memory, stress responsiveness, and the regulation of emotionality.

We will outline the phenotypic and age differences in the expression of various CAMs in the hippocampus of neonate, adolescent, and adult bHRs and bLRs. We will show that CAMs can be expressed strongly at certain time points in development while exhibiting low expression at other times. We will describe the phenotypic specificity of these developmental trajectories. Our findings suggest that emotional reactivity, which contributes to susceptibility to mood disorders, may depend in part on the temporally regulated expression of specific CAMs during development.

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330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 330.21/RR4

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Increased diversity in microbial communities after chronic social defeat stress in mice

Authors: *K. MCGAUGHEY¹, D. A. CRUZ², N. ELSAYED², T. YILMAZ-SWENSON², R. RODRIGUIZ², W. WETSEL², M. KRITZER-CHEREN², O. MUELLER³, D. E. WILLIAMSON^{2,4}

²Psychiatry & Behavioral Sci., ³Mol. Genet. & Microbiology, ¹Duke Univ., Durham, NC; ⁴Durham VA Med. Ctr., Durham, NC

Abstract: Abstract:

Modulation of the stress response by the interaction between the hypothalamic-pituitary-adrenal (HPA) axis and gut microbiota has been shown to have implications in major depressive disorder (MDD). Significant shifts in microbial composition can play a critical role in the stress response and the microbiome may serve a modulatory role between stressor exposure and onset of MDD. Chronic social defeat stress in mice is an established preclinical model of MDD and results in subpopulations of defeated mice that are susceptible or resilient to further stress. This study examines the structural and functional shifts in microbial composition as a result of chronic social defeat stress in mice and examines whether these differences in the microbiota map onto susceptible or resilient subpopulations. To produce a defeated phenotype, 36 male C57BL/6J mice were exposed to chronic social defeat conditioning and a social interaction paradigm that enabled profiling for susceptibility and resilience to stress. Fecal samples were collected 24 hours post-social interaction. DNA was isolated from the fecal samples and the gut microbiota was profiled using 16S rRNA metagenomic sequencing on the Illumina miSeq platform. Chronic social defeat induced behavioral changes that were associated with distinct shifts at the level of operational taxonomic units (OTUs) across phyla and families ($t_{34} = 6.28$, p < 0.001) as well as increased richness and diversity of the gut microbial community compared to controls ($t_{34} = 6.02$, p < 0.001). The OTUs that differed between defeated and control mice largely belonged to an unclassified family of Bacteroidetes (S24-7; 68 OTUs) or an uncharacterized phylum (TM7; 3 OTUs), limiting the precision in identifying specific taxa. In examining the role of chronic social defeat in creating subpopulations of susceptible or resilient mice, 10% of mice fell into the resilient category. The OTUs of susceptible and resilient mice were significantly different from controls, but not different from each other. This study indicates that exposure to chronic social stress is associated with complex OTU- level shifts in microbiota. While defeated mice present increases in overall diversity and richness, this diversity might not necessarily confer good health, particularly given evidence implicating the role of S24-7 and TM7 in modulating

inflammatory conditions and the interconnectivity of gut microbiota and macrophage activity. Additional studies would aid in the understanding of the role of microbial diversity in chronic stress paradigms and their contribution to MDD.

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Poster

330. Animal Models for Affective Disorders: Mechanisms I

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Program#/Poster#: 330.22/RR5

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NARSAD Young Investigator Grant #2510

Title: Changed endocannabinoid signaling in the medial prefrontal cortex is related to chronic pain induced depression

Authors: *B. PAN¹, Z. ZHANG¹, C. J. HILLARD³, Q. H. HOGAN² ¹Anesthesiol., ²Med. Col. of Wisconsin, Milwaukee, WI; ³Med. Col. Wisconsin, Milwaukee, WI

Abstract: Patients with chronic pain often suffer from depression, but the underlying mechanisms are still unclear. The medial prefrontal cortex (mPFC) is a key brain area regulating depression. There are critical links of mPFC synaptic function to depression, with signaling through the endocannabinoid (eCB) system, whereby eCBs 2-Arachidonoylglycerol (2-AG) and N-arachidonylethanolamide (AEA) pre-synaptically suppress GABAergic inhibitory input to mPFC pyramidal neurons via their receptors (CB1Rs), thereby keeping the mPFC active. Anatomical and electrophysiological studies also show that afferent nociceptive pathways connect to the mPFC. eCB signaling is activity dependent. So, it is possible that noxious stimuli acutely activate and chronically depress eCB signaling in the mPFC, which results in depression. The present study was designed to address this hypothesis. We found that spared nerve injury (SNI) reliably induces neuropathic pain and significantly decreases performance of rats on behavioral tests relevant to depression, including the sucrose preference test, novelty-suppressed feeding test, and forced swimming test. Thus, SNI was followed by development of multiple indicators of a depression phenotype. Levels of 2-AG but not AEA in mPFC were elevated 3 days after SNI surgery, but normalized by 34 days. Electrophysiological recordings showed that CB1Rs in GABAergic presynaptic terminals are desensitized 34 days after SNI surgery, which results in increased inhibitory innervation of mPFC pyramidal neurons. Together, these data indicate that depressed eCB signaling in the mPFC may contribute to chronic pain induced

depression, and that eCB signaling is a potential target for treating depression induced by chronic pain.

Disclosures: B. Pan: None. Z. Zhang: None. C.J. Hillard: None. Q.H. Hogan: None.

Poster

330. Animal Models for Affective Disorders: Mechanisms I

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Program#/Poster#: 330.23/RR6

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Ibaraki University Cooperation between Agriculture and Medical Science (IUCAM) (The MEXT, Japan)

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Council for Science, Technology and Innovation (CSTI), Cross-ministerial Strategic Innovation Promotion Program (SIP), "Technologies for creating next-generation agriculture, forestry and fisheries" (Bio-oriented Technology Research Advancement Insti

Title: Diet purity impacts on the cecum microbiome but not depressive-like behaviors and plasma corticosterone levels in C57Bl/6J mice

Authors: ***A. TOYODA**¹, H. SHIMONISHI¹, M. SATO², K. USUDA⁴, K. NAGAOKA³ ¹Agr., Ibaraki Univ., Ibaraki, Japan; ³Vet. Med., ²Tokyo Univ. of Agr. and Technol., Tokyo, Japan; ⁴Vet. Sci., United Grad. Sch. of Vet. Science, Gifu, Tokyo, Japan

Abstract: (Introduction) For the animal experiments, appropriate diets should be used to obtain the reproducible results, however the information about the suitable diets for neuroscience researches is limited. Recently, we reported that vulnerability in chronic social defeat model of mice is affected by feed qualities (Goto et al., Nutr Neurosci, 2016). The purified diet (AIN-93G) increased susceptibility to chronic social defeat stress compared with non-purified diet (MF). In this study, we observed the effects of chronic feeding with AIN-93G and MF on depressive-like behaviors, plasma corticosterone, and cecum microbiome in mice. (Materials and Methods) C57BI/6J male mice (8 weeks, SLC, Shizuoka, Japan) were divided two groups. Group 1 was fed MF (Oriental Yeast, Tokyo, Japan), and group 2 was fed AIN-93G (Oriental Yeast) for 4 weeks. Homecage activity, food intake, and water intake were constantly measured by "3 Points Meter" (O'hara, Tokyo, Japan). After that, all animals were subjected to behavioral tests including open field, elevated plus maze, tail suspension, and forced swimming. Finally, blood plasma and cecum contents were collected. Cecum microbiome was analyzed using MiSec sequencing technology (illumina, San Diego, CA). Plasma corticosterone was measured by radio immunoassay. (Results and Discussion) Total food intake in group 1 was higher compared with group 2, however there were no significant difference between two groups in total calorie intake, water intake, behavioral tests, and plasma corticosterone. In the cecum microbiome, there were several differences between two groups, especially genus *Allobaculum* was predominant in group 2 compared to group 1. These microbiome differences in the digestive tract may affect brain functions and behaviors under stressful conditions. Actually, our previous study showed that diet quality and chronic social defeat stress affect metabolome in cecum, liver, and plasma (Goto et al., J Proteome Res, 2017). Conclusively, the interaction between diets and behaviors should be comprehensively studied in future. (Reference) Goto T, Kubota Y, Toyoda A, Effects of diet quality on vulnerability to mild subchronic social defeat stress in mice. Nutritional Neuroscience, 19(7), 284-289, 2016 Goto T, Tomonaga S, Toyoda A, Effects of diet quality and psychosocial stress on the metabolic profiles of mice. Journal of Proteome Research in press

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Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 330.24/RR7

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NARSAD Young Investigator Grant

DFG

CIHR

Title: Adult hippocampal neurogenesis increases preference for delayed rewards

Authors: *D. R. SEIB, D. ESPINUEVA, O. PRINCZ-LEBEL, E. CHAHLEY, R. QI YU, S. B. FLORESCO, J. S. SNYDER

Dept. of Psycholgy, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: There is growing evidence that reduced adult neurogenesis plays an important role in depression. However, clear proof from animal models is still missing. We combine a novel rat model in which we can specifically block adult neurogenesis (GFAP-TK) and complex behavioral operant testing paradigms to elucidate if characteristics of depressive behaviors can be mimicked by loss of hippocampal neurogenesis, for example assigning less value to a delayed future reward.

We first tested rats on a delay discounting paradigm, where animals must choose between a low

reward lever that delivers 1 sugar pellet immediately and a high reward lever that delivers 4 pellets after a delay. Compared to intact wild types (WT), GFAP-TK rats showed a decreased preference for the high reward option with increasing delay times, indicating that adult neurogenesis increases the subjective value of future rewards. We were able to replicate this finding by ablating adult hippocampal neurogenesis with irradiation in WT rats. On the contrary, increasing neurogenesis by voluntary running for four weeks led to increased preference for delayed high rewards.

Second, we employed a probabilistic reversal learning task where rats have to show optimal behavior in the face of an uncertain reward. Here, we test for behavioral flexibility and sensitivity towards negative feedback, behaviors that are known to be impaired in depression. Third, we performed an effort-based decision-making task, since depression is often associated with reduced energy and motivation. Here, the number of lever presses required to receive the high reward option increases across blocks. We did not find any difference in the performance of WT and GFAP-TK animals in these two other tasks, indicating that functions for neurogenesis in reward-based decision-making are specific for immediate vs. delayed rewards.

Ongoing experiments are investigating cellular mechanisms by which new neurons influence valuation of future rewards. Preliminary results indicate that decreased neuronal activity in the ventral dentate gyrus in the GFAP-TK rat is associated with a lower preference for delayed rewards.

This project combines a novel rat model with complex behavioral tasks to study the impact of reduced neurogenesis on various phenotypes observed in psychiatric disorders with a focus on depression. It suggests that increased neurogenesis, for example by exercise, could benefit depressed patients. Results from this project can also be transferred to other psychiatric disorders, where neurogenesis is affected and similar behavioral phenotypes can be observed, such as addiction, Alzheimer's disease, and schizophrenia.

Disclosures: D.R. Seib: None. D. Espinueva: None. O. Princz-Lebel: None. E. Chahley: None. R. Qi Yu: None. S.B. Floresco: None. J.S. Snyder: None.

Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 330.25/RR8

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH Grant R01MH106500

Title: Reduced enkephalin signaling in the nucleus accumbens D2-MSN circuit regulates depression-like phenotype in social defeat stress

Authors: *H. NAM, R. CHANDRA, T. FRANCIS, M. LOBO Dept. of Anat. and Neurobio., Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: Enkephalins are primary endogenous ligands for delta opioid receptors (DORs) and are highly enriched in D2-medium spiny neurons (MSNs) in the nucleus accumbens (NAc) and dorsal striatum. Enkephalins are highly implicated in depression, as preproenkephalin knockout and DOR knockout mice display anxiety- and depression-like phenotypes. Further, enkephalinase inhibitors can act as antidepressants. However, the specific the role of enkephalins, especially in the D2-MSN microcircuit in NAc - ventral pallidum (VP) pathway, in depression is not fully investigated. To provide insight into enkephalin function in this circuit we use an animal model of depression, the 10-day chronic social defeat stress paradigm. Following the defeat sessions, animals were either categorized as susceptible (displaying depression-like behavior) or resilient to defeat stress based on their performance in a social interaction test. Compared to the control and the resilient animals, the susceptible animals showed reduction in enkephalin levels in the NAc and VP. To determine if the reduced enkephalin levels cause depression-like behavior through disrupted Enk - DOR signaling, we treated the animals that experienced chronic social defeat stress with a DOR agonist SNC80. Our results indicate that SNC80 can reverse the depression-like conditions in susceptible animals. To investigate the mechanisms that reduce levels of enkephalins in the depressed conditions, we analyzed levels of different enzymes that can degrade or produce enkephalins. We demonstrate that the decrease in enkephalin levels may be due to increased levels of enkephalinases, such as angiotensinconverting enzyme (ACE) and analyl aminopeptidase (ANPEP), and decreased levels of proprotein convertase 1 (PC1) in susceptible animals. Overall, our data implicate that depressionlike behavior induced by social defeat stress is caused by reduced DOR signaling resulting from lowered level of enkephalins, which may be mediated through elevated expression of enkephalinases and decreased proprotein convertases.

Disclosures: H. Nam: None. R. Chandra: None. T. Francis: None. M. Lobo: None.

Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 330.26/RR9

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Age specific transcriptional changes in the neuronal cell types forming the canonical cortical microcircuit

Authors: ***R. SHUKLA**¹, T. D. PREVOT², L. FRENCH⁴, B. ROCCO⁴, M. BANASR³, R. ISSERLIN⁵, G. BADER⁵, E. SIBILLE⁶

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and Aging, ²Ctr. For Addiction and Mental Hlth., Toronto, ON, Canada; ⁴Camh, Toronto, ON, Canada; ⁵Univ. of Toronto, Toronto, ON, Canada; ⁶CAMH - Univ. of Toronto, Toronto, ON, Canada

Abstract: Aging is characterized by a decline in motor, sensory, cognitive, and mood functions which are associated with subtle changes in the Excitation/Inhibition balance of the canonical cortical microcircuitry composing of excitatory: pyramidal and inhibitory: Sst, Pvalb, and Vip positive interneurons. The age-associated long-term changes in these neurons of the microcircuitry we hypothesize are regulated through the transcriptomic changes within and across these neuronal cell types. To address this hypothesis, we first assessed the age-associated changes in anxiety-like behavior using Elevated plus maze and open field tests and cognitive abilities using novel object recognition and Y-maze alternation tests in young (2 months, n=9) and old (22 months, n=12) male mouse. Cell type samples for PYC, *Pvalb*, *Sst*, and *Vip* neurons obtained from the frontal cortex, using laser capture microdissection were sequenced for total RNA and the expression profile of each cell type was correlated with the first three principal components of the principal component analysis performed on all the test results. Using ontological approach we show that (1) Pyramidal neurons, with age, show upregulation of pathways related to energy production which can be associated with high activity and protective thus vulnerable nature of these neurons. There was a prominent downregulation of pathways related to synaptic activity in these neurons (2) VIP neurons have increased transcriptional control whereas SST neurons have increased translational control with age. (3) PV neurons seem to be least vulnerable of the four neurons as suggested by relatively increased photolytic clearance and synaptic activity with age. Together our data suggests that different cell types have a different vulnerability, which has a collective effect towards aging. We propose an order of neuronal vulnerability, which may lead to a dominos like fall of neuronal circuitry components and their functions with normal aging. The order of vulnerability has specific consequences and change in this order may lead to a state of psychiatric disorder.

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Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

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Program#/Poster#: 330.27/RR10

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NARSAD

BioHaven

Servier

Title: Linking dynamic GABAergic, astroglial and synaptic dysfunctions to stress-induced depressive-like endophenotype: Importance of astroglial integrity

Authors: *T. D. PREVOT¹, K. A. MISQUITTA^{1,2}, C. J. FEE^{1,2}, D. NEWTON^{1,2}, H. OH^{1,3}, E. SIBILLE^{1,2,3}, M. BANASR^{1,2}

¹Ctr. For Addiction and Mental Hlth., Toronto, ON, Canada; ²Dept. of Pharmacol., ³Dept. of Psychiatry, Univ. of Toronto, Toronto, ON, Canada

Abstract: Major depressive disorder (MDD) is a severe mental condition affecting multiple cellspecific pathways that interact to produce heterogeneous phenotypes. MDD is associated with molecular changes within GABA cells, astrocytes and synapses in the prefrontal cortex (PFC), and similar changes are reported in stress-based rodent models of depression. Despite their intricate functional roles, the cellular alterations observed have been studied independently, when the pathology is already established. To identify the sequence of events affecting each cell compartment we characterized the time-dependent (i.e. onset and trajectory) cell-specific changes during chronic stress exposure and their correlation with depressive-like behaviors to characterize potential key features in the onset of pathological state. Using the chronic restraint stress (CRS) paradigm (1h restraint stress, 2x day) in male and female C57/BL6 mice, we determined CRS effects on behavior and on the expression of GABAergic, astrocytic and synaptic markers in the PFC at different time points (1 to 5 weeks). We assessed CRS effects on anxiety-like behavior (response to a 1h spotlight stressor in home cage-like setting), anhedonialike deficits (reduced sucrose consumption) and coat deterioration. The GABAergic, astroglial and synaptic alterations induced by CRS were characterized using qPCR and western blot analysis. CRS-exposed mice exhibited increased anxiety-like behavior regardless of the duration of CRS but showed a progressive and significant increase in anhedonia-like behavior starting on the 3rd week of CRS-exposure. Both anxiety- and anhedonia-like phenotypes were observed for the following weeks. At the cellular level, GABAergic marker expression (GAD67 and vGAT) reductions were identified as early as the first week of CRS exposure. Astrocytic marker (GFAP, glutamine synthetase (GS), GLT1) expression levels were altered after 3 weeks of CRS exposure and preceded a decrease in synaptic (PSD-95, Syn1) markers. Interestingly, while anxiety-like behaviors correlated with GABAergic marker expression, astrocytic marker expression profile correlated with both anhedonia- and anxiety-like deficits, suggesting that astroglial dysfunction coincides with the emergence of the maladaptive response to stress. We confirm here that chronic stress is a multiphasic process where time-dependent cell-specific alterations are linked to various behavioral outcomes. Altogether, our results suggest that astroglial dysfunction would be a potential turning-point in the response to stress leading to the pathological state associated with MDD and other stress-related illnesses.

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Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 330.28/RR11

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIMH R01 Grant MH077159

Title: Acute and chronic chemogenetic silencing of somatostatin-expressing GABA interneurons induces rapid, robust, and lasting elevation of depressive- and anxiety-like behaviors

Authors: *C. J. FEE^{1,2}, K. MISQUITTA^{1,2}, R. BRAD¹, T. D. PREVOT¹, M. BANASR¹, E. SIBILLE^{1,2,3}

¹Ctr. For Addiction and Mental Hlth., Toronto, ON, Canada; ²Dept. of Pharmacol. & Toxicology, ³Dept. of Psychiatry, Univ. of Toronto, Toronto, ON, Canada

Abstract: Major depressive disorder (MDD) studies consistently demonstrate reduced level and function of somatostatin (SST)-expressing GABAergic interneurons, pronounced in brain regions that regulate cognitive-emotional functions. Past mouse models, using SST gene deletion or frontal cortex-specific silencing, supported a causal role for SST cell deficits in depressive-like behavior. However, these do not recapitulate key features of human pathology, where SST cell deficits are widespread and potentially include altered GABA function. Here, using a brainwide designer receptors exclusively activated by designer drugs (DREADD) approach, we addressed this issue by developing and characterizing a novel mouse model of chronically inducible, global, and cell-specific SST neuron silencing.

C57BL/6J SST^{Cre} and floxed-hM4Di mice were crossed to generate mice with inhibitory DREADD expression specifically in SST neurons (confirmed by immunohistochemistry). *Acute Silencing:* adult SST^{DREADD} or SST^{Cre} mice were administered veh (0.9% saline) or clozapine-N-oxide (CNO 5mg/kg) i.p.30 min prior to behavioral tests (*n*=12/group; 50% female). Z-scoring summarized changes across tests measuring similar outcomes. While SST^{Cre} (±CNO) and SST^{DREADD} (Veh) groups did not differ significantly across tests, SST^{DREADD} (CNO) mice showed elevated emotionality, indexed by increased Z-scores for anxiety (open field, elevated plus maze, Phenotyper tests) and anhedonia/helplessness (sucrose consumption, forced-swim tests), and decreased Z-cognition (object recognition, Y-maze), compared to all other groups. SST^{DREADD} (CNO) mice showed hypolocomotion and reduced food/water intake, suggesting a potential motivational confound. Measures were excluded (food tests) or adjusted (% line crosses) to account for this. *Chronic Silencing:* mice received water or CNO (~5mg/kg/day) *ad libitum* for 5 weeks and behavioral tests were performed weekly. Compared to all other groups, SST^{DREADD} (CNO) mice showed chronically elevated emotionality, unlike past models of regional SST silencing, but unchanged cognition. Where sex was a significant covariate,

between-group relations were unchanged.

This data supports chronic, global, SST neuron silencing as a model to recapitulate depressivelike behavior. Further cellular/behavioral model validation is underway. This model represents a potentially valuable tool to probe the role of SST pathology in MDD, identify cellular adaptations for target discovery, and screen for novel antidepressants.

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Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 330.29/RR12

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NFSC81671180

Title: Behavioral, inflammatory and neurochemical disturbances in LPS and UCMS-induced mouse models of depression

Authors: *J. MA, K. FAN, X. YANG, G. LIU Anat., Dalian Med. Univ., Liaoning, China

Abstract: The immuno-inflammatory activation triggered by various stresses play an important role in pathophysiology of depression. The immune responses display differential pathological characters in different stresses. However, comparative data and analysis on behavioural, inflammatory and neurochemical changes in different stress-induced depression is limited. To imitate different stressful situations, in this study, mice were subjected to a single injection of LPS (0.5 mg/kg, i.p.) and UCMS (4 week period), respectively. LPS-stressed mice showed more immobility time in FST and TST, as well as more time in periphery in OFT than UCMS-stressed mice. Further, LPS-stressed mice showed more robust expression and release of TNF-a, IL-1β and IL-6 in serum and depression-related brain areas (prefrontal cortex, hippocampus and striatum) as compared to UCMS-stressed mice. The ELISA results showed that IDO expression was significantly increased following LPS and UCMS stresses, but more increased IDO expression was observed in prefrontal cortex and hippocampus of LPS-stressed mice. The decrease of 5-HT and BDNF was detected only in hippocampus of LPS-stressed mice, but in overall all the brain areas assessed in UCMS-stressed mice as compared to control. The data indicate that LPS induced more severe depressive-like behaviours and more robust immune activation than UCMS. Our study strongly imply that hippocampus is probably most vulnerable to acute inflammatory challenge in depression, while chronic psychological stress more likely to

resemble the multidimensional symptoms of clinical depression. Our findings provide more insight into pathophysiology in stress-induced depression.

Disclosures: J. Ma: A. Employment/Salary (full or part-time):; Anatomy Department of Dalian Medical University. K. Fan: None. X. Yang: None. G. Liu: None.

Poster

330. Animal Models for Affective Disorders: Mechanisms I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 330.30/RR13

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Cariplo Foundation Grant 2014-0972

Telethon Foundation Grant GGP14074

Title: How the environment shapes our behavior: The role of LSD1 and SRF in adaptation and vulnerability

Authors: *F. S. RUSCONI¹, B. GRILLO², A. LONGARETTI³, C. FORASTIERI³, E. TOFFOLO³, L. GEROSA⁵, M. PASSAFARO⁵, M. POPOLI⁴, E. BATTAGLIOLI² ¹Med. Biotech. and Traslational Med., Univ. of Milan, Segrate, Italy; ³Med. Biotech. and Translational Med., ⁴Pharmacol. and biomolecular Sci., ²Univ. of Milan, Milan, Italy; ⁵CNR Inst. of Neurosci., Milan, Italy

Abstract: In mammals, different forms of stress including psychosocial stress can impact on several aspects of human health, fostering mood and anxiety disorders. However, very little is known about the mechanisms underlying brain physiology of stress response, hindering development of new therapeutic strategies. We uncover a role for transcriptional corepressor Lysine Specific Demethylase-1 (LSD1) and its dominant-negative splicing isoform neuroLSD1, in the modulation of emotional behavior. In mouse hippocampus, LSD1 and neuroLSD1 interacting with transcription factor Serum Response Factor (SRF) and SRF Δ 5 participate as molecular transducers of stress stimuli. Indeed, also SRF is modulated by a dominant negative alternative splicing isoform devoid of transactivation domain. Psychosocial stress acutely decreases neuroLSD1 expression through a splicing-based modulation that entails increasing amount of LSD1, while the relative ratio between SRF and SRF Δ 5 is sensitive to both ASDS and CSDS. Moreover, SRF Δ 5 shows a SUS-restricted downregulation that might contribute to shaping psychosocial stress vulnerability, through interfering with homeostatic mechanisms underlying stress resiliency. All these data suggest involvement of the dual system LSD1/neuroLSD1 and SRF/SRF Δ 5 in adaptive response to stress.

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Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 331.01/RR14

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant P20GM103442

NIH Grant P50DA018165

NIH Grant R24AA020245

Title: Depression like symptoms associated with acute and prolonged methamphetamine withdrawal in a mouse model for binge methamphetamine intake

Authors: ***S. SHABANI**¹, B. GHIMIRE¹, B. SCHMIDT¹, E. MOJICA¹, S. HOULTON¹, T. PHILLIPS-RICHARDS^{2,3}

¹Biol., Minot State Univ., Minot, ND; ²R&D 32, VA Med. Ctr., Portland, OR; ³Behavioral Neurosci., Oregon & Hlth. Sci. Univ., Portland, OR

Abstract: Binge MA users have higher MA use and depression like symptoms associated with acute withdrawal and lower success rates in abstaining from MA use. Drug acute withdrawal sequela are thought to contribute greatly to this type of compulsive drug use. A genetic animal model for binge MA use has recently been developed, though the strength of MA withdrawal after different time points of abstention from chronic MA intake is missing. We used the selectively bred MA high drinking (MAHDR) line to explore sensitivity to MA withdrawal after chronic binge like MA intake at different abstention time points, as well as the resumption of MA intake after prolonged abstention. For comparison data were also collected on the MAHDR line's progenitor inbred strain DBA/2J (D2). In all studies, a multiple bottle choice procedure that had an access ratio of 3 MA to 1 water tube in home cagetops was used as the method for measuring voluntary MA intake and total fluid intake. MA concentration was increased in 20 mg/l increments every four days from 20 to 80 mg/l and at 80 mg/l was kept for 16 days. In two studies, depression like symptoms in the two strains were measured in a tail suspension test (TST) and a subsequent forced swim test (FST) after acute withdrawal of 6 hour (6h), and 30h from the 28 days of chronic MA intake. Similar study in only the MAHDR line, measured the same depression symptoms after prolonged abstention of 1 week and 2 weeks from chronic MA intake procedure. In addition, MA intake was also measured after 1 and 2 week MA abstention.

The MAHDR and D2 mice had similar escalation of MA intake during gradual increase of MA concentration, however MAHDR mice overall MA intake was substantially higher than D2 mice. Furthermore, MAHDR mice escalated fluid consumption from the MA tubes, but not D2 mice. Depression symptoms in chronic MA intake animals as compared to drug naïve animals was significantly higher after acute withdrawal and was dependent on genotype. MA acute withdrawal was more robust in MAHDR mice than in D2 mice. Depression symptoms in MAHDR mice than in D2 mice. Depression symptoms in MAHDR mice were absent after 1 and 2 week abstention. Similarly, MA intake in these animals after 1 and 2 week abstention also dropped significantly. The absolute MA intake after prolonged abstention however remained robust in MAHDR mice. This genetic mouse model has strong face validity for human binge MA use and withdrawal sequela associated with abstention. Furthermore genetic risk factors associated with MA intake and withdrawal can be teased apart to advance our understanding about the emergence of binge MA use like patterns.

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Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 331.02/RR15

Topic: G.08. Drugs of Abuse and Addiction

Support: Psychological Health and Well-being grant, Bishop's University

Title: Prescription stimulant misuse in undergraduate students: Prevalence and predictors

Authors: A. JONES-LAVALLÉE, N. VAN DEN BERG, M. LAFOREST, G. GOODING, C. GOLDFARB, S. LINDEN-ANDERSEN, A. MENDREK, *S. HOOD Psychology, Bishop's Univ., Sherbrooke, QC, Canada

Abstract: Evidence suggests that prescription stimulant misuse is increasing in prevalence on college and university campuses in North America (e.g., McCabe et al., 2014). Using an anonymous survey, we assessed prescription stimulant use and misuse in a sample of undergraduate students at a small Canadian university (N = 726, mean age = 21 years \pm 0.15 (S.E.), 66% female). Overall, 12% (87/726) of the sample reported having taking prescription stimulants at least once in the past year. Of students who had ever taken prescription stimulants, the majority (125/179, or 70%) indicated not having a prescription. Previous reports have identified demographic predictors of prescription stimulant misuse in post-secondary students such as male gender, Caucasian ethnicity, having poorer academic standing, and reporting higher levels of stress and depressive symptoms (Benson et al., 2015; Teter et al., 2010; McCabe et al., 2014). In our sample, however, demographic variables including gender, age, year of study, and

self-reported quality of academic performance were not associated with prescription stimulant misuse. Furthermore, students who misused did not report higher levels of anxiety, perceived stress, or depressive symptoms compared with non-misusing students. In contrast, students who misused were more likely to have tried other types of stimulants in the past 12 months, including cocaine or amphetamine (χ^2 (2) = 54.320, p < 0.001, odds ratio [OR] 6.78); caffeinated energy drinks (χ^2 (2) = 19.061, p < 0.001, OR 2.32); and energy shots (χ^2 (2) = 15.906, p < 0.001, OR 3.64), compared with students who did not misuse. Taken together, these results suggest that misuse represents a large proportion of total prescription stimulant consumption in undergraduate students. Students who have taken these drugs without a prescription in the past year are more likely to also have consumed other kinds of stimulants, including illicit drugs such as cocaine or amphetamine. These findings may contribute to understanding the pattern of prescription stimulant use by undergraduate students, and to increasing awareness of potential risks that misusers may face from taking multiple types of stimulants.

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Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 331.03/RR16

Topic: G.08. Drugs of Abuse and Addiction

Support: USPHS grants R01 DA042211

T32 AA007456

Title: Comparison of the potency and efficacy of the novel cathinone α -PPP, α -PVP and pentedrone: Self-administration and locomotor stimulation in male and female rats

Authors: *M. JAVADI PAYDAR, S. VANDEWATER, M. TAFFE Dept. of Neurosci., The Scripps Res. Inst., LA Jolla, CA

Abstract: Cathinone is a close structural relative of amphetamine and a recent proliferation of cathinone derivatives (CATHS) produce varying central stimulant actions and represent a new class of drugs of abuse. The CATHS seem to produce their major effects via release or reuptake inhibition of monamine neurotransmitters with differences in vivo that depend upon the nature of their terminal amine, α substituent and/or aryl substituents. **OBJECTIVES:** This study compared the efficacy and potency of α -PPP, α -PVP, Pentyloneand Pentedrone in rodent models to further determine the role of common substituent moieties. **METHODS:** Groups of

male and female Wistar rats were trained in the intravenous self-administration (IVSA) of α -PVP (0.05 mg/kg/infusion), α -PPP (0.3 mg/kg/infusion), or pentedrone (0.2 mg/kg/infusion) under a fixed-ratio 1 schedule of reinforcement. Subsequent randomized dose substitution (0.025-0.3 mg/kg/infusion) was used to evaluate potency and efficacy of α -PVP, α -PPP, pentylone or pentedrone. Additional groups were assessed for locomotor activity and body temperature responses to noncontingent administration of α -PVP, α -PPP, Pentylone or Pentedrone (0.5-10 mg/kg, i.p.). **RESULTS:** Acquisition of α -PVP, α -PPP, or pentedrone IVSA resulted in a consistent drug intake and discrimination for the drug-paired lever. Dose substitution under a fixed-ratio 1 schedule confirmed that potency and efficacy was higher in rats trained on α -PVP compared to those trained on α -PPP, or pentedrone. Across training groups, potency reflected α -PVP > pentedrone $\approx \alpha$ PPP > Pentylone. Peak locomotor responses to α -PVP were observed after the 1.0 mg/kg, i.p. dose and lasted ~ 2 h; however, maximum activity response to α -PPP, pentylone or pentedrone were recorded following a 10 mg/kg, i.p. dose. Body temperature changes were negligible (<0.5 °C) for these compounds. **CONCLUSIONS:** The potency and efficacy of α -PPP and pentedrone were very similar across multiple assays. Pentylone was comparatively less potent and α -PVP was more potent.

Disclosures: M. Javadi Paydar: None. S. Vandewater: None. M. Taffe: None.

Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 331.04/RR17

Topic: G.08. Drugs of Abuse and Addiction

Title: Binge alcohol exposure attenuates both the development and extinction of amphetamine conditioned place preference

Authors: *L. O'LOUGHLIN¹, C. IRVING², L. NORENA², A. PAZMINO², A. SAPERSTEIN², E. WALSH², T. PHAN², R. DUSSAULT³, J. SCHROEDER² ¹Behavioral Neurosci., Connecticut Col., Waterbury, CT; ²Behavioral Neurosci., Connecticut Col., New London, CT; ³Wheeler High Sch., North Stonington, CT

Abstract: The binge use of alcohol and abuse of amphetamine in the form of prescription ADHD medication is on the rise among young adults. According to the gateway hypothesis, repeated exposure to alcohol, particularly binge use of alcohol may predispose individuals to use and become addicted to other drugs of abuse including psychostimulants. Alcohol abuse may also make it more difficult to recover from dependency on other drugs of abuse. This study investigated the effects of binge alcohol pre- and/or post-exposure on the development and extinction of amphetamine conditioned place preference in young adult Sprague-Dawley rats. Animals pre-exposed to alcohol received 6 total alcohol (1.5 g/kg, sc of 20% alcohol in saline)

injections on 2 consecutive days at 48-hour intervals for a 10-day period one week prior to undergoing a 6 day, biased amphetamine conditioned place preference (CPP) paradigm. Contrary to the gateway hypothesis, binge alcohol exposure reduced the development of amphetamine CPP. However, binge alcohol pre-exposure attenuated extinction to amphetamine CPP. Exposure to alcohol following amphetamine CPP (post-exposure) only affected extinction in animals that also underwent alcohol pre-exposure. These results suggest that binge alcohol exposure may dampen the development of the association between amphetamine reward and contextual cues, but may extend the extinction of amphetamine reward behavior. Immunohistochemical evaluation of nucleus accumbens c-Fos expression was performed in a follow-up study in an attempt to correlate activity patterns in brain reward regions with reward behavior.

Disclosures: L. O'Loughlin: None. C. Irving: None. L. Norena: None. A. Pazmino: None. A. Saperstein: None. E. Walsh: None. T. Phan: None. R. Dussault: None. J. Schroeder: None.

Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

Location: Halls A-C

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Program#/Poster#: 331.05/RR18

Topic: G.08. Drugs of Abuse and Addiction

Support: DoD Grant W23RYX2111N603

Title: CNS mechanisms underlying the suppression of stress-induced methamphetamine seeking by oxytocin in female rats

Authors: *C. E. O'NEILL¹, R. J. NEWSOM², J. L. HOPKINS³, V. GRINEVICH⁴, J. F. MCGINTY⁵

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Abstract: High comorbidity of substance use disorders (SUDs) has been observed in patients with post-traumatic stress disorder (PTSD). Stimulants, such as methamphetamine (METH), are among the most highly abused substances by patients with comorbid PTSD. Previous work from our lab found that male rats exposed to the predator odor, 2,4,5-dihydro-2,5-trimethylthiazoline (TMT), exhibit exacerbated levels of stress-induced reinstatement (RST) and 10 days of systemic oxytocin (OXT) injections prevented this effect (Ferland et al, 2016). We also found that female rats pre-exposed to TMT daily for 5 days showed a similar exacerbation of METH-seeking. However, repeated OXT treatment decreased stress-induced RST regardless of prior stress experience in females. A key question is whether stimulation of CNS OXT neurons is sufficient to suppress stress induced METH-seeking. In this experiment, the paraventricular hypothalamic

nuclei (PVN) of female rats were infused bilaterally with an AAV1/2 vector encoding an excitatory DREADD (hM3Dq) driven by the Oxt promoter. Rats then underwent METH selfadministration and extinction training. Prior to TMT-induced RST, rats were injected with clozapine-N-oxide (CNO, 3 mg/kg, i.p.) or vehicle. CNO-induced activation of PVN OXT neurons suppressed stress-induced RST, indicating that stimulation of the endogenous OXT system in the CNS is sufficient to affect relapse similar to the effects of systemic OXT administration. Another goal of the current study is to further elucidate the neural mechanisms underlying the effects of OXT on stress-induced relapse. Thus, we exposed female rats to TMT or saline for 5 days followed by 10 days of OXT (1.0 mg/kg, i.p.) or saline. Rats then underwent METH self-administration, extinction, and TMT-induced RST. Repeated OXT treatment prevented increased expression of heteronuclear corticotropin-releasing hormone (Crh) mRNA in the PVN and bed nucleus of the stria terminalis as well as prevented a decrease in Oxt mRNA, as revealed by rtPCR in METH-treated female rats following stress-induced RST. Ongoing RNAscope in situ hybridization aims to reveal whether OXT treatment following stress alters cfos mRNA expression in Crh-, Oxt-, or Oxt receptor-expressing neurons of stress-related structures. Together these data suggest that OXT may be considered as a therapeutic agent for reducing relapse susceptibility in patients exhibiting comorbid SUD and PTSD.

Disclosures: C.E. O'Neill: None. R.J. Newsom: None. J.L. Hopkins: None. V. Grinevich: None. J.F. McGinty: None.

Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

Location: Halls A-C

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Program#/Poster#: 331.06/RR19

Topic: G.08. Drugs of Abuse and Addiction

Support: GM-64783

HRD-1302873

Title: Dose-dependent effects of methamphetamine on positive and negative affect in rats

Authors: *B. C. CORTES¹, A. ESCOBEDO², K. A. TRUJILLO³ ¹Biol., California State University- San Marcos, San Marcos, CA; ³Dept. of Psychology, ²California State Univ. San Marcos, San Marcos, CA

Abstract: Methamphetamine is a powerful psychomotor stimulant that is widely abused due to its rewarding and addicting properties. Though there is much research on the effects of methamphetamine, few studies have examined how different doses might produce different affective responses. The present study examined ultrasonic vocalizations (USVs) and locomotor

activity in response to different doses of methamphetamine (METH; 0.3, 1.0 or 3.0 mg/kg) in Sprague-Dawley rats. USVs are indicative of affective states, with 50 kHz USVs reflecting positive affect and 22 kHz USVs reflecting negative affect. Locomotor activity assesses the stimulant response to the drug, which may be indirectly related to the affective response. It was hypothesized that there would be an inverted U-shaped dose response, with the greatest number of reward-related (50 kHz) USVs at 1.0 mg/kg, and a reduction at 3.0 mg/kg due to aversive side-effects of the drug. The results supported our hypothesis: 1.0 mg/kg of METH produced the greatest number of 50 kHz USVs. In addition, there was an increase in aversive (22 kHz) USVs at 3.0 mg/kg (but not lower doses), demonstrating the dysphoric side-effect of METH at this dose. For locomotor activity, we found that the medium and high dose of METH produced a similar locomotor effect. The inverted-U response found with USVs gives insight into the affective responses to METH across doses, and suggests that users will moderate dosing to achieve the greatest reward while avoiding aversive side-effects. A better understanding of dose sensitivity enables for more accurate models for addiction, as addicts strive to achieve the "ideal" high. More research is necessary to develop suitable methods of prevention and treatment.

Disclosures: B.C. Cortes: None. A. Escobedo: None. K.A. Trujillo: None.

Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

Location: Halls A-C

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Topic: G.08. Drugs of Abuse and Addiction

Support: NSF HRD-1302873

NIH GM-64783

Title: Does sensitization to the rewarding effects of methamphetamine develop with repeated administration?

Authors: *T. T. TOWNER, V. GUTIERREZ, T. ZAFAR, K. A. TRUJILLO California State Univ. San Marcos, San Marcos, CA

Abstract: Behavioral sensitization is an increase in a behavior after repeat exposure to a stimulus and has been widely studied in regards to administration of drugs of abuse. Research suggests that sensitization may play a significant role in the development of addiction. The current studies examined sensitization to the rewarding effect of methamphetamine (METH). Locomotor behavior is a well-established (albeit indirect) measure of drug reward whereas ultrasonic vocalizations (USVs) are a more direct measure of affect and reward. Two experiments were performed to determine the rewarding effect of METH following a single

injection and sensitization following repeated injections, using USVs and locomotor activity in Sprague-Dawley rats. In Experiment 1, animals were injected with METH (1.0 mg/kg) or saline every other day for six days. In Experiment 2, animals were injected with METH (0.5 mg/kg) or saline once daily for ten consecutive days. Both studies had a METH challenge day at the end in which all animals received METH regardless of previous exposure. Initial exposure to METH induced a locomotor stimulant effect as well as an increase in 50-kHz USVs, showing that METH produces rewarding effects. METH induced successive increases in locomotor behavior and 50-kHz USVs across days of treatment, which is consistent with the development of sensitization. However, when all animals were challenged with METH, saline-pretreated and METH-pretreated animals showed similar increases in locomotor behavior and 50-kHz USVs. The current results suggest that sensitization to the rewarding effects of METH did not occur. Instead, it appears that repeated exposure to the experimental environment led to an increase in the response to METH. The fact that the same results were obtained with two different approaches strengthens the conclusions. These findings point toward the need for a challenge test in drug sensitization studies to rigorously test whether or not sensitization develops with repeated administration.

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Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: NSF HRD-1302873

NIH GM-64783

NIH GM-08807

Title: Differences between adolescents and adults in the behavioral response to amphetamines

Authors: *V. ESPINOZA¹, A. ROCHA², K. A. TRUJILLO² ²Dept. of Psychology, ¹California State Univ. San Marcos, San Marcos, CA

Abstract: Amphetamines are synthetic psychomotor stimulants and common drugs of abuse. According to the 2013 National Survey on Drug Use and Health, nearly 600,000 Americans 12 years of age or older were current users of amphetamines. Initiation of drug use during adolescence has been linked to the formation of drug addiction later in life. However, relatively little is known about the effects of amphetamines in adolescents when compared to adults. Research to date demonstrates mixed results in laboratory animal models, with some studies showing adolescents exhibiting an enhanced behavioral response when compared to adults, and other studies showing a reduced behavioral response. Given these differences, more evidence is necessary to further understand potential differences between adolescents and adults. In this study, the locomotor response to methamphetamine (METH), dextroamphetamine (D-AMPH), and methylphenidate (MPH) were examined in adolescent Sprague Dawley rats at 30 days of age and adult Sprague Dawley rats at 60 days of age. Animals were administered METH (1.0 mg/kg s.c.), D-AMPH (1.0 mg/kg s.c.), or MPH (1.5 mg/kg s.c.) and locomotor activity was assessed with a Kinder Scientific Motor Monitor (a photocell-based system). METH, D-AMPH, and MPH produced an increase in activity in adolescents and adults. Differences between adolescents and adults depended on the specific type of locomotor behavior examined. For example, adults showed a greater response when examining fine movements (indicative of stereotypy), while adolescents showed a greater response when examining ambulations (indicative of forward locomotion). These results show that differences between adolescents and adults in the response to amphetamines are dependent on the specific type of locomotor behavior assessed. Since forward locomotion is associated with drug reward, while stereotypy is associated with aversion, the results suggest a more rewarding response in adolescents. Further studies will explore other behaviors related to METH, D-AMPH, and MPH in adolescents and adults.

Disclosures: V. Espinoza: None. A. Rocha: None. K.A. Trujillo: None.

Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH GM-64783

NIH GM-08807

Title: Ketamine inhibits methamphetamine-induced ultrasonic vocalizations and locomotor activity in rats

Authors: *A. ESCOBEDO¹, A. ROCHA², C. GARCIA³, K. A. TRUJILLO² ²Dept. of Psychology, ¹California State Univ. San Marcos, San Marcos, CA; ³The Med. Affairs Co., Saint Louis, MO

Abstract: Polydrug abuse is common among drug users. Individuals combine drugs in order to increase the rewarding effects (e.g. "speedball"), to decrease aversive effects (e.g. withdrawal), and/or to experience unique psychoactive effects. A combination that has received little scientific exploration is the combination of a dissociative drug (such as ketamine) with a psychomotor
stimulant (such as methamphetamine). Both classes of drugs are abused in the dance club and rave scene leading to the opportunity for combined use. Additionally, studies have shown that psychomotor stimulants, like methamphetamine (METH), are regularly used simultaneously with dissociative drugs such as ketamine (KET) in these settings. The present study examined the effects of combinations of KET with METH using ultrasonic vocalizations (USVs) and locomotor activity in rats. USVs have been found to reflect affective states, including positive affect (50 kHz USVs) and negative affect (22 kHz USVs). For example, 50 kHz USVs are emitted under the influence of methamphetamine and other drugs of abuse, while 22 kHz USVs are emitted during drug withdrawal. It was hypothesized that the combination of KET with METH would induce a greater rewarding effect compared to either of the drugs alone. The responses to KET (5.0, 10.0 or 30.0 mg/kg s.c.) and METH (0.3 or 1.0 mg/kg s.c.) were assessed alone or in a "cocktail" combination in adult Sprague-Dawley rats. Contrary to our hypothesis, KET inhibited 50 kHz USVs evoked by METH and reduced METH-induced locomotor activity. The results illustrate the complex effects of combinations of KET with METH and raise questions as to why individuals abuse combinations of these drugs.

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Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

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Program#/Poster#: 331.10/RR23

Topic: G.08. Drugs of Abuse and Addiction

Title: Prelimbic dopamine facilitates the extinction of amphetamine conditioned place preference

Authors: *E. LATAGLIATA¹, G. CHIACCHIERINI³, F. FIOCCHI³, G. COCCIA³, S. PUGLISI-ALLEGRA^{3,2}

¹Santa Lucia Fndn., Roma, Italy; ²Santa Lucia Fndn., Rome, Italy; ³Dept. di Psicologia and Ctr. Daniel Bovet, Sapienza Univ., Rome, Italy

Abstract: Environmental stimuli or discrete cues paired with addictive drugs are major factors able to trigger drug-seeking and drug-taking behaviors. Repeated and unreinforced re-exposure to cues, to induce the extinction, is a method to decrease the magnitude and the frequency of the conditioned response (CR) induced by them. Dopaminergic transmission in medial prefrontal cortex (mPFC) has been critically involved in the modulation of highly salient memories and in particular in the extinction of the CR induced by them (Abraham et al. Neurobiol Learn Mem. 2014 Feb;108:65-77). Manipulation of dopamine (DA) levels in this area, such as selective depletion produces a delay in the extinction of fear CR (Morrow et al. Neuroscience. 1999;92(2):553-64.). Instead, treatments that increase DA transmission in mPFC or that

stimulate D1 dopaminergic receptors in prelimbic (PL) cortex foster the extinction of drug seeking behavior (Brenhouse et al.Neuroscience. 2010 Aug 25;169(2):628-36; Nesbit et al. Behav Brain Res. 2017 Mar 15;321:223-231). In the present study, we investigated a possible different involvement by D1 and D2 dopaminergic receptors in the PL cortex of C57BL/6J mice, in the extinction of drug associated memories. Thus, we evaluated the effects of a single PL infusion of D1 (SKF38393), D2 (Quinpirole) or both (D1 and D2) dopaminergic agonists on the expression and the extinction of amphetamine-induced conditioned place preference (CPP). Single treatment with D1 or D2 dopaminergic agonists did not alter either the expression or the extinction of the amphetamine CPP, whereas treatment with both D1 and D2 dopaminergic agonists produced an early extinction of the CPP without alter the expression of the CR the day of infusion. These results confirm the involvement of PL DA transmission on the extinction of CR, suggesting that a coordinated activity of D1 and D2 receptors in this area is necessary to promote the extinction.

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Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: National Institute on Drug Abuse DA011064

Title: Effects of a 5-HT1B receptor agonist on the acquisition and Expression of Methamphetamine-conditioned place preference in C57BL/6 MALE mice

Authors: *J. L. NEISEWANDER, T. DER-GHAZARIAN, 85282, D. CHARMCHI, S. NOUDALI, A. MAHMUD Sch. of Life Sci., Arizona State Univ., Tempe, AZ

Abstract: We previously reported that the 5-HT_{1B} receptor (5-HT_{1B}R) agonist CP94253 enhances cocaine self-administration and seeking in rats tested during daily self-administration sessions, but inhibits these behaviors when tested after a period of forced abstinence. Interestingly, when we replicated the experiment using methamphetamine (METH) we showed that CP94253 reduced METH intake both before and after an abstinence phase. This study investigated the effect of CP94253 on the expression as well as the acquisition of METH conditioned place preference (CPP) in c57 male mice. Conditioning took place in a 2compartment apparatus. First we assessed baseline preference during a 15-min free-access test, repeated on days 1-3. In the Expression experiment, conditioning occurred on days 4-7 with daily 30-min exposure to one compartment immediately after saline injection, and 5 h later exposure to the other compartment immediately after METH, 1 mg/kg, IP (i.e., total of 4 METH sessions). Subsequently, we verified CPP acquisition from a preference test on day 8, and then we assessed the effects of CP94253 on CPP expression on day 11. On the expression test day, mice were pretreated with either saline or CP94253 (10 mg/kg, IP) and given a preference test 30 min later. The Acquisition experiment followed the same procedure for conditioning and testing, except that only one daily 30-min exposure was given alternating between compartments across days (i.e., total of 2 METH 3 mg/kg, IP sessions). Also on each conditioning day, mice were pretreated with saline or CP94253 (10 mg/kg, IP) 30 min prior to the sessions. We found that CP94253 blocked the expression of METH CPP, but did not significantly alter acquisition. These findings suggest that CP94253 attenuates motivational effects of a METH-associated environment without requiring a period of abstinence and supports the idea that 5-HT_{1B}R agonists may be useful for treating METH dependence.

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Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: DA003906

DA012513

DA015369

Title: Integrins and focal adhesion kinase as a signaling pathway for MMP-9 induction of transient synaptic plasticity in cocaine relapse

Authors: *C. GARCIA-KELLER, *C. GARCIA-KELLER, D. NEUHOFER, M. SCOFIELD, A. BOBADILLA, S. SPENCER, S. VARANASI, C. MONFORTON, T. REEVES, P. W. KALIVAS Neurosci., Med. Univ. of South Caroline, Charleston, SC

Abstract: Cocaine use elicits neuroplasticity within the nucleus accumbens core (NAcore) that leads to increased vulnerability to relapse, even after protracted abstinence. Matrix metalloproteinases (MMPs) are inducible endopeptidases that degrade extracellular matrix (ECM) proteins (such as fibronectin, laminin and thrombospondin) as well as non-ECM signaling molecules, and reveal an RGD domain that binds and signals through integrins.

Integrins are heterodimeric receptors composed of $\alpha\beta$ subunits, and their primary signaling kinases are the focal adhesion kinase (FAK) and integrin linked kinase (ILK). Previous results show that $\beta3$ integrin is upregulated after cocaine self-administration and MMP-9 activity is increased during cued-reinstatement of cocaine and promotes transient synaptic plasticity (t-SP: increases in spine head diameter (d_h) and AMPA/NMDA (A/N)). Here we endeavor to understand if $\beta3$ integrin signaling through FAK and cofilin (actin depolymerization factor) is necessary to promote synaptic growth and increased AMPA/NMDA ratio during t-SP produced during drug-seeking.

To study the increases of d_h and A/N induced by MMP activation we use an antisense morpholino to reduce the expression of β 3 Integrin during cued-reinstatement. β 3 Integrin downregulation reduced cued-reinstatement, d_h and A/N compared with scramble morpholino. Additionally, microinjection of a FAK inhibitor into the NAcore blocked cue-reinstated behavior, while ILK inhibitor was without effect on reinstatement. Importantly, neither the FAK inhibitor nor β 3 Integrin morpholino impacted cued sucrose-seeking following intra-NAcore microinjection. Immunohistochemistry on NAcore labeled spines with AAVC2-hSYN-ChR2-YFP virus, revealed increased p-FAK and p-Cofilin localization in dendritic spines of reinstated cocaine animals compared to extinguished and yoked-saline. Moreover, using tissue plasminogen activator (t-PA) that increase MMPs activation, MMP-9 in particular, induced increase cued-reinstatement that was blocked with β 3 Integrin morpholino pre-treatment. These data propose that β 3 Integrin, FAK and Cofilin constitute a signaling pathway downstream of MMPs activation that is involved in promoting transient synaptic growth induced by cocaineconditioned cues that reinstate drug seeking.

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Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

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Program#/Poster#: 331.13/RR26

Topic: G.08. Drugs of Abuse and Addiction

Title: The effects of different environmental conditions and abstinence periods on sucrose seeking and glutamate receptors

Authors: *B. A. HUMBURG, E. J. GARCIA, A. N. BEESLEY, M. E. CAIN Psychological Sci., Kansas State Univ., Manhattan, KS

Abstract: Long periods of abstinence following repeated drug use results in an increase in drug seeking behavior. This increase in drug seeking is termed incubation of drug craving. Grimm et

al. (2008) found that an enrichment intervention after sucrose self-administration decreased sucrose seeking compared to the control rats. Recent data from our laboratory indicate that enrichment throughout the experiment reduces amphetamine seeking but does not change AMPA of mGlur expression. The current experiment examined if continuous enrichment housing could reduce sucrose seeking behavior when compared to isolated housing after 1 day and 40 days of forced abstinence. Additionally, we examined AMPA and metabotropic glutamate receptors (mGur) to determine if they changed in accordance with sucrose seeking in differently reared rats. Male Sprague-Dawley arrived at 21 days of age and reared for 30 days in either an isolated (IC) or an enriched condition (EC). The IC rats were housed in metal cages with one rat per cage and did not get handled during the 30 day rearing period. The EC rats were housed with cohorts and handled every day for about a minute with daily toy changes. After rats came of age they began lever press training with 20% sucrose on an FR-1 schedule. Once the rats reached stable responding, they began sucrose self-administration. They self-administered 20% sucrose for 15 days with 1-hr sessions each day on an FR-1 schedule. After the 15th day of self-administration, rats rested for 1 day or 40 days were then tested for sucrose seeking test. Rats were presented with a cue associated with sucrose reinforcement and active lever presses resulted in a cue but no sucrose. Immediately following each sucrose seeking test the brains were removed and the nucleus accumbens was dissected. A western blot was used to determine if there were any difference in the AMPA subunits (GluA1 and GluA2) and mGluR5 receptor expression. Results indicate that IC rats had more seeking after 1 day when compared to EC rats. After 40 days, sucrose seeking was not different between EC and IC rats. EC and IC rats did not express GluA1 and GluA2 AMPA subunits differently at any time point. After 40 days mGlur5 increased in both EC and IC rats. These results indicate that AMPA subunit plasticity in the nucleus accumbens may be specific to psychomotor stimulants. The mGlur5 expression increase is attributable to reduced reward seeking. Future research will examine different abstinence periods to determine how differential rearing alters seeking and receptor expression across varying abstinence periods.

Disclosures: B.A. Humburg: None. **E.J. Garcia:** None. **A.N. Beesley:** None. **M.E. Cain:** None.

Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: American Psychological Association Dissertation Award

Kansas State University Arts and Sciences small research grant

Title: The effects of differential rearing and abstinence period on amphetamine seeking and glutamate receptors

Authors: *E. J. GARCIA, M. E. CAIN Kansas State Univ., Manhattan, KS

Abstract: Cues associated with drug reinforcement can invigorate drug seeking behavior. The magnitude of invigoration changes depending on abstinence period length. The time-dependent increase in drug seeking is termed the incubation of drug craving. Following cocaine and methamphetamine self-administration and prolonged abstinence, there is also a time-dependent increase in Ca²⁺ permeable AMPA receptors (CP-AMPA). Blocking CP-AMPA receptors or stimulating metabotropic glutamate 1 receptors mitigates cue-induced drug seeking behavior. Environmental enrichment (EC) also decreases cue-induced drug seeking. Enrichment during abstinence can also reduce the incubation of drug craving. However, many of these studies have examined cocaine. In the present study, we used amphetamine (0.1 mg/kg/infusion) and reared rats in the EC or isolated condition (IC) to determine if early life development changes the propensity to show an increased drug seeking response motivated by drug-associated cues. Male Sprague-Dawley rats were housed in an EC or IC condition beginning at postnatal day 21 and remained in their conditions throughout behavioral testing. At day 51, rats were trained to press a lever and a week later, were implanted with indwelling jugular catheters. Rats were allowed to self-administer amphetamine or saline for 1h for 16 sessions. After the last session, rats went through a 1 day abstinence period and were tested in a cue-induced seeking test. The nucleus accumbens (NAc) was dissected from half the rats and prepared for western blot. The other half of rats had a 40-day abstinence period and were tested again in the cue-induced seeking test followed by NAc dissection. GluA1 and GluA2 AMPA subunits and mGlur1 and mGlur5 were quantified using western blot. Results indicated that EC and IC rats had equivalent amphetamine self-administration across the sessions. Following 1 day of abstinence, IC rats had significantly more drug seeking during the cued seeking test. This significant increase in IC rats was also present on day 40, indicating that IC rearing significantly increases drug seeking motivation following short and long abstinence periods. There were not significant differences between any of the glutamate receptors measured, suggesting that amphetamine seeking may not result in similar AMPA receptor and mGlur receptor trafficking as cocaine. In conclusion, our results clearly indicate that EC rearing significantly reduces amphetamine seeking behavior at short and long abstinence intervals, even when baseline amphetamine exposure is equal. Thus, the propensity to show enhanced susceptibility to drug cues is moderated by early life development.

Disclosures: E.J. Garcia: None. M.E. Cain: None.

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 331.15/RR28

Topic: G.08. Drugs of Abuse and Addiction

Title: The impact of sex and exercise on methamphetamine preference in a rat animal model

Authors: *M. PURPURA¹, P. VIEIRA³, C. BARKAS², J. ADAMS², T. E. KIPPIN⁴ ¹Psychological and Brain Sci., ²Psychological & Brain Sci., Univ. of California Santa Barbara, Santa Barbara, CA; ³Col. of Natural and Behavioral Sci., California State University, Dominguez Hills, Los Angeles, CA; ⁴Dept Psychololgical and Brain Sci., Univ. California, Santa Barbara, Santa Barbara, CA

Abstract: Methamphetamine (METH) abuse remains an extremely serious problem in the United States. METH has a long duration of action, and therefore leads to prolonged stimulant effects. Currently, there is limited information on the individual differences in vulnerability to substance use disorder (SUD) and how subject factors may impact the selection of METH over a competing reinforcer. To explore factors contributing to addiction vulnerability for METH in an animal model, we compared sex, dose (0.05 and 0.1 mg/kg/inf IV), short (20 s) or long (10min) inter-trial intervals (ITI) and the effects of exercise on the behavioral allocation of effort on drug taking. If the reward for exercise and drug taking have overlapping circuitry, then engaging in exercise may reduce the need for drugs. Rats were allowed voluntary exercise in Whamann wheels (6 hr/day, 4 weeks) followed by assessment of choice between METH (1 mg/kg/inf IV, FI20s) or food (2-3x45 mg, FI20s) reinforcement in standard operant chambers. There was a significant interaction between sex and dose (F,145)=5.765, p=0.021. ITI of 20 s and dose of 0.1 mg/kg/inf was the most preferred combination and females exhibited higher and more plastic (i.e. dose dependent) selection between METH over a competing reinforcer. There was a negative correlation between running wheel rotations/hour and preference for METH, and a significant effect of treatment (exercise versus control) amongst the METH preferring rats F(1,11)=5.133, p=0.045. Thus engagement of voluntary exercise may decrease vulnerability and increase resilience to the development of a SUD amongst a subpopulation that is initially METH preferring. Generally, the results of this study are consistent with the growing body of clinical and preclinical evidence demonstrating that females exhibit higher addiction vulnerability for stimulant drugs of abuse.

Disclosures: M. Purpura: None. **P. Vieira:** None. **C. Barkas:** None. **J. Adams:** None. **T.E. Kippin:** None.

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 331.16/RR29

Topic: G.08. Drugs of Abuse and Addiction

Support: Department of Education Award #P031S150199

Title: Cue-Induced methamphetamine seeking behavior is reduced by disruption of memory reconsolidation through the NMDA receptor antagonist memantine

Authors: *M. HANNA, R. CARPENTER, M. JESKE, C. KEITH, L. RIZKALLA Vanguard Univ., Costa Mesa, CA

Abstract: Relapse rates for those struggling with drug addictions are relatively high due to strong craving induced by exposure to cues that have been previously associated with drug administration. Drug associated memories are formed through the process of consolidation, the conversion of memories from a fragile state to a stable state. Reconsolidation claims that when a stable, long-term memory is reactivated, it undergoes consolidation again in which the memory engram is in an unstable form. It has been shown that consolidation requires the activation of the glutamate receptor NMDAR to initiate cellular processes that leads to stable synapses. In this study we examined whether the NMDA receptor plays a role in the reconsolidation of long-term drug-associated memories. To address this question, rats were injected with the NMDA receptor antagonist memantine in a conditioned place preference paradigm. Multiple injections of memantine immediately after reactivation of drug-paired memories attenuated preference for the drug-associated compartment. Our data also showed that the attenuating effects of memantine on cue-induced methamphetamine seeking behavior lasted for several weeks. Finally, we also show that the time frame for reconsolidation interference with memantine is between 0 to 6 hours. Our data suggest that the reconsolidation of methamphetamine-associated memories are dependent on NMDA receptor activation.

Disclosures: M. Hanna: None. R. Carpenter: None. M. Jeske: None. C. Keith: None. L. Rizkalla: None.

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA033877

NIH Grant GM100829

Title: Effects of nicotine exposure on oral methamphetamine self-administration, extinction, and reinstatement in adolescent rats

Authors: *Z. R. HARMONY, E. M. ALDERSON, I. GARCIA, L. D. BITUIN, C. A. CRAWFORD

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Abstract: Adolescence is a vulnerable developmental period, especially in regard to the use of addictive drugs. Nicotine is particularly problematic, as there are clinical studies indicating that early nicotine exposure increases the likelihood of later illicit drug use. Recently, we found that adolescent exposure to a low dose of nicotine increased the intake of methamphetamine (METH) in adult male rats. The purpose of the present study was to determine if METH intake during adolescence would also be altered by nicotine exposure. Male and female Sprague-Dawley rats were pretreated with saline or nicotine (0.16 or 0.64 mg/kg, sc) for 10 consecutive days beginning on postnatal day (PD) 25. On PD 35, rats in the 0.16 and 0.64 mg/kg pretreatment conditions were evenly divided and assigned to groups that either received saline or continued to receive the same nicotine dose that they were given as adolescents. Rats that had received saline as adolescents either continued to receive saline or were injected with 0.16 or 0.64 mg/kg nicotine. These late adolescent drug treatments started on PD 35 and continued until the end of the experiment. Thus, there were a total of 7 groups: SAL-SAL, 0.16-0.16, 0.16-SAL, SAL-0.16, 0.64-0.64, 0.64-SAL, SAL-0.64. On PD 35, all rats began nose poke training. Rats were exposed to a METH fade in, sucrose fade out procedure across 5 different METH-sucrose combinations. This procedure resulted in exposure to a 40 mg/l METH solution for 3 consecutive days on a FR2 schedule. Following the last day of METH self-administration, rats were given extinction training. Once the extinction criteria were met, rats were given a priming injection of METH (1.0 mg/kg, ip). Rats given continuous nicotine exposure (0.16-0.16) during self-administration had attenuated METH intake, whereas rats only pre-exposed to nicotine during early adolescence (i.e., the 0.16-SAL group) did not show this effect. Conversely, pre-exposure to a high dose of nicotine (i.e., the 0.64-0.64 and 0.64-SAL groups) augmented METH intake during selfadministration. This enhancement was larger in female rats than males. Lastly, neither dose of

nicotine altered behavior during extinction or reinstatement. These data suggest that exposure to a high dose of nicotine during early adolescence enhances METH intake later in adolescence.

Disclosures: Z.R. Harmony: None. **E.M. Alderson:** None. **I. Garcia:** None. **L.D. Bituin:** None. **C.A. Crawford:** None.

Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 331.18/RR31

Topic: G.08. Drugs of Abuse and Addiction

Title: Neural basis of methamphetamine anticipation in a volitional self-administration paradigm

Authors: *R. SILVER^{1,3,4,2}, C. JUÁREZ-PORTILLA³, R. D. KIM⁴, M. PITTER⁴, P. PATEL⁴, R. A. LEDESMA², J. LESAUTER⁴

²Psychology, ¹Columbia Univ., New York, NY; ³Investigaciones Biomédicas, Univ. Veracruzana, Xalapa, Mexico; ⁴Psychology, Barnard Col., New York, NY

Abstract: Background: The ability to sense time and anticipate events is critical for survival in nature. Not surprisingly, anticipatory behaviors occur prior to availability of numerous reward stimuli, including food, water, sex, and drugs of abuse, such as methamphetamine (MA). When available daily, at a regular time of day, animals exhibit anticipatory behaviors, and waken from sleep hours prior to the availability of rewards such as food. Goal: In the present study we evaluated whether animals that have voluntary daily access to MA in early or late day, would anticipate the availability of the drug. We also analyze c-FOS expression in the brain to identify the neural circuitry associated with anticipation of reward availability. Method: To this end, C57/BL6 mice were given free access to nebulized MA or water, for one hour daily either in the early or late day for 14 days. On day 15, at the usual time of availability of nebulized MA, animals were euthanized, and brains were removed and processed for c-FOS immunoreactivity. **Results**: The results indicate that anticipatory behaviors occur in animals with access to MA, but not water (controls), with more anticipatory activity during the early day than in the late day. In parallel, during the anticipatory interval, prior to exposure to MA, c-FOS expression was greater in the early versus the late day. Importantly, FOS-ir was greater in the MA groups than in controls at both early and late times. The brain regions associated with anticipatory activity have greater expression of FOS in early vs. late day, and in animals having access to MA rather than water. Conclusion: In summary, mice that voluntarily choose to self-administer nebulized MA will anticipate its availability by self-awakening and increasing their locomotor activity. The results also point to the importance of time of day, with substantially more anticipatory behavior early vs late in the day. Finally, this work delineates brain regions implicated in anticipatory behavior.

Disclosures: R. Silver: None. C. Juárez-Portilla: None. R.D. Kim: None. M. Pitter: None. P. Patel: None. R.A. Ledesma: None. J. LeSauter: None.

Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 331.19/RR32

Topic: G.08. Drugs of Abuse and Addiction

Title: Co-administration of GABAB receptor agonist baclofen with 5-HT2C receptor agonist Ro 60-0175 has additive effects on the expression of amphetamine-induced locomotor sensitization in rats

Authors: *L. N. CEDILLO ZAVALETA, J. C. JIMENEZ, I. R. RUIZ-GARCIA, A. I. BARRIENTOS-NORIEGA, F. MIRANDA-HERRERA UNAM, FES Iztacala, Estado De México, Mexico

Abstract: Drugs of abuse, such as cocaine, amphetamine (AMPH) and methamphetamine, share the ability to activate the mesolimbic dopamine (DA) system. The behavioral effects of AMPH are largely mediated by increased DA neurotransmission in the nucleus accumbens which is an important locus for regulation of motor function and for rewarding influence of psychostimulants drugs. Recent evidence suggests a potential role for γ -aminobutyric acid B (GABAB) receptor in modulating some of the behavioral effects of psychostimulants. We previously reported that GABAB receptor agonist baclofen (BCF) reduced the discriminative stimulus properties of AMPH and the development and expression of AMPH-induced locomotor sensitization. Although it has also been reported that BCF produces adverse side effects such as musclerelaxant properties, sedative and hypothermic effects, which limit its widespread application as a therapeutic agent in humans and as a tool for behavioral research. On the other hand, several lines of evidence suggest that 5-HT2C receptor ligands modulate the behavioral effects of psychostimulants. For example, it has been reported that 5-HT2C receptor agonist Ro 60-0175 (Ro) reduced the behavioral effects of psychostimulants. One strategy for attempting to overcome the side effects of BCF is to use lower dosages to reduce unwanted effects of the BCF, in combination with 5-HT2C receptor agonist, such as Ro. This study was designed to examine the effects of co-administration of BCF and Ro on the expression of AMPH-induced locomotor sensitization. On development phase rats were treated with AMPH (1 mg/kg, ip, experimental groups) or saline (control group) for 5 days. On the test days (expression phase), separate groups of sensitized rats (n=10 for each dose) were treated with saline or BCF (2.0, 3.0 y 4.0 mg/kg) + AMPH (1.0 mg/kg), Ro (2.0, 3.0 y 4.0 mg/kg) + AMPH (1.0 mg/kg) or the co-administration of BCF (3.0 mg/kg) and Ro (2.0, 3.0 y 4.0 mg/kg) + AMPH (1.0 mg/kg). The results showed that BCF and Ro produced a dose-dependent prevention of the expression of AMPH-induced

locomotor sensitization. Furthermore, Ro increased the effects of a lower dose of BCF on the expression of AMPH-induced locomotor sensitization. These data provide further evidence that GABA-B receptor ligands in combination with 5-HT2C receptors ligand may modulate psychostimulant-induced behaviors.

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Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA013476

Title: Methamphetamine exposure and withdrawal impact gut microbiota in rats

Authors: S. FOROUZAN¹, *O. OHIA-NWOKO², T. A. KOSTEN¹ ¹Psychology, ²Dept. of Psychology, Univ. of Houston, Houston, TX

Abstract: Methamphetamine (MA) is one of the most frequently used amphetamine-type stimulants in the United States. Individuals who repeatedly abuse MA can develop MA use disorder (MuD)—a chronic, relapsing condition often triggered by withdrawal symptoms that develop following cessation of use. Given the lack of effective treatments for those with MuD, novel therapeutic targets must be considered. One potential target is the gut microbiome, which has an important influence on brain, behavior, and health as a part of the gut-brain axis. In this study, we evaluated the effects of MA administration (2 mg/kg, s.c.) on withdrawal- induced behaviors and gut bacteria in female Sprague-Dawley rats (n=8). Saline (s.c.) was administered twice daily for 14 days, during which baseline behaviors (elevated plus maze (EPM) for anxietylike behavior; forced swim test (FST) for depressive-like behavior) were assessed. Next, rats were given twice daily injections of MA for 14 days, followed by 4 days of withdrawal, during which performance on the EPM and FST were assessed. Fecal collections for microbiome analyses occurred at baseline (before saline administration), on day 5 of saline administration, day 14 of MA administration, and day 4 of withdrawal. Results indicated that MA withdrawal increased depressive-like behavior, with an increase in immobility time in the FST (p<0.05). Anxiolytic-like behavior was increased during withdrawal, indicated by a decrease in time spent in the closed arms of the EPM (p<0.05). Fecal microbiome analyses revealed MA administration and withdrawal significantly changed the relative abundances of several bacterial phyla including an increase in *Firmicutes* (p<0.05, vs. saline) and *Actinobacteria* (p<0.05, vs. saline) and a decrease in Cyanobacteria (p<0.05, vs. saline). No significant changes in Shannon

diversity were observed. Taken together, our observations indicate MA exposure and withdrawal produce specific and lasting changes in gut bacteria. Such information will guide the choice of target therapeutics for MuD to test in future studies.

Disclosures: S. Forouzan: None. O. Ohia-Nwoko: None. T.A. Kosten: None.

Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

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Program#/Poster#: 331.21/RR34

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R15DA041618

Title: Behavioral characterization of outbred mice for amphetamine withdrawal: A GWAS pilot study

Authors: ***J. T. DO**¹, *J. T. DO¹, R. AMBACHEW¹, P. KUMAR¹, E. MASIAS¹, K. SHARIF¹, M. THOMAS¹, K. WILSON¹, J. YUAN², C. C. PARKER³

¹Program in Neurosci., ²Program in Mol. Biol. and Biochem., ³Dept. of Psychology and Program in Neurosci., Middlebury Col., Middlebury, VT

Abstract: Negative mood states that characterize drug withdrawal are partly under genetic control and have been associated with craving and relapse to drug use in humans. Mice can be used to model aspects of the negative mood states associated with amphetamine (AMP) withdrawal and offer a number of advantages relative to studies in humans. Here, we investigated negative mood states associated with AMP withdrawal using commercially available, outbred CFW mice to evaluate their usefulness as a mapping population for future quantitative trait locus (QTL) studies. Outbred mice are powerful populations for fine mapping of QTLs because each individual possesses a large number of recombinations over the course of many generations of outbreeding. These recombinations break down linkage disequilibrium between the QTL and surrounding markers, allowing high resolution mapping of the variants that influence quantitative traits. Mice were tested in the Elevated Zero Maze (EZM), Porsolt Forced Swim Test (FST), and Sucrose Preference Test to assess changes in anxiety-like behavior, dysphoria, and anhedonia following 14 consecutive days of 2.5 mg/kg AMP administration via intraperitoneal injection. A paired-samples t-test was conducted for each of these assessments. There was a significant increase in FST immobility time from before AMP administration (M = 125.75, SD = 65.46) to after (M = 166.43, SD = 44.34), t (45) = -6.45, p < 0.0001, suggesting the mice exhibited dysphoric behavior due to AMP withdrawal. Interestingly, EZM results showed a significant decrease in the time spent in closed quadrants from baseline (M = 222.08, SD =48.70) to withdrawal (M = 180.13, SD = 76.55), t (45) = 3.42, p < 0.001, suggesting a reduction

in anxiety-like behavior after AMP administration. A significant difference was also found in the animals' corrected sucrose preference from before administration (M = 52.56, SD = 25.10) to after (M = 52.55, SD = 26.40), t (42) = -3.19, p < 0.003, indicating a similarly unexpected reduction in anhedonic behavior. Importantly, we observed tremendous variation in all three traits which enables genetic mapping of naturally occurring genetic variation that is associated with trait variation. We predict that the phenotypic diversity displayed by this highly recombinant population will facilitate the discovery of genes and biological pathways underlying drug use disorders in humans.

Disclosures: J.T. Do: None. R. Ambachew: None. P. Kumar: None. E. Masias: None. K. Sharif: None. M. Thomas: None. K. Wilson: None. J. Yuan: None. C.C. Parker: None.

Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 331.22/RR35

Topic: G.08. Drugs of Abuse and Addiction

Title: Voluntary physical exercise abolishes the development of conditioned hyperactivity and behavioral sensitization in mice

Authors: *A. S. RAUHUT, A. STASIOR

Psychology, Dickinson Col., Carlisle, PA

Abstract: This experiment, using an animal model of drug addiction (i.e. behavioral sensitization), examined the impact of voluntary physical activity (i.e., home-cage wheel running) on the acquisition, extinction and behavioral sensitization in male, Swiss Webster mice. The experiment consisted of 4 phases: initial exercise, acquisition, extinction, and tests for behavioral sensitization. Upon arrival, half of the mice either were given access to home-cage running wheels (runners) or not (sedentary) for 3 weeks prior the start of the acquisition phase. During the acquisition phase, mice received either an injection (subcutaneous, s.c.) of physiological saline (vehicle) or methamphetamine (0.25 or 1.0 mg/kg) immediately prior to 5 daily locomotor activity sessions. During the extinction phase, all mice received daily injections (s.c.) of physiological saline prior to 4 locomotor activity sessions. Locomotor activity sessions were 30 minutes in duration for the acquisition and extinction phases. The tests for sensitization occurred following the extinction phase. At this time, all mice received a once daily injection of methamphetamine according to an escalating drug-dose regimen (0.25, 0.5 and 1.0 mg/kg) and tested for 60 minutes. Distance traveled served as the dependent measure of locomotor activity.

Support: This research was supported financially by Student-Faculty Research funds provided by Dickinson College

The results showed that methamphetamine dose-dependently increased locomotor activity in sedentary mice during acquisition; however, runners showed a blunted hyperactive response to the high methamphetamine dose (1.0 mg/kg) following either acute (Day 1) or repeated (Day 5) methamphetamine administration. During the extinction phase, conditioned hyperactivity was observed initially for the sedentary mice conditioned with either the low or high methamphetamine doses and quickly extinguished. However, the runners did not show conditioned hyperactivity following conditioning with any methamphetamine dose. Finally, sedentary mice showed a dose-dependent sensitized response, with the low and moderate methamphetamine doses inducing greater locomotor activity compared to sedentary vehicle control mice. However, runners did not differ following any methamphetamine challenge dose on the tests for sensitization. These results suggest that voluntary physical exercise may have a neuro-protective effect and blunt the unconditioned, conditioned and sensitized locomotor effects of methamphetamine.

Disclosures: A.S. Rauhut: None. A. Stasior: None.

Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 331.23/RR36

Topic: G.08. Drugs of Abuse and Addiction

Title: Investigation of the cellular mechanisms of the discriminative stimulus effects and hyperlocomotion induced by methamphetamine using the "On cell" analysis

Authors: *T. MORI, A. KAN, C. IWASAWA, M. SASAKI, M. WATANABE, Y. HAMADA, M. NARITA, N. KUZUMAKI, M. NARITA Hoshi University/Department of Pharmacol., Tokyo, Japan

Abstract: Although the analysis of the behavioral effects induced by methamphetamine could provide evidences for the pharmacological mechanism of its behavioral effect of methamphetamine, specific activated cells and/or cellular interactions following methamphetamine treatment is not fully elucidated yet. In the drug discrimination study, the D₁-receptor agonist SKF82958 fully substituted for the discriminative stimulus effects of methamphetamine in mice, indicating that activation of D₁-receptors is critical for the discriminative stimulus effect of methamphetamine. To pin-down the cellular mechanism of methamphetamine-induced discriminative stimulus effects, we performed the "On cell" analysis. Methamphetamine-induced hyperlocomotion was suppressed by an optical suppression of the nucleus accumbens in cFos-eNpHR mice, which had been exposed by methamphetamine to express eNpHR. Furthermore, the D₁-receptor agonist SKF82958-induced c-fos positive "On cells" in the nucleus accumbens were mostly merged to those induced by methamphetamine. We

are now investigating the cellular identification of methamphetamine-activated "On cells" to produce the methamphetamine-induced discriminative stimulus effects and hyperlocomotion. Investigation of the cellular mechanisms of the behavioral effect of methamphetamine using the "On cell" analysis".

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Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

Location: Halls A-C

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA036582

Title: DREADD-mediated modulation of the subthalamic nucleus alters locomotor sensitization to amphetamine in rats

Authors: *K. G. NAKATA^{1,2}, E. YIN³, S. M. FERGUSON^{1,2,4}

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Abstract: Addiction to stimulant drugs like amphetamine is a debilitating neuropsychiatric disorder that carries with it substantial medical, social, and monetary costs for both addicted individuals and society. It is well established that the basal ganglia play essential roles in the development and maintenance of addiction-related behavior. However, although the subthalamic nucleus (STN) serves as a significant interface within basal ganglia circuitry and is a critical node of the indirect striatal pathway, its role in addiction behavior has been relatively understudied. In addition, studies to date have reported conflicting findings, likely due to the methods for targeting and disrupting cellular activity within the STN. To address these issues and further characterize the role of the STN and its afferents in addiction to stimulant drugs, we used viral expression of DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) to transiently modulate activity of either STN neurons or cortical afferents to STN (i.e., the hyperdirect pathway) during the development of amphetamine-induced locomotor sensitization in adult male Sprague Dawley rats. Stimulation of STN neurons (via Gq-coupled DREADDs (AAV-hSyn-hM3Dq) and clozapine-N-oxide (CNO)) had no effect on the acute locomotor response to amphetamine; however, this manipulation completely blocked the development and persistence of locomotor sensitization. In addition, repeated STN stimulation induced mild hyperactivity in amphetamine-free DREADD controls. In contrast, inhibition of

STN neurons (via Gi/o-coupled DREADDs (AAV-hSyn-hM4Di) and CNO) enhanced amphetamine-induced locomotor sensitization, though this effect did not persist on an amphetamine challenge following two weeks of drug abstinence. In addition, inhibition of STN neurons had no effect on the acute locomotor response to amphetamine, nor did it alter locomotor activity in controls. Next, we used a Cre-recombinase-dependent dual-virus approach to target DREADDs to STN afferents originating in prelimbic cortex (PL). Preliminary data suggest that although transient inhibition of PL-STN afferents with CNO has no effect on the development of amphetamine sensitization during the induction phase, it does attenuate the persistence of this form of behavioral plasticity. As with STN inhibition, this manipulation in afferents had no effect on the initial locomotor response to amphetamine or on locomotion in controls. These results suggest that the STN and its cortical afferents play important, but complex, roles in the regulation of addiction-related behavior and could be relevant targets for future addiction treatments.

Disclosures: K.G. Nakata: None. E. Yin: None. S.M. Ferguson: None.

Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 331.25/SS2

Topic: G.08. Drugs of Abuse and Addiction

Support: University of Vermont

Title: Effects of estradiol and methamphetamine exposure on habit formation in rats

Authors: *H. SCHOENBERG¹, E. SOLA¹, J. MLCUCH¹, A. P. KIRSHENBAUM², D. J. TOUFEXIS¹

¹Psychological Sci., Univ. of Vermont, Burlington, VT; ²Psychology, St. Michael's Col., Colchester, VT

Abstract: One aspect of drug addiction may be an accelerated progression from goal-directed behavior to stimulus-directed (habitual) behavior. The psychostimulant methamphetamine (METH) changes dopaminergic pathways in the dorsolateral striatum (DLS), which mediates habitual motor behavior. It has been previously shown that methamphetamine pre-exposure accelerates habit formation in males, and this was related to increased dopamine availability in the DLS. Data also suggests that changes in estrogen levels across the estrous cycle can also increase dopamine availability in the DLS in females. Thus, we hypothesized that accelerated habit formation would be induced by pre-exposure to METH in ovariectomized (OVX) female rats with cyclic estradiol replacement compared to OVX females with no replacement. We tested habitual nose-poking for sucrose following 8 days of pre-exposure to METH or vehicle and sub-

threshold operant training. The replacement group received bolus injections of proestrus levels of estradiol every four days to mimic naturally cycling estradiol. Following acquisition, one half of each group received five days of reward-devaluation (RD) by pairing lithium chloride (LiCl) with sucrose availability. The remaining rats received five days of LiCl unpaired with sucrose. Rats were then tested in extinction. Our results indicate that, at this low level of training, methamphetamine with or without cycling estrogen was not sufficient to accelerate habit formation, as all animals exhibited sensitivity to devaluation of sucrose. This evidence suggests a potential sex difference in habit formation, since methamphetamine exposure has previously been found to push males into habit at the same degree of operant training.



Figure 1. Within each group, all paired and unpaired animals differed significantly at the .05 level

Disclosures: H. Schoenberg: None. E. Sola: None. J. Mlcuch: None. A.P. Kirshenbaum: None. D.J. Toufexis: None.

Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 331.26/SS3

Topic: G.08. Drugs of Abuse and Addiction

Support: P20 MD0022290

Title: Environmental enrichment: Role in anxiolysis and resilience to amphetamine

Authors: *S. DONALDSON¹, C. CALHOUN¹, S. FISCHER¹, S. KELSEY¹, B. PLOTKIN² ¹Developmental and Brain Sci., ²Developmental and Brain Sciences, Psychology Dept., Univ. of Massachusetts Boston, Boston, MA

Abstract: Few have examined trait anxiety as a risk factor for amphetamine (AMPH) sensitivity in pre-clinical models or evaluated whether environmental enrichment (EE) impacts this vulnerability. We investigated trait anxiety rats' response to anxiogenic stimuli and AMPH, and measured dendritic arborization. Filial 5 outbred high anxiety (HAn) and low anxiety (LAn) male rats (N=60) were housed in: EE, standard environment (SE) and isolated environment (IE) for 30 days. Anxiogenic responses were measured using the elevated plus maze (EPM) and open field activity (OFA); locomotor (LMA) responses were recorded after saline (0.9% NaCl IP) or AMPH (0.5 mg/kg IP). At the end of testing, all animals were transcardially perfused with saline followed by 8% paraformaldehyde and the brains were extracted, quick-frozen, and stained using the Golgi-Cox protocol for Sholl analysis on six neurons per region (e.g., medial prefrontal cortex, basolateral amygdala, and the paraventricular nucleus of the hypothalamus). Neurons were imaged and analyzed using light microscopy and CellTarget software; both the Branching Index (BI), and Schoenen Ramification Index (SRI) were employed for all analyses. Behavioral results revealed that EE significantly reduced anxiety like behavior (p < 0.001) on the EPM and OFA, and attenuated AMPH hyperactivity (p<0.0001) in HAn lines. Interestingly, EE increased BI and SRI in the BLA of LAn and HAn lines (p<0.05) in the BLA. These findings demonstrate a role for EE in mitigating the effects of trait anxiety on anxiety-like behavior and AMPH sensitivity. EE may exert these benefits, at least in part, as a consequence of promoting neuroplasticity in the BLA.

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Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIMH T32-MH016804

NIMH RO1-MH101180

Title: Diazepam reverses anxiety-like behavior, social anhedonia and dopamine deficit following acute amphetamine withdrawal

Authors: *M. RINCÓN-CORTÉS, A. A. GRACE

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Abstract: Psychostimulants, including amphetamine (AMPH), increase dopamine (DA) release from ventral tegmental area (VTA) neurons, which is associated with their acute reinforcing actions. However, this positive state is followed by a negative affective state each time the drug is taken (i.e. opponent process theory). Indeed, AMPH-withdrawal is accompanied by symptoms of anxiety and depression, which are commonly associated with DA system dysfunction in humans and animal models. Importantly, although most studies have focused on the negative affective state after withdrawal from long-term (i.e. chronic) drug administration, this negative affective state appears even after a drug is taken for the first time in both humans and rodents. In male rats, acute AMPH-withdrawal increases FST immobility and decreases the number of spontaneously active VTA DA neurons up to 48 hours post-withdrawal. Here we extended these findings by assessing social anhedonia in the three-chambered social approach test and anxietylike behavior in the elevated plus maze (EPM) following acute AMPH withdrawal within this period. Acute AMPH withdrawal induced social anhedonia (i.e. less sniff time), and increased anxiety-like behavior in the EPM (p<0.05, n=6-9 per group), suggesting a link between increased anxiety and impaired social interaction. Given that benzodiazepines are commonly used to treat anxiety disorder and can treat post-acute amphetamine withdrawal syndrome in humans, we tested the effects of diazepam on anxiety-like and social behaviors and conducted extracellular recordings of VTA DA neurons. We show that an acute (5mg/kg) dose of diazepam circumvents the neurobehavioral effects resulting from acute AMPH-withdrawal, as demonstrated by decreased anxiety-like behavior, normal social behavior and VTA DA activity comparable to controls (i.e. non AMPH-withdrawn rats) (p<0.05, n=6-8 per group). These data suggest that negative affective states resulting from AMPH-withdrawal can be modulated by diazepam administration and a highlight a window of time during which effective treatment may be able to prevent neurobehavioral changes that promote transition into chronic use.

Disclosures: M. Rincón-Cortés: None. A.A. Grace: 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.; Lundbeck. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Lilly, Autofony, Johnson and Johnson. F. Consulting Fees (e.g., advisory boards); Johnson and Johnson, Lundbeck, Pfizer, GSK, Merck, Takeda, Dainippon Sumitomo, Otsuka, Lilly, Roche, Asubio, Abbott.

Poster

331. Amphetamines: Reinforcement, Seeking, and Reinstatement

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 331.28/SS5

Topic: G.08. Drugs of Abuse and Addiction

Support: The University of St. Thomas Startup Laboratory Funding

Title: The effects of methamphetamine exposure on anxiety-like behavior and corticosterone levels in adolescent and adult mice

Authors: J. H. WEISS, K. H. STRUNTZ, *J. A. SIEGEL Psychology and Neurosci., The Univ. of St. Thomas, Saint Paul, MN

Abstract: Methamphetamine (MA) has neurotoxic effects on the adult brain that can lead to deficits in behavior and changes in the stress response system. The effects of MA are modulated by age, and while much research has examined the effects of MA in adults, relatively little research has examined the effects of MA in adolescents. As the brain is developing during adolescence, the effects of MA may differ from the effects in adults. Previous research shows that acute MA exposure in adolescence increases anxiety-like behavior while acute MA exposure in adults decreases anxiety-like behavior. Acute MA exposure in adolescence does not alter plasma corticosterone levels while acute MA exposure in adults increases corticosterone levels. Furthermore, age modulates the effects of MA on the brain dopamine system, with adolescent rodents showing relative resistance to the neurotoxic effects of MA on the dopamine system compared to adults. To the best of our knowledge, no study has directly compared the acute effects of MA on anxiety-like behavior and corticosterone levels in both adolescent and adult mice together. In order to assess age differences in the response to MA, this research examines the acute effects of MA exposure during adolescence and adulthood on anxiety-like behavior, plasma corticosterone levels, and the brain dopaminergic system in adolescent and adult male C57BL/6J mice. Current experiments are ongoing to study the effects of adolescent and adult MA exposure on behavior in the open field test to evaluate locomotor activity and anxiety-like behavior. Plasma corticosterone and tyrosine hydroxylase levels will be measured following behavioral testing. Based on previous data, we expect MA to cause an increase in anxiety-like behavior in adolescent mice and a decrease in anxiety-like behavior in adult mice, and to have no effect on plasma corticosterone levels in adolescent mice and increase plasma corticosterone levels in adult mice. Furthermore we predict that MA will have no effect on tyrosine hydroxylase levels in adolescent mice, but will decrease tyrosine hydroxylase levels in adult mice. These findings will contribute to a greater understanding of how MA alters behavior, the stress response system, and the dopamine system and how these effects of MA may differ between adolescent and adult mice.

Disclosures: J.H. Weiss: None. K.H. Struntz: None. J.A. Siegel: None.

332. Cocaine and Behavior

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 332.01/SS6

Topic: G.08. Drugs of Abuse and Addiction

Support: VA BLR&D IK2BX002531

Title: Acute sleep deprivation accelerates the development of locomotor sensitization to cocaine

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Abstract: With respect to drugs of abuse, sensitization is a progressive increase in drug effect following repeated drug exposures thought to be due to adaptations in the mesocortical dopamine system. Repeated administration of cocaine results in a conditioned increase in locomotor activity. There is some contradictory evidence that sleep disruption, either REM deprivation or total sleep deprivation, can influence locomotor sensitization to amphetamine. We have preliminary data indicating that acute sleep deprivation increases the acquisition and expression of conditioned place preference (CPP) to cocaine and now we are testing whether acute sleep deprivation increases locomotor sensitization to cocaine.

Adult male C57BL/6 mice underwent a locomotor sensitization procedure in which there were 3-5 saline administration days (habituation phase), 5 cocaine administration days (initiation phase), and 1 additional cocaine administration day a week after the initiation phase (challenge phase). Mice were injected intraperitoneally with saline on the habituation days and cocaine (15mg/kg) on each cocaine administration day. After injection, mice were placed into activity boxes (Med Associates) for 1 hour and activity was assessed by infrared beam breaks. Habituation criteria consisted of a decrease in beam breaks from the first to last habituation days and from the second-to-last to last habituation days. Mice that did not meet these criteria after 5 days were excluded. After habituation, mice were divided into two groups. One group of mice was sleep deprived via the treadmill method of forced, slow walking for 4 hours immediately prior to each cocaine trial, while the other group was undisturbed.

We found that mice that were sleep deprived prior to the cocaine-paired trials showed an increase in sensitization during the initiation phase, but sensitization was similar between groups during the challenge phase. Furthermore, day to day comparisons suggest that the increase in sensitization following sleep deprivation is due to a large increase in activity from the first to second cocaine exposures. These data suggest that sleep deprivation may speed the development of sensitization.

Disclosures: T.E. Bjorness: None. R.W. Greene: None.

332. Cocaine and Behavior

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 332.02/SS7

Topic: G.08. Drugs of Abuse and Addiction

Support: R21 DA042599

Title: Contribution of sex chromosome complement to sex differences in cocaine induced locomotor sensitization

Authors: *M. MARTINI¹, W. J. LYNCH², E. F. RISSMAN¹

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Abstract: Repeated exposure to drugs of abuse such as cocaine, enhances their motor-stimulant response, a phenomenon termed behavioral sensitization. Females show a greater locomotor response to cocaine and more robust behavioral sensitization than males. This sex difference is attributed to circulating estrogens. In our study, we aim to examine sex differences induced by cocaine from a different perspective. We use a novel mouse model, the four core genotype (FCG) mouse, that has a spontaneous mutation in the sex determining Sry gene and a transgene that replaces its function. By breeding wild type females to males with the mutation and the transgene we obtained four types of offspring: XX-males, XY-males, XX-females, XY-females. This model allows us to separate the actions of gonadal hormones from the potential effects of sex chromosome genes on cocaine induced locomotor sensitization and to test the novel hypothesis that sex chromosome complement underlies sex differences in cocaine effects. Cocaine-HCl (20mg/kg) was dissolved in saline (0.9%) and administered i.p. in a volume of 0.1 ml/10 g body weight (BW). Mice were tested between 60-80 days of age. Sensitization to locomotor responses induced by repeated cocaine treatment was evaluated using Open Field (OF) boxes. For each genotype, Group 1 (n = 7, cocaine group, Coc) received cocaine (20) mg/kg) for 5 days and a single cocaine challenge (20mg/kg) on day 15 after a 10-day drug-free period. Group 2 (n = 7, control group, Sal) received saline for 5 days and a single cocaine challenge (20 mg/kg) on day 15. Mice were routinely habituated to the test cage (OF) for 30 min, then saline and cocaine injections were administered and locomotor activity was recorded for 1 h. The comparison between locomotor activity on day 1 and day 5 after cocaine administration determines the induction phase of behavioral sensitization. The response to cocaine challenge 10 days after discontinuation of saline/cocaine administration (day 15) was compared to i) the response to cocaine on day 5, and ii) the response of saline controls that will receive cocaine challenge for the first time on day 15.

Using the FCG mouse, we are examining the independent contribution of chromosomal sex to

cocaine sensitization. If sex chromosome complement is implicated, this will open up a new source of genetic studies on drug addiction.

Disclosures: M. Martini: None. W.J. Lynch: None. E.F. Rissman: None.

Poster

332. Cocaine and Behavior

Location: Halls A-C

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Program#/Poster#: 332.03/SS8

Topic: G.08. Drugs of Abuse and Addiction

Support: FAPEAL/PPSUS

CAPES

Title: Crack cocaine inhalation decreases the threshold for epileptic seizures in rats subjected to a subconvulsive dose of pilocarpine

Authors: *C. D. CAVALCANTE^{1,2}, J. F. SANTOS², A. L. D. PACHECO², I. S. MELO², M. A. COSTA², J. M. SILVA³, S. S. MACHADO³, A. U. BORBELY², O. W. CASTRO² ¹UFAL, Maceió, Brazil; ²Inst. of Biol. Sci. and Hlth., ³Inst. of Chem. and Biotech., Federal Univ. of Alagoas, Maceió, Brazil

Abstract: Propose: Crack cocaine is a powerful addictive stimulant drug. Animal studies have been shown that cholinergic system may play a role in neurotoxicity induced by cocaine or its active metabolites inhalation. Behavioral alterations of using crack cocaine include hyperactivity, depressed mood, and decreased seizure threshold. Here we evaluate the acetylcholinesterase (AChE) activity, behavior and the threshold for epileptic seizures in rats exposed to crack cocaine smoke followed by a subconvulsive dose of pilocarpine. Methods: Experimental procedures were approved by the Ethical Committee for Animal Research of UFAL. Male Wistar rats (n=48 [240-340g]) were submitted to stereotaxic surgery for implantation of a cannula into the hippocampus. Animals received microinjections of subconvulsive pilocarpine (PILO [0.75mg/µL]) or vehicle (VEH [saline 0.9%]). Immediately after PILO administration animals were individually exposed to air (O2 + PILO) or 400 mg of crack cocaine smoke (VEH + CRACK and PILO + CRACK) for 10 minutes. We analyzed, the number of Wet Dog Shakes (WDS), frequency, duration and severity of limbic seizures according to Racine's Scale (1972), during 120 min after crack cocaine exposure. Acetylcholinesterase activity (n=24) was performed after 60 min of crack cocaine exposure in cell-free extracts of brain tissue as described by Ellman et al. (1961) and total contents of the extracts were determined according to the method of Bradford (1976). Results were expressed as mean±SEM, compared by one-way ANOVA followed by post-hoc test Student-Newman-Keuls. Results: Animals subjected to PILO

+ CRACK demonstrated increased severity of limbic seizures (stages 0-4) in comparison to O2 + PILO and VEH + CRACK (stages 0-2). PILO + CRACK (107.7 \pm 24.41) displayed higher seizure frequency than O2 + PILO (24.83 \pm 6.44) and VEH +CRACK (11.43 \pm 2.44). Similarly PILO + CRACK revealed increase in duration of seizures (2138 \pm 723,9) when compared with O2 + PILO (442,0 \pm 198,5) and VEH +CRACK (11.43 \pm 2.44). Nonetheless, number of WDS decreased in CRACK+ VEH (8,00 \pm 2,646) and O2 + PILO (127,3 \pm 52,73) compared to PILO + CRACK (162,79 \pm 45,40). The AChE activity showed reduction in PILO + CRACK (0,0475 \pm 0,0030) when compared to O2 + PILO (0,07827 \pm 0,0019) and VEH +CRACK (0,0697 \pm 0,0021) indicating inhibition of AChE. **Conclusion**: Together, these preliminary data suggest that crack cocaine inhalation decreases the threshold for epileptic seizures in rats submitted to a subconvulsive dose of pilocarpine, supposedly through the inhibition of acetylcholinesterase.

Disclosures: C.D. Cavalcante: None. J.F. Santos: None. A.L.D. Pacheco: None. I.S. Melo: None. M.A. Costa: None. J.M. Silva: None. S.S. Machado: None. A.U. Borbely: None. O.W. Castro: None.

Poster

332. Cocaine and Behavior

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 332.04/SS9

Topic: G.08. Drugs of Abuse and Addiction

Title: Dopamine transporter knockout rats as a model of dopamine transporter hypofunction

Authors: *M. PARDO¹, S. IZENWASSER², P. ILLIANO¹, R. R. GAINETDINOV³, D. C. MASH¹

²Psychiatry & Behavioral Sci., ¹Univ. of Miami, Miami, FL; ³Inst. of Translational Biomedicine, St. Petersburg State Univ., St. Petersburg, Russian Federation

Abstract: Dysregulated dopamine transporter function contributes to the pathophysiology of many psychiatric disorders, including substance abuse, attention deficit disorder, and bipolar mania. Functional hyperdopaminergia has been characterized in dopamine transporter (DAT) knock-out mice. DAT knock-out mice exhibit extreme phenotypes, including decreased dopamine uptake, hyperactivity, exaggerated responses to psychostimulants and altered parameters of dopamine homeostasis. Rodent models of hyperdopaminergia related to genetic deletion of the DAT have been studied only in mice (Gainetdinov et al., 1999). Here, we report on a novel rat model of functional hyperdopaminergia. Behavioral and neurochemical studies demonstrate a consistent hyperdopaminergic state in DAT knock-out rats. We studied DAT hypofunction rats in early (PND35) and mid adolescence (PND48). Homozygous DAT-KO rats (KO) have increased basal locomotor activity due to increased levels of synaptic dopamine. Basal locomotor activity in heterozygous DAT knock-out rats (HET) was unchanged from wild-

type (WT) rats. However, HET rats showed an exaggerated locomotor response compared to WT rats following acute administration of cocaine (10 and 20 mg/kg). This effect was more robust at early adolescence (PND35) compared to mid adolescence (PND48). Sex differences were observed in HET rats following cocaine administration with acute cocaine treatment producing an earlier time to peak locomotor response in female HET rats at PND35, compared to WT rats. In contrast, male HET rats had a sustained increase in locomotion after acute cocaine treatment that remained elevated for at least 60 minutes. In Homozygous DAT knock-out rats, acute cocaine produced dose dependent changes in locomotor activity, since cocaine increased locomotion at 10mg/kg and reduced locomotion at a higher dose 20mg/kg. However, both doses decreased locomotion in homozygous DAT knock-out female rats. Studies are underway to examine the neuroplastic changes in dopamine homeostasis that occur in DAT knock-out rats with chronic cocaine self-administration. We hypothesize that DAT knock-out heterozygous rats may show exaggerated responses to cocaine that model the loss of transporter function observed in people at risk for cocaine-related excited delirium syndrome. This neuropsychiatric disorder is characterized by dysregulation of dopamine, defective regulation of DAT and a functional state of hyperdopaminergia (Mash et al., 2009). Reverse translational strategies will explore the role of DAT mutation in response to stress and psychostimulants to develop a rodent model of excited delirium syndrome.

Disclosures: M. Pardo: None. **S. Izenwasser:** None. **P. Illiano:** None. **R.R. Gainetdinov:** None. **D.C. Mash:** None.

Poster

332. Cocaine and Behavior

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA032789

Title: Sex differences in cocaine-induced ultrasonic vocalizations are modulated by the medial preoptic area

Authors: *J. R. MARTZ, N. MITTAL, C. L. ROBISON, C. L. DUVAUCHELLE, J. M. DOMINGUEZ

The Univ. of Texas At Austin, Austin, TX

Abstract: Evidence indicates that the medial preoptic area (mPOA) in the hypothalamus plays a modulatory role in the regulation of cocaine response, both neural and behavioral activity, as evidenced by conditioned-place preference and microdialysis experiments. The mPOA is also a major regulator of sex-sensitive behavioral differences across various species and for a variety of

behaviors. In rodents, a commonly employed approach for inferring affective states involves measuring their ultrasonic vocalizations (USVs). In this set of experiments we tested whether the mPOA modulates cocaine-induced changes in USVs and possible sex differences that may occur in response to drug administration. To this end, Sprague-Dawley rats received either a lesion or sham lesion of the mPOA. Baseline USV measures were obtained, over 3-days, followed by subsequent sessions of cocaine administration, and USV recordings. Results indicate that females displayed a greater number of vocalizations compared to males; however, this sex difference disappeared with lesions of the mPOA. Cocaine increased USVs in both males and females, compared to baseline. As with baseline, this increase was greater in females than in males, but also disappeared with lesions of the mPOA. These findings point to the mPOA as a modulator of affective response to cocaine and, moreover, as a modulator of sex differences that manifest after cocaine administration.

Disclosures: J.R. Martz: None. N. Mittal: None. C.L. Robison: None. C.L. Duvauchelle: None. J.M. Dominguez: None.

Poster

332. Cocaine and Behavior

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 332.06/SS11

Topic: G.08. Drugs of Abuse and Addiction

Title: The antioxidant Tempol abolished the expression of cocaine conditioned reward

Authors: *T. BEISER, R. NUMA, R. KOHEN, R. YAKA The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: Cocaine abuse induces toxic effects in many organs, including the brain. In the last two decades it has been discovered that oxidative stress (OS) is the inducer and augmenter of cocaine toxicity and cocaine-induced addictive behaviors. In order to study the mechanism and find pharmacotherapy to drug addiction, it is essential to understand how cocaine-induced OS interferes with behavior and which pharmacological agent can prevent this interference. In previous studies we demonstrated that acute or chronic administration of cocaine generates substantial OS in main areas of the reward system, such as the pre frontal cortex (PFC) and the nucleus accumbens (NAc). In addition, we found that i.p administration of the antioxidant Tempol prior to cocaine injection attenuated cocaine-induced elevation of OS in these areas. Moreover, we found that administration of Tempol in this manner attenuated cocaine-induced psychomotor sensitization in rats and prevented the increase of OS in the PFC and NAc. In the present study we hypothesized that Tempol negatively affects the rewarding properties of cocaine. Therefore, we aimed at testing the effects of Tempol using conditioned place preference (CPP) paradigm. We found that treatment with Tempol during conditioning decreased cocaine-

induced OS in the PFC and NAc. In addition we found that in rats treated with Tempol, a significant decrease in the expression of CPP was evident. Further, we show that repeated injections of Tempol (7 days) following withdrawal from cocaine CPP abolished the expression of CPP. Finally, we show that the cocaine-induced CPP decreases the phosphorylation of the eukaryotic elongation factor 2 (eEF2) in the NAc which was remarkably restored following Tempol treatment. Taken together, this data suggests that Tempol given during conditioning and following withdrawal can interfere with reward processing in the mesolimbic system in a mechanism that may involve the translation machinery. Further, our results pave the way for the development of new therapy based on reducing OS to prevent cocaine addiction.

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Poster

332. Cocaine and Behavior

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Topic: G.08. Drugs of Abuse and Addiction

Support: Mark Diamond Research Fund Grant

Title: Neuropeptide Y blocks the expression of a Pavlovian-conditioned locomotor response in rats with a 3-week history of cocaine exposure

Authors: *M. SUAREZ¹, A. C. THOMPSON²

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Abstract: Our lab has been exploring the hypothesis that NPY may be a target for cocaine dependence treatment. We have found that intracerebroventricular (ICV) administration of NPY, in rats with a history of cocaine exposure, decreases the expression of cocaine-induced conditioned place preference (CI-CPP). Furthermore, this decrease is long-lasting; a single ICV NPY injection blocked the expression of CI-CPP on a subsequent test one week later. Here we tested whether the acute and long term effect of ICV NPY on the expression of CI-CPP generalizes to another conditioned response: cocaine-induced Pavlovian conditioned locomotor response (CI-PCLR). We tested the hypothesis that ICV NPY will block the expression of CI-PCLR in rats with a history of cocaine exposure. One week later, rats were then tested again for the expression of CI-PCLR, every other day for 5 days, to determine whether the initial single ICV NPY exposure induces a persistent inhibition of CI-PCLR. Male Long-Evans (hooded) rats were exposed to daily injections of cocaine for 21 days using an escalating cocaine dose regimen (5 to 30 mg/kg, IP). CI-PCLR training occurred over the last 4 days of cocaine treatment and involved 2 conditioning sessions a day: one session with cocaine and one session with saline. We

used an opaque Plexiglas Locomotor Box, (LMB; 39 cm X 39 cm X 30.5 cm) as a unique training context. On training days half of the rats received cocaine in the LMB (i.e. the Paired group) and saline in a familiar context, while the other half received saline in the LMB and cocaine in a familiar context (i.e., the Unpaired group). NPY (0, 0.1, or 1 nmol in 5μ l) was microinjected 30 min before a 30-min, cocaine-free, test session in the LBM (i.e. first CI-PCLR test) 24 h after the last training day. As expected, rats that received cocaine in the LMB demonstrated a robust CI-PCLR, compared to saline controls, and showed extinction with repeated testing. Both doses of NPY treatment attenuated the CI-PCLR at test 1 in the Paired group but had no effect on rate of extinction (i.e. did not produce a persistent effect). NPY had no effect on the locomotor response of the Unpaired group at any dose on any test day. These results support the idea that central administration of NPY decreases the expression of at least two cocaine-induced conditioned behaviors, but that the persistent decrease seen in CI-CPP does not generalize to CI-PCLR. Consideration of the differences in learning and behavioral expression between these two conditioning paradigms may suggest a more specific role of NPY in the treatment of cocaine dependence.

Disclosures: M. Suarez: None. A.C. Thompson: None.

Poster

332. Cocaine and Behavior

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Title: Male offspring of cocaine-experienced sires have impaired cocaine-induced behavioral sensitization

Authors: *A. S. ELLIS¹, M. E. WIMMER¹, F. M. VASSOLER², S. L. WHITE³, H. D. SCHMIDT⁵, S. SIDOLI⁴, Y. HAN⁴, B. A. GARCIA⁵, C. PIERCE³ ¹Psychology&Neuroscience, Temple Univ., Philadelphia, PA; ²Dept. of Biomed. Sci., Tufts Univ. Cummings Sch. of Vet. Med., North Grafton, MA; ³Ctr. for Neurobio. and Behavior, Dept. of Psychiatry, ⁴Epigenetics Institute, Dept. of Biochem. and Biophysics, Perelman Sch. of Med. at the Univ. of Pennsylvania, Philadelphia, PA; ⁵Dept. of Biobehavioral Hlth. Sci., Sch. of Nursing, Univ. of Pennsylvania, Philadelphia, PA

Abstract: In a 2015 survey, 1.5 million Americans were reported to be using cocaine. Environmental stimuli, including cocaine, produce heritable changes to the genome via epigenetic mechanisms. Studies investigating transgenerational effects of drugs have mostly focused on maternal use and prenatal exposure. Our lab and others have recently focused on paternal phenotype transmission. We used a rat model of paternal cocaine self-administration to examine cocaine-induced behavioral plasticity in male and female offspring of sires with a history of cocaine taking. Additionally, we assessed changes in histone post-translational modifications (PTMs), an epigenetic process that regulates gene expression by controlling the accessibility of genes to transcriptional machinery. Previously, our group found that paternal cocaine exposure reduced the reinforcing effects of cocaine in male, but not female offspring. Here, we examined cocaine-induced locomotor sensitization. Repeated exposure to cocaine produces behavioral sensitization, which is characterized by increased locomotor response to subsequent stimulant challenge injection. We found that male, but not female, progeny show deficits in cocaine sensitization, indicating a resistance in the long-lasting behavioral responses following cocaine exposure. Paternal cocaine experience has been shown to change histone modifications in the hippocampus and prefrontal cortex. Here we used a mass spectrometrybased approach to survey histone PTMs in the nucleus accumbens (NAc), a brain region critical for the rewarding effects of cocaine. Five histone PTMs were found to be consistently different between saline- and cocaine-sired males, suggesting that targeted epigenetic remodeling is responsible for the behavioral effects observed in F1 male progeny. We also examined cocaine self-administration and sensitization in grand-offspring (F2 generation) of cocaine-exposed sires to further investigate the transmission of altered behavioral responses to cocaine. Importantly, no effect of ancestral cocaine experience was found on sensitization or self-administration in the F2 generation. These data suggest that paternal cocaine exposure remodels the epigenome of F1 male progeny, resulting in blunted responses to cocaine.

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Poster

332. Cocaine and Behavior

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Topic: G.08. Drugs of Abuse and Addiction

Support: KAKENHI Grant 16K10197

Title: Fingolimod attenuate cocaine induced locomotor activity by decreasing PKA/DARPP32 signaling in striatal D₁-type medium spiny neurons

Authors: *K. UEMATSU¹, T. SHUTO², N. UCHIMURA¹, A. NISHI² ¹Cognitive and Mol. Res. Inst. of Brain Dis., ²Dept. of Pharmacol., Kurume Univ. Sch. of Med., Kurume, Fukuoka, Japan

Abstract: Fingolimod (FTY720) is an agonist of sphingosine-1-phosphate receptors (S1PRs) and a new oral drug for multiple sclerosis. It has been reported that S1PRs are expressed in many cell types including neurons and glias in the brain. In this study, we investigated the effect of fingolimod on cAMP/PKA signaling in mouse striatal slices by monitoring the phosphorylation states of an intracellular phosphoprotein, dopamine- and cAMP-regulated phosphoprotein of M_r 32 kDa (DARPP-32) at Thr34 (PKA-site). In striatal slices prepared from D₁-DARPP-32-Flag/D₂-DARPP-32-Myc transgenic mice, fingolimod (100 nM) decreased P-Thr34 DARPP-32 in D₁-type/striatonigral neurons at 30 min to 60% of control. The effects of cocaine and a D1 receptor agonist were examined after 30 min of preincubation with fingolimod. The cocaine (100 μ M)- and a dopamine D₁ agonist (±)-SKF-81297 (1.0 μ M)-induced increases in P-Thr34 DARPP-32 were abolished by pre-treatment of slices with fingolimod (100 nM). In behavioral studies, pretreatment with fingolimod (1.0 mg/kg, i.p.) attenuated cocaine (20 mg/kg, i.p.)- and R(+)-SKF-81297 (0.7 mg/kg, i.p.)-induced locomoter activities. Thus, fingolimod inhibits cAMP/PKA signaling in striatonigral neurons, resulting in the suppression of psychostimulant-induced activation of dopamine signaling and behavior.

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Poster

332. Cocaine and Behavior

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Topic: G.08. Drugs of Abuse and Addiction

Support: Psi Chi Undergraduate 2016 Spring Research Grant

Title: The long-term effects of repeated cocaine exposure on impulse inhibition

Authors: *N. MACK, M. BLOSSON, C. COWAN, M. CREWE, K. PONDER, S. SEQUERIA, D. HOLT, J. DYCHE James Madison Univ., Harrisonburg, VA

Abstract: The correlation between cocaine use and impulsivity has been well established but the causal relationship remains unclear. Are drug addicts innately more impulsive, or does drug use cause long lasting changes in impulsive behavior? It is established that repeated cocaine use results in long-term deficits to delayed reinforcement, but other facets of impulsivity have not been studied in depth. This experiment analyzes the long-term effects of chronic cocaine

exposure on impulse inhibition using differential reinforcement of low rates of responding (DRL) methodology. Sprag Dawley rats (N=16) were trained in operant chambers on a DRL-20 task and measured on efficiency. Following stabilization, each rat received two daily injections of either 15 mg/kg cocaine HCl or saline solution for 14 days total. Rats were re-tested on the DRL-20 on days 7, 14, and 21 following drug sensitization. A mixed ANOVA revealed no differences in DRL-20 efficiency across the study, f(1,4) = 0.008, p = 0.929. The results suggest that there are no short term nor long term effects on impulse inhibition following repeated cocaine exposure. The results are discussed considering validity of DRL methodology and implications of behavioral therapy for addicts. Future work includes analyzing inter-response time profiles as different measure of impulse inhibition and immunohistochemical analysis of D2 receptor expression in the nucleus accumbens core.

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Poster

332. Cocaine and Behavior

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Topic: G.08. Drugs of Abuse and Addiction

Support: NSERC

Title: Synaptic zinc modulates the effects of chronic cocaine exposure

Authors: *S. E. THACKRAY, S. S. BRYDEN, N. BIHELEK, R. H. DYCK, V. LOVIC Psychology, Univ. of Calgary, Calgary, AB, Canada

Abstract: Zinc (Zn) is a divalent metal that plays critical roles in many biological processes throughout the body. In the brain, it is packaged into vesicles and released into synapses by a subset of neurons that also release glutamate. Synaptic Zn acts as a potent neurotransmitter/neuromodulator, exerting its action by binding to Zn-specific sites on several neurotransmitter receptors. In addition, Zn can enter post-synaptic cells and affect intracellular signaling cascades. Most zincergic neurons are in the forebrain, almost exclusively in cerebral cortex, hippocampus and amygdala. Although the striatum itself does not house zincergic neurons, it is densely innervated by zincergic afferents. Cortico-striatal circuits are important in learning and control of actions, including responses to drugs of abuse. As a first step to understanding the role of Zn signaling in striatal function, we assessed acute and chronic effects of cocaine exposure in zinc transporter 3 (ZnT3) knockout (KO) mice. ZnT3 is the sole protein responsible for packaging zinc into synaptic vesicles. In normal mice, acute cocaine exposure leads to increased locomotor activity, while repeated administration, additionally, results in

behavioral sensitization and alterations in morphology of medium spiny neurons (MSNs) in the striatum. Locomotor activity was recorded and quantified in female ZnT3 wildtype (WT) and KO mice in response to cocaine administered at 6 doses compared to control animals administered vehicle. Specifically, the mice in cocaine groups were administered 5 mg/kg cocaine i.p. on experimental day (E) 1& 2, 10 mg/kg on E3 & 4, 15 mg/kg on E5, 20 mg/kg on E6 & 7, 25 mg/kg on E8, and 30mg/kg on E9 &10. All mice were left untreated for 14 days, then challenged with a dose of 10mg/kg cocaine to assess sensitization (% change from initial exposure). The recordings were screened for instances of stereotypy. The mice were euthanized and the brains processed for Golgi-Cox staining to examine MSN morphology. All mice exhibited dose-related increases in locomotor activity, however ZnT3 KO mice traversed significantly lower distances at higher doses of cocaine (≥ 20 mg/kg) compared to WT mice. These differences were not attributed to differential expression of non-ambulatory behaviors/stereotypies (eg. grooming, digging, circling). Both ZnT3 WT and KO mice exhibited behavioral sensitization with no genotype by drug interaction. In addition, dendritic length of MSNs in ZnT3 KO and WT mice was increased in the cocaine groups compared to control groups. In conclusion, synaptic zinc is important for modulating cocaine-induced locomotor activity, but does not appear to affect sensitization to cocaine.

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Poster

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Title: Error monitoring deficits in cocaine addicted individuals with high trait anger

Authors: *M. A. PARVAZ, R. Z. GOLDSTEIN, N. ALIA-KLEIN Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Compromised error processing is a hallmark of substance use disorders and of heightened trait anger. However, it is not known whether this deficit is exacerbated in addicted individuals with high trait anger and whether such comorbid symptomology influences drug taking behavior. To fill this knowledge gap, we recruited 33 individuals with cocaine use

disorders (CUD) and 34 healthy controls (HC) who were further stratified into low (LA) and high anger (HA) groups by way of a median split on the State-Trait Anger Expression Inventory trait anger scores with comparable non-verbal IQ and education but significantly different age [HC-LA (n=18; 43.9±6.9 years); HC-HA (n=16; 37.5±9.2 years); CUD-LA (n=16; 50.9±6.8 years); and CUD-HA (n=17; 47.3±7.3 years)]. Drug use severity was assessed using the Severity of Dependence Scale in all CUD. All participants performed a Simon's Flanker Task during the acquisition of event-related potentials (ERPs). The response-locked error-related negativity (ERN; quantifies early subliminal error monitoring), error positivity (Pe; quantifies more conscious error sensitivity) and the total number of errors were used as dependent variables. Although drug use severity did not differ between CUD-LA and CUD-HA, there was a preponderance of comorbid alcohol and other drug use in the CUD-HA (12/18) compared to the CUD-LA (4/16). For dependent variables, a 2 (Cocaine: CUD, HC) \times 2 (Anger: LA, HA) ANOVA, with age as covariate, revealed significant Cocaine (p=.004), and Anger (p=.008) main effects such that the ERN was reduced and number of errors were increased in CUD (vs. HC, p < .014) and in HA (vs. LA, p < .043); there was also a trend for reduced Pe in CUD (vs. HC, p=.074). A follow-up one-way ANOVA across all 4 groups (HC-LA, HC-HA, CUD-LA, and CUD-HA, to explore the additive effect of cocaine use and high anger), also with age as covariate, revealed significant linear effects for all dependent variables. That is, HC-LA showed the highest ERN and Pe amplitudes and least number of errors, CUD-HA showed the most reduced ERN and Pe amplitudes and highest number of errors, while HC-HA and CUD-LA showed comparable effects. Together, these results indicate that while both cocaine use and high trait anger negatively impact error-processing, their comorbid symptomology further worsens these impairments. Because these individuals also present an increased proclivity for using other substance of abuse, results underscore the potential contribution of deficits in error processing and underlying neural underpinnings to the exacerbation of symptoms in addiction, calling for the development of targeted intervention approaches for this subgroup of CUD.

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Poster

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DA039821

DA043030

Title: Cannabidiol attenuates expression of cocaine conditioned place preference with a U-shaped dose-response profile

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Abstract: Cannabidiol (CBD), the major non-addictive and non-toxic component of cannabis sativa, may have potential for relapse prevention in addiction. Our previous findings demonstrated that a transdermal cannabidiol preparation (tCBD) attenuated both cue- and stressinduced drug seeking. These findings suggested that tCBD may reverse neuroplasticity that underlies perseverative drug seeking. These longitudinal studies included only one dose of tCBD such that no dose-response information was available. However, measures of plasma and brain CBD levels revealed that maximal attenuation of drug seeking occurred before CBD in plasma and brain reaches maximal levels, and that at maximal plasma and brain levels CBD did not further increase the reduction of drug seeking. We therefore sought to characterize the doseresponse profile of tCBD's attenuation of drug seeking by establishing the effects of five tCBD doses on the expression of cocaine conditioned place preference (CPP). Male Wistar rats were initially habituated to a two-chamber CPP apparatus for seven days to measure the animals' inherent bias toward one chamber over the other. The rats then underwent ten daily conditioning sessions, alternating between chambers each day, for a total of five sessions per chamber. Following conditioning, rats entered a 4-day treatment phase during which they received one of five tCBD doses or vehicle (0.0mg/kg, 2.5mg/kg, 5.0mg/kg, 7.5mg/kg, 10.0mg/kg, 15.0mg/kg). Expression of CPP was measured on the fourth day of treatment. Consistent with the hypothesis, tCBD suppressed the expression of CPP along a U-shaped dose-response profile, with maximal efficacy at 7.5 mg/kg. These findings continue to support potential for CBD in the attenuation of drug seeking and relapse prevention. Although CBD's dose effects were U-shaped with significant suppression of drug seeking at intermediate doses, the highest tCBD dose was associated simply with a lack of effect, but not aggravation of CPP (i.e. drug seeking). It is not clear presently whether U-shaped effects apply to other actions of CBD such as amelioration of anxiety or cue- or stress-induced reinstatement of drug seeking, and these questions remain for future research.

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Poster

332. Cocaine and Behavior

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA R01-DA031734

Title: Stress-induced cocaine bingeing in rats: Role of mesolimbic dopamine

Authors: *M. Z. LEONARD, M. H. DAWES, J. F. DEBOLD, K. A. MICZEK Tufts Univ., Medford, MA

Abstract: Intermittent exposure to brief episodes of social defeat stress precipitates long-term modulation of mesolimbic dopamine activity in rats. These adaptations are mediated via CRF activity within the VTA, and are associated with an intense cocaine-taking phenotype; whereby rats with a history of social stress exhibit an extended duration of cocaine self-administration under continuous access conditions. Factors that contribute to the maintenance and termination of these binge-like episodes are not well-understood. As mesolimbic DA signaling appears to be critical to the maintenance of self-administration behavior, we aim to determine the extent to which augmented dopaminergic response to cocaine might promote sustained bingeing in stressed rats. To that end, rats were exposed to social defeat stress intermittently over 10 days, or handled (Ctrl). After one week, rats were implanted with microdialysis guide cannulae targeting the NAcSh, and trained to self-administer cocaine on a fixed-ratio (FR5) schedule of reinforcement. In vivo microdialysis was conducted throughout a 24-hour cocaine 'binge' (FR5, 0.3mg/kg), where dialysate was collected in 20 min. fractions and analyzed for DA content. These ongoing experiments gather compelling evidence to suggest a pattern of DA efflux that predicts the maintenance/termination of cocaine bingeing in both stressed, and non-stressed animals. Parallel studies also examine the acute contributions of VTA-CRF as a driver of extended cocaine binges through modulation of DA activity during self-administration. Together, these studies approach a mechanism by which upregulated CRF signaling in the VTA might potentiate binge-like cocaine consumption in individuals with a history of stress exposure.

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Poster

332. Cocaine and Behavior

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant R01 DA006886-19

Title: The emergence of negative affect as motivation for drug taking in rats chronically selfadministering cocaine
Authors: *S. D. KLEIN¹, J. KULIK², N. BEACHER², A. P. PAWLAK¹, M. O. WEST³ ²Behavioral Neurosci., ³Psychology, ¹Rutgers Univ., Piscataway, NJ

Abstract: A large body of work examining ultrasonic vocalizations (USVs) emitted by rats in relation to psychostimulant drug administration has demonstrated that rising drug level leads to an increase in 50-kHz calling (positive affect), while acute withdrawal induces an increase in 22kHz calls (negative affect). In the context of self-administration studies, the role of negative affect as a motivational factor in animal models of drug addiction has been underexplored. The present investigation explored the relationship between the magnitude of affective response, and the quantity of cocaine consumption. Rats underwent a 14-day self-administration (SA) paradigm in which cocaine availability was indicated by the presentation of a 30-second tone cue. 50-kHz calls were measured prior to the introduction of cocaine, during the load-up period of days 1 and 14 of SA, and during an equivalent time period during the first day of abstinence. 22-kHz calls were measured prior to the introduction of cocaine, during the entire first day of abstinence, and a 2-hour window following day 14 of the SA paradigm dubbed "Withdrawal". Experimental results showed a highly statistically significant increase in 50-kHz calling between baseline and the load-up periods of self-administration. Results also showed a highly statistically significant increase in 22-kHz calling between baseline and forced abstinence, but not between baseline and withdrawal. Taken together, the results suggest that negative affect emerges during acute withdrawal when animals are expecting cocaine. Our findings that an increase in negative affect when deprived of drug was directly related to drug intake, while concurrently, the direct relationship of positive affect to drug intake decayed, provides strong empirical evidence from an animal model for the widely hypothesized shift from positive to negative affect as the salient motivational factor in drug addiction as the disease progresses. These observations have implications for understanding the role of hedonic dysregulation in preclinical models of drug addiction.

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Poster

332. Cocaine and Behavior

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Topic: G.08. Drugs of Abuse and Addiction

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Title: Environmental enrichment during abstinence from cocaine self-administration alters cocaine-seeking behavior and addiction-related gene expression in female rats

Authors: *G. L. POWELL¹, M. ST. PETER², T. CHAUDHURY², D. ALCAZAR², R. M. BASTLE³, R. J. OLIVER, JR⁴, N. I. PERRONE-BIZZOZERO⁴, J. L. NEISEWANDER² ¹Sch. of Life Sciences, Dept. of Psychology, ²Sch. of Life Sci., Arizona State Univ., Tempe, AZ; ³Dept. of Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; ⁴Dept. of Neurosciences, Univ. of New Mexico HSC, Albuquerque, NM

Abstract: Our lab found that rats express an increase in cocaine-seeking following abstinence from drug and that this effect can be blunted when the rats are housed in environmental enrichment (EE) during abstinence. Much research has investigated changes in addiction-related genes associated with cocaine-seeking and EE; however, little of this work has been done in female rats, despite preclinical and clinical data suggesting sex differences in cocaine consumption and relapse patterns. Therefore, we aimed to investigate the effects of EE on cocaine-seeking behavior and addiction-related gene expression following abstinence in adult, female Sprague-Dawley rats. The rats were trained to press a lever for cocaine (0.75 mg/kg/infusion i.v., paired with a light + tone cue) during 2-hr sessions with a minimum of 21 sessions, during which they were individually housed. Upon stabilization of cocaine intake, animals were placed into abstinence for 21 days and either remained in isolated housing or were placed into EE chambers that contained 3-6 rats, a running wheel, tubes, toys, and communal food and water. After 21 days of abstinence, all animals returned to their self-administration cages for a 1-hr cue-reactivity test session, where cocaine-paired cues were presented responsecontingently but no cocaine was available. Rats were immediately sacrificed after the session, and brain tissue punches of the nucleus accumbens core (NAcore) and shell (NAshell) were collected and processed for RNA analysis using qRT-PCR. EE significantly blunted cocaineseeking behavior following 21 days of abstinence, comparable to results seen in male rats housed in EE vs. isolation for 21 days of abstinence. Additionally, qRT-PCR analysis of female rats housed in EE vs. isolation demonstrated significant alterations in multiple addiction-related genes: 1) Arc and Camk2a, both plasticity-associated genes, were significantly reduced in the NAcore but not the NAshell, in enriched animals; 2) miR-495, a known regulator of addiction related genes including Arc and Camk2a, demonstrated a trend toward an increase (p<0.06) in NAcore in enriched animals. These results represent the initial stages of discerning sex differences in cocaine-seeking behavior and the mechanisms that potentially modulate that behavior. We are now in the process of comparing gene profile changes in male and female rats using RNAseq with the aim of revealing new potential targets to reduce cocaine-seeking behavior.

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Poster

332. Cocaine and Behavior

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Support: CURE 2015

Title: Erk1/2 phosphatase (MKP3) expressed in dopaminergic neurons affects cocaine induced conditioned place preference and self-administration

Authors: *S. LEWANDOWSKI¹, D. L. BERNSTEIN², R. A. ESPAÑA², O. V. MORTENSEN¹ ¹Pharmacol. & Physiol., ²Neurobio. and Anat., Drexel Univ., Philadelphia, PA

Abstract: Psychostimulant addiction is a chronic relapsing disorder that is thought to occur in vulnerable individuals and is characterized by compulsive drug use despite consequences. Approximately 2.5 million people abuse cocaine every year, with only 800,000 of them being treated. Cocaine's primary molecular mechanism is to block the dopamine transporter (DAT), leading to excess dopamine (DA) in the synaptic cleft, and thus resulting in the euphoric "high" that is sought after during addiction. There are currently no FDA approved pharmacotherapies for cocaine use disorders, and therefore it is imperative to develop more viable treatment options. The ERK1/2 Map kinase signaling pathway has been implicated in the locomotor stimulant effects of psychostimulants but also cocaine induced psychomotor sensitization and cocaine induced conditioned place preference. However, limitations exist in these previous studies as they were based on systemic or local administration of kinase inhibitors and thus lack the anatomical specificity that is needed for a more detailed investigation of the role of ERK1/2 signaling in specific cell types. This is critical to identify specific downstream targets of this pathway to reveal novel therapeutic targets for treating cocaine use disorders. In this study we describe the modulation of the ERK1/2 pathway in vivo by expressing the ERK1/2 phosphatase MKP3 only in dopaminergic cells. We have generated adeno-associated viral (AAV) vectors with Cre-recombinase (Cre)-dependent expression of MKP3 resulting in a decrease of ERK1/2 signaling in dopaminergic cells in the ventral tegmental area (VTA). This construct was injected into the VTA of Long Evans rats expressing Cre in tyrosine hydroxylase positive cells (Th-Cre rats). We used synaptosomal uptake assays to evaluate the effect of this overexpression on DAT uptake function and sensitivity to cocaine, as well as examined the effects of this overexpression on cocaine induced conditioned place preference, a Pavlovian conditioning paradigm that assesses the rewarding and aversive properties of drugs. Animals underwent a pre-test, three days of saline/cocaine conditioning and a post-test to determine preference. Finally, a different cohort of animals underwent self-administration, an operant model of addictive behavior that

investigates the reinforcing properties of drugs. Results from the present study demonstrate specific relationships between ERK1/2 signaling in DA neurons and cocaine induced behaviors that model human substance abuse. We believe these studies could have potential for identifying novel therapeutic targets for the treatment of cocaine use disorders.

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Poster

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Title: Retinoic acid signaling regulates medium spiny neuron activity in the nucleus accumbens shell and downstream targets regulate depression-like and addiction-related behaviors

Authors: ***E. J. CROFTON**¹, M. N. NENOV², Y. ZHANG³, O. FOLORUNSO⁴, S. KOSHY⁵, D. LI⁵, J. D. HOMMEL², F. LAEZZA⁶, T. A. GREEN⁵

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Abstract: Psychiatric disorders including depression and addiction have proven difficult to treat, therefore, novel targets are critical for future development of effective treatments. We previously identified retinoic acid (RA) signaling as a novel regulator of behavior in preclinical models. Our previous transcriptomic identified the RA pathway as a likely mediator of the protective depression and addiction phenotypes conferred by environmental enrichment. In addition, a previous topographic transcriptomic analysis found that several genes in the RA pathway have selectively enhanced expression in the NAc shell (NAcSh). We also previously demonstrated

that manipulation of RA in the NAcSh by in vivo knockdown of cytochrome p450, family 26, subfamily b, polypeptide 1 (CYP26b1), which degrades RA, altered anxiety-like, depressionlike, and addiction-related behaviors. Here we aimed to provide further evidence for the role of RA signaling in the NAcSh by investigating the downstream signaling partners of retinoic acid through patch-clamp electrophysiological analysis of medium spiny neurons (MSNs) as well as behavioral analysis. Cellular retinoic acid binding protein 2 (CRABP2) and fatty acid binding protein 5 (FABP5) both bind RA, delivering RA to one of two downstream signaling pathways, either retinoic acid receptor (through CRABP2) to induce transcription through retinoic acid receptor response elements or peroxisome proliferator receptor beta/delta (through FABP5) to signal through peroxisome proliferator response elements. Knockdown of CRABP2 in the NAcSh decreased action potential threshold and increased the number of evoked action potentials while knockdown of FABP5 decreased the number of action potentials without altering threshold. CRABP2 knockdown also potentiated sEPSC amplitude but neither knockdown altered sEPSC frequency. Evaluation of the effects of acute retinoic acid on medium spiny neurons in the NAcSh are ongoing. In behavioral tests, CRABP2 knockdown decreased depression-like behaviors with no effect on addiction-related behaviors, while FABP5 knockdown decreased cocaine self-administration with no effect on depression-like behaviors. These compelling results are the first demonstration of a role of RA signaling in controlling the input and output strength of MSNs and provides insight into the neurobiological basis of addiction and depression.

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Poster

332. Cocaine and Behavior

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Title: Acute cocaine evoked behavior develops independently from ventral tegmental area long term potentiation

Authors: A. VAQUER-ALICEA¹, J. C. VICENTY-PADILLA², R. VAZQUEZ-TORRES³, K. BOSQUE- CORDERO⁴, T. M. DOMÍNGUEZ- GONZÁLEZ⁵, L. A. APONTE-COFRESI⁴, T. M. ORTIZ-ITHIER⁶, L. F. CENTENO-MATOS⁷, *C. A. JIMENEZ-RIVERA⁸ ¹UPR Med. Sci. Campus, San Juan, Puerto Rico; ²Section of Neurosurg., UPR, Med. Sci. Campus-SOM, San Juan, Puerto Rico; ³Physiol., Univ. of Puerto Rico, San Juan, Puerto Rico; ⁴Biol., ⁵Chem., Univ. of Puerto Rico, Rio Piedras Campus, San Juan, Puerto Rico; ⁶Biol., Univ. Sagrado Corazón, San Juan, Puerto Rico; ⁷Psychology, Univ. of Puerto Rico Cayey Campus, Cayey, Puerto Rico; ⁸Physiol., Univ. of Puerto Rico, Physiol. Dept., San Juan, PR

Abstract: Cocaine is known to induce synaptic plasticity in the brain reward system. Persistent phosphorylation of protein kinases PKM² mediates late-phase LTP maintenance, and it can be inhibited by the myristoylated PKMzeta inhibitory peptide (ZIP). Using the cocaine behavioral sensitization model, we previously showed that persistence of cocaine evoked long term potentiation (LTP) in the ventral tegmental area (VTA) is necessary for synaptic alterations in the Nucleus Accumbens (NAc), which suggests a temporal sequence of neuroplastic changes in these two areas. However continuous disruption of LTP in VTA had a behavioral effect only after chronic micro-infusions of ZIP. In order to further characterize the role of LTP in cocaine elicited-behavior, we explored if ZIP had an acute effect in cocaine-induced plasticity in the VTA. For this, male Sprague-Dawley rats (250g) were stereotaxically implanted with bilateral cannulas into the VTA. The animals were given a cocaine injection and a ZIP micro-infusion 6 hours later. Whole cell patch clamp recordings of the VTA DA cells and NAc MSN were made 24 hours after ZIP microinjection. Results showed that a single ZIP micro-infusion after an acute cocaine exposure, effectively inhibited VTA LTP. Taken together with our previous results this suggests that initial behavioral response develops independently from VTA plasticity. Moreover this implies a more important role of plasticity during later phases of cocaine sensitization.

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Poster

332. Cocaine and Behavior

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Title: Inherent variability in dopamine uptake in the medial dorsal striatum is associated with subsequent motivation for cocaine

Authors: *J. K. SHAW¹, P. ALONSO¹, S. I. LEWANDOWSKI², R. A. ESPAÑA¹ ¹Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA; ²Pharmacol. & Physiol., Drexel Univ., Philadelphia, PA

Abstract: Cocaine addiction is a chronic, relapsing brain disease characterized by the heavy use of, aberrant motivation for, and an inability to maintain abstinence from cocaine. Considerable effort has been directed towards understanding the factors underlying the risk for cocaine addiction, however the mechanisms that predispose individuals to develop the disease remain unclear. The mesolimbic dopamine system has been particularly implicated in the reinforcing effects of cocaine, as cocaine enhances extrasynaptic DA in this system primarily by inhibiting DA uptake. Recent evidence suggests that inherent variability in DA neurotransmission may influence the risk for developing cocaine addiction. To determine the relative contributions of DA release and uptake to the vulnerability to use cocaine, we employed a novel application using fast scan cyclic voltammetry in anesthetized male rats to construct a neurochemical profile of DA signaling in the medial dorsal striatum (mDS) and ventral striatum of individual animals under baseline, drug-naïve conditions prior to any behavioral testing. Following recovery, rats were allowed to self-administer cocaine or were induced to develop cocaine conditioned place preference (CPP). Results suggest that there is no significant relationship between DA release or uptake and the acquisition of either CPP or operant self-administration, nor did we identify a significant relationship between DA signaling and the consumption of cocaine. We did, however, identify a strong, linear relationship between DA uptake in the mDS and the motivation for cocaine under both progressive ratio and threshold schedules of reinforcement-implicating DAT function in the risk for aberrant motivation associated with cocaine addiction. Preliminary results in an ex vivo model suggest that variability in DA uptake may be positively associated with sensitivity to the uptake inhibiting effects of low concentrations of cocaine. Examinations of the effects of viral-mediated alterations of DA uptake in the mDS on the motivation for and neurochemical response to cocaine are ongoing. Together, these data suggest that inherent variability in the mesolimbic DA system may underlie behavioral components associated with cocaine abuse.

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Poster

332. Cocaine and Behavior

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Title: Role of acid-sensing ion channel-1a in cocaine-induced synaptic plasticity

Authors: *S. C. GUPTA^{1,2}, R. J. TAUGHER², J. A. WEMMIE², R. T. LALUMIERE³ ¹Univ. of Iowa, Iowa, IA; ²Univ. of Iowa, Iowa City, IA; ³Psychological and Brain Sci., Univ. of Iowa, Iowa, IA

Abstract: Acid-sensing ion channel-1A (ASIC1A) is a cation channel expressed in dendritic spines that contributes to synaptic plasticity and is activated by extracellular acidosis. ASIC1A is particularly abundant in the nucleus accumbens (NAc) and genetic disruption of ASIC1A increased cocaine-conditioned place preference and altered cocaine-evoked synaptic plasticity. Loss of ASIC1A increased the ratio of AMPA receptor to NMDA receptor (A/N) at synapses in NAc medium spiny neurons (MSNs) resembling cocaine withdrawal, and like a cocainewithdrawn mice, the change in A/N in Asic1a^{-/-} mice was reversed by a single dose of cocaine. In this study we sought to test whether this cocaine-induced effect on A/N in Asic1a^{-/-} mice is permanent or transient. We administered a single dose of cocaine or saline, and assessed A/N at 6 hours, 24 hours, and 7 days after injection. Although the cocaine-evoked effect was not observed at 6 hours following injection, it was evident by 24 hrs. Moreover, by 7 days the A/N had returned to the elevated baseline state in the $Asic1a^{-/-}$ mice. Together these observations suggest that the cocaine-evoked plasticity in the Asic1a^{-/-} mice is transient and reversible. Next, we sought to determine whether the effect of ASIC1A on the A/N was cell autonomous or might be due to ASIC1A outside of the NAc. We injected AAV2/1-CMV-Cre mixed with AAV2/1-CMV-eGFP (AAV-Cre) into the NAc of Asic1a^{loxP/loxP} mice to disrupt ASIC1A specifically in a subpopulation of neurons in the NAc. Littermates were injected with AAV2/1-CMV-eGFP (AAV-eGFP) as a control. After allowing three weeks for viral transduction, we studied the A/N in both transduced (eGFP+) and non-transduced (eGFP-) NAc MSNs. We found that in the AAV-Cre injected mice, A/N was elevated in transduced cells relative to non-transduced cells. There was no difference in A/N between transduced and non-transduced cells in the AAV-eGFP group. Thus, the ASIC1A-dependent increase in A/N is cell autonomous and NAc specific. Together, these studies help discern when and where the interaction between cocaine and

ASIC1A disruption takes place, and set the stage for further investigation into the cellular mechanisms for the interaction.

Disclosures: S.C. Gupta: None. R.J. Taugher: None. J.A. Wemmie: None. R.T. LaLumiere: None.

Poster

332. Cocaine and Behavior

Location: Halls A-C

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Support: NIH Grant P01 DA031656

Title: Using the intermittent access self-administration procedure to assess sex differences in the motivation for cocaine

Authors: *A. KAWA, T. E. ROBINSON Univ. of Michigan, Ann Arbor, MI

Abstract: Studies assessing addiction-like behaviors in rats using the Intermittent Access (IntA) cocaine self-administration procedure have produced findings suggesting that the temporal pattern of cocaine consumption (in the case of IntA, characterized by intermittent spiking of brain cocaine concentrations) is a critical factor for addiction-like behavior. This pattern of intermittency is suggested to more closely resemble the temporal pattern of cocaine consumption in humans suffering from addiction and thus can serve as an asset in animal models of addictionlike behavior. To this point, studies using the IntA procedure have involved male rats, exclusively. However, work in humans and in animals- using the traditional "Long Access" (LgA) model, has suggested that females may be more susceptible to develop addiction-like behavior. For example, female rats are more likely to acquire self-administration and do so more rapidly than males, and escalate their cocaine intake to a greater degree than male rats. In humans, females more rapidly progress from casual cocaine use to cocaine addiction, suggesting some sex difference that predisposes them to suffer from addiction. Previous work has shown that IntA cocaine experience produces robust incentive-sensitization in male rats, but it is not known if this occurs in female rats. In this study, we directly compared the effects of IntA selfadministration on the motivation to self-administer cocaine in male and female rats measured using behavioral economic metrics. Our results reinforce previous findings regarding addictionlike behavior in males, as males exhibited an escalation in drug consumption, as well as an increase in motivation to self-administer cocaine over the course of the 30 day IntA procedure. In female rats, we observed an even greater escalation in drug consumption as well as stronger

motivation to consume cocaine throughout IntA experience. Finally, a 14-day abstinence period led to greater incentive-sensitization in female rats than male rats.

Disclosures: A. Kawa: None. T.E. Robinson: None.

Poster

332. Cocaine and Behavior

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Topic: G.08. Drugs of Abuse and Addiction

Support: PHS grant PO1 DA031656

Title: Relapse depends on the type of cue and the type of brain: A cue that signals cocaine availability reinstates drug-seeking more readily in goal-trackers than sign-trackers, and depends on basal forebrain cholinergic activity

Authors: *K. PITCHERS, K. B. PHILLIPS, J. L. JONES, T. E. ROBINSON, M. SARTER Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: The most predictable outcome of a first diagnosis of addiction is a high chance for relapse. When addicts encounter cues previously associated with drug administration, their attention is unduly attracted to such cues, and these cues can evoke motivational states that instigate and maintain drug-seeking behavior. There is, however, considerable variation in the motivational properties of such cues, both as a function of the individual and the nature of the cue. Some individuals (sign-trackers, STs) are especially susceptible to cues paired with reward delivery, perhaps because they are prone to process information via dopamine-dependent, cuedriven, incentive salience systems. Other individuals (goal-trackers, GTs) are better able to incorporate higher-order contextual information, perhaps because they have better executive/attentional control over behavior, which requires frontal cortical cholinergic activity. We hypothesized, therefore, that a cue that 'sets the occasion' for drug-taking (a discriminative stimulus, DS), which shares properties of contextual stimuli, would renew cocaine-seeking more readily in GTs than STs, and that this would require intact cholinergic neurotransmission. To this end, STs and GTs were trained to self-administer cocaine using an Intermittent Access schedule with periods of cocaine availability and unavailability signaled by a DS+ and a DS-, respectively. After this, half the rats received an immunotoxic lesion that destroyed ~50% of basal forebrain cholinergic neurons, and later, after extinction training, tested for the ability of non-contingent presentations of the DS+ to renew cocaine-seeking behavior. The DS+ was much more effective in renewing cocaine-seeking in GTs than STs, and this effect was abolished by the cholinergic lesion, despite the fact that all rats continued to orient to the DS+. While STs were previously demonstrated to exhibit greater relapse vulnerability for localizable Pavlovian drug cues, here we

demonstrate that their counterparts, the GTs, are more vulnerable if drug cues act to signal drug availability, and that the basal forebrain cholinergic system mediates such vulnerability. We conclude that vulnerability to relapse depends on individual cognitive-motivational biases that interact with the form of the drug cue encountered. Given the importance of contextual cues for triggering relapse and the human cognitive-cholinergic capacity for the processing of such cues, GTs model essential aspects of relapse vulnerability.

Disclosures: K. Pitchers: None. **K.B. Phillips:** None. **J.L. Jones:** None. **T.E. Robinson:** None. **M. Sarter:** None.

Poster

332. Cocaine and Behavior

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Topic: G.08. Drugs of Abuse and Addiction

Support: PHS grant PO1 DA031656

Title: Motivational-dopaminergic versus cognitive-cholinergic processing of a Pavlovian cocaine cue in sign-versus goal-tracking rats

Authors: *L. F. KANE¹, K. K. PITCHERS², Y. KIM³, T. E. ROBINSON⁴, M. SARTER⁵ ¹Biopsychology, Univ. of Michigan, Baltimore, MD; ²Psychology, ³Univ. of Michigan, Ann Arbor, MI; ⁴Dept Psychol, ⁵Psychol, Univ. of Michigan Dept. of Psychology, Ann Arbor, MI

Abstract: Pavlovian reward cues are known to have greater motivating properties, or acquire greater incentive salience, in some animals (sign trackers; STs) compared to others (goal trackers; GTs). Conversely, GTs have a greater capacity for the processing of more complex contextual cues, reflecting their relatively superior bias for goal-directed cue processing. Prefrontal circuitry is essential for the processing of cues, including for influencing the motivational-attentional processes that determine the efficacy with which cues exert control over behavior. Here we investigated the activity of two major prefrontal neuromodulatory input systems, dopamine (DA) and acetylcholine (ACh), in response to a Pavlovian cue that was previously paired with cocaine administration in STs and GTs. Rats underwent 15 days/sessions of training in which each session consisted of 9 light-cue presentations that were either paired or explicitly unpaired with an intravenous cocaine infusion (0.4 mg/kg/infusion). Consistent with previous findings using similar training conditions, STs and GTs exhibited similar rates of orienting toward the paired cue. Following a 10-day abstinence period, prefrontal, 4-min dialysates were collected in STs and GTs during Pavlovian cue presentations in the absence of cocaine. We used benzoyl chloride derivatization (Song et al., 2011) and HPLC-mass spectrometry to simultaneously measure several neurotransmitters and metabolites from these

collections. In STs, the previously paired cocaine cue significantly increased prefrontal DA levels. DA levels remained elevated over baseline across multiple cue presentation blocks. ACh levels remained unaffected. In contrast, in GTs, presentations of the cocaine cue did not affect DA levels but increased prefrontal ACh levels. In STs, elevated DA levels coincided with the propensity of these rats to approach the previously paired cue. GTs did not exhibit such cue approach behavior and thus elevated ACh levels did not correlate with these rats' behavior during cue presentation. These results suggest that, in STs, dopaminergic modulation of prefrontal circuitry contributes to the expression of the motivational state evoked by the drug cue. In contrast, in GTs, cholinergic modulation of prefrontal circuitry mediates a "colder", or more cognitive, analysis of the stimulus and extinction context, not yielding cue-directed behavior. The attraction of STs to, and the neutrality of GTs toward, a Pavlovian cocaine cue is mediated via dopaminergic versus cholinergic modulation of prefrontal circuitry.

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Poster

332. Cocaine and Behavior

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Topic: H.01. Animal Cognition and Behavior

Support: P50DA037844

Title: Environmental complexity increases tendency to attribute incentive salience to a food cue, but does not affect cocaine conditioned "cue" preference in Heterogeneous Stock (HS) rats

Authors: ***J. A. TRIPI**¹, A. M. GEORGE², C. D. MARTIN¹, K. ISHIWARI³, A. A. PALMER⁵, J. B. RICHARDS², P. J. MEYER⁴

¹Psychology, Univ. at Buffalo Dept. of Psychology, Buffalo, NY; ²Res. Inst. On Addictions, Buffalo, NY; ³Res. Inst. on Addictions, Univ. at Buffalo, Buffalo, NY; ⁴Psychologu, Univ. at Buffalo, Buffalo, NB; ⁵Psychiatry, UCSD, La Jolla, CA

Abstract: Environmental complexity produces an array of neurobehavioral effects in preclinical models, including altering responsivity to novel and reward-related stimuli. Studies in outbred rat strains have indicated that environmental complexity may reduce addiction-like behaviors. However, there is limited research addressing the interaction between genetic variability and the environment on responsivity to rewards and their cues. To address this, we used a genetically heterogeneous stock (HS) rats to assess the effects of environmental complexity on response to a food cue using Pavlovian conditioned approach (PavCA) and conditioned reinforcement (CRf), and response to cocaine in conditioned "cue" preference (CCP). Male HS rats (30-40 days

postnatal) were reared in pairs in a standard housed cage (SH; n=100) or in groups of 16 in an environmentally complex cage (EC; n=32) throughout testing. EC housing consisted of a multilevel cage filled with pet toys. Subjects were first tested in PavCA, a task used to assess the attribution of incentive salience to a food cue. Subjects learned to associate a lever cue with the delivery of a food pellet into a food cup. We measured approach to the lever ("sign-tracking") and the food cup ("goal-tracking") over five sessions (25 lever-pellet trials per session). Rats were then tested in CCP, during which a tactile floor (grid or hole) was paired with cocaine (10 mg/kg, *i.p.*) and the opposite floor was paired with saline. After eight conditioning trials rats were tested for their floor preference. Data analysis of PavCA behavior revealed a main effect of housing type on goal-tracking, with EC rats displaying more food cup entries (p < .001). Additionally, EC rats sign-tracked more than SH rats, indicating an increased tendency to attribute incentive salience to the food cue (p < .001). During a conditioned reinforcement test, in which rats could snout-poke into an active port to gain lever access, EC rats produced significantly more active pokes and subsequent lever presses (p < .001). During CCP trials, EC rats had lower levels of injection-induced locomotion (p < .05). However, both groups showed similar preference for the cocaine-paired floor and conditioned locomotion. These studies indicate a robust effect of environmental complexity on reward learning and locomotor sensitivity, but not to Pavlovian drug learning. This suggests that environmental complexity facilitates incentive salience attribution to food but not drug cues. Yet, others have observed contrasting effects of environmental complexity; this may be due to factors including the genetic background of the HS rats and the specificities of the housing conditions.

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Poster

332. Cocaine and Behavior

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant P50DA037844

Title: Effects of environmental complexity on novelty seeking, impulsivity and sustained effortful attention in heterogeneous stock (HS) rats

Authors: *A. M. GEORGE¹, C. D. MARTIN², K. ISHIWARI¹, P. J. MEYER², A. A. PALMER³, J. B. RICHARDS¹ ¹Res. Inst. On Addictions, Buffalo, NY; ²Psychology, Univ. at Buffalo Dept. of Psychology, Buffalo, NY; ³Psychiatry, UCSD, La Jolla, CA **Abstract:** Most laboratories use standard shoebox housing for behavioral studies, despite evidence that complex or enriched environments can profoundly change behavior. We sought to compare the effects of different housing conditions on behavioral measures of novelty seeking, impulsivity and effort. Five-hundred male heterogeneous stock (HS) rats in social housing with standard plastic cages, and sixty-four male HS rats housed in a complex environment were tested on locomotor, light reinforcement and reaction time procedures, to measure individual differences in novelty seeking, impulsivity and sustained effortful attention, respectively. In the locomotor behavior task, fine movements, total distance, and rearing are used to quantify exploration in a novel environment. Standard rats had higher and more variable activity, distance, and rearing scores than the complex housed rats. Complex housed rats habituated more rapidly on all measures, suggesting they were hypoactive with an ability to distinguish an irrelevant novel sensory stimulus.

Using a light reinforcement paradigm, we have previously shown, on average, HS rats demonstrate an initial increase in responding to a novel light stimulus followed by monotonic decrease in responding as the reinforcing effects of the novelty habituate. The rate of habituation for the complex housed rats was more rapid than the standard housed rats. Additionally, some standard housed rats show a sensitization pattern, rather than habituation, while this phenomenon is absent in the complex housed rats.

The number of trials completed in the reaction time task is the primary measure of sustained attention. Standard housed rats were less able to sustain attention than complex housed rats, completing fewer trials throughout the 18-minute test session. Premature responding is considered a measure of motor impulsivity and represents a failure of inhibitory control. Premature responding may be analogous to false alarms in human vigilance tasks. Using this measure, standard housed rats were more impulsive, completing more false alarms per opportunity, than complex housed rats.

Together, these results indicate that the large behavioral differences reflect environmental influences in which genes are differentially expressed as a function of their housing environment. Furthermore, these large effects of housing on behavior in rats may question traditional standard housing conditions and support transitioning towards complex environments to optimize translational validity. Future studies will explore whether genetic effects of these tasks interact linearly or epistatically with differences in housing conditions.

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Poster

333. Cocaine and Neurotransmission

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Topic: G.08. Drugs of Abuse and Addiction

Support: Estonian Research Council Grants ETF9262 and PUT1686

Title: The essential role of DNA methylation and demethylation in cocaine withdrawal

Authors: *K. ANIER¹, M. URB¹, T. MATSALU¹, K. KIPPER¹, K. HERODES¹, T. TIMMUSK², A. ZHARKOVSKY¹, A. KALDA¹ ¹Univ. of Tartu, Tartu, Estonia; ²Tallinn Univ. Technol., Tallinn, Estonia

Abstract: An increasing number of reports have provided crucial evidence that epigenetic modifications, such as DNA methylation, may be involved in initiating and establishing psychostimulant-induced stable changes at the cellular level by coordinating the expression of gene networks, which then manifests as long-term behavioral changes. Recent discoveries suggest that ten-eleven translocation enzymes (TET1-3) participate in the DNA demethylation process and might also play a role in cocaine action. However, to date there are no studies that have focused on the complex role of both DNA methylation and demethylation in the mechanisms of psychostimulant-induced addiction. Our aim of this study was to investigate the effect of non-specific DNMT inhibitor (RG108) and selective Dnmt3a inhibition (AAV-Dnmt3a shRNA) on the expression of cocaine-induced behavioral sensitization in mice. Using the behavioral sensitization model, we evaluated the effect of cocaine withdrawal on the expression and activity levels of Dnmt1, Dnmt3a, Dnmt3b and Tet1-3 in the nucleus accumbens (NAc) of male C57BL/6NTac mice. We also assessed the specificity of these alterations in the NAc with changes in peripheral blood cells. Our qPCR data showed that cocaine withdrawal upregulates mRNA levels of DNA methyltransferases (Dnmts) and downregulates mRNA levels of Tets in the nucleus accumbens (NAc) of mice and that these changes correlate tightly with Dnmts and Tets mRNA levels in peripheral blood cells. Our data show that cocaine withdrawal increases DNMT activity but decreases TET activity in the NAc and that these changes are associated with enhanced global DNA methylation levels. In addition, bilateral intra-NAc injection of a nonspecific inhibitor of DNMT (RG108) during withdrawal from cocaine decreases DNMT activity levels in the NAc and diminishes the expression of cocaine-induced behavioral sensitization. In contrast, long-term selective Dnmt3a silencing in the NAc did not affect DNMT activity levels or the expression of cocaine-induced behavioral sensitization. These data indicate that cocaine withdrawal may disturb the equilibrium between DNA methylation and demethylation processes in the NAc and cause global changes in DNA methylation.

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Poster

333. Cocaine and Neurotransmission

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Topic: G.08. Drugs of Abuse and Addiction

Support: R00 DA033372

Title: PKMz knockout enhances cocaine self-administration and reinstatement through sex dependent mechanisms

Authors: *A. MCGRATH, J. LENZ, L. BRIAND

Temple Univ., Philadelphia, PA

Abstract: PKM^{\zet} is a constitutively active form of protein kinase C, once believed to be critical for the maintenance of memory. However, PKMζ knockout (KO) mice show no impairments in multiple forms memory or long term potentiation. Although very few behavioral phenotypes have been found in PKM² KO mice, they do exhibit increased alcohol drinking. This suggests that this protein may play a role in addictive behavior. The current study examined the effect of constitutive PKMζ KO on food and cocaine self-administration and cocaine reinstatement. We found that the PKMC KO mice exhibited increased food and cocaine self-administration as well as increased cocaine seeking during cue-induced reinstatement. This enhanced cocaine seeking could not be attributed to an increase in cocaine-induced locomotor activity or an increased preference for novelty. We next examined whether these effects were due to the actions of PKMC in the nucleus accumbens utilizing floxed PKM KO mice. When PKM was knocked down specifically in the nucleus accumbens, we did not see any effects on food self administration. Further, we saw an increase in cocaine self-administration and reinstatement that was only present in the male mice. Ongoing experiments are examining the role of circulating hormones in the sex-specificity of the effect of accumbal PKM² KO. Furthermore, as the constitutive PKM² KO female mice show enhanced cocaine seeking and the accumbens specific PKMC KO females do not; additional studies are underway to determine what brain regions are responsible for the effect seen in the constitutive KO females. Together these data suggest that PKM² plays a role in inhibiting cocaine consumption and seeking. A better understanding of the role of PKM² in addiction could one day lead to novel therapeutic options for people struggling with cocaine addiction.

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Poster

333. Cocaine and Neurotransmission

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Topic: G.08. Drugs of Abuse and Addiction

Support: R00 DA033372

Center for Substance Abuse Research, Temple University

Title: The effect of prefrontal GRIP deletion on drug-seeking behavior

Authors: *M. M. WICKENS, A. Q. FOSNOCHT, L. A. BRIAND Psychology, Temple Univ., Philadelphia, PA

Abstract: Disrupted glutamatergic signaling is a component in the development and maintenance of addiction. The glutamatergic pathway from the prefrontal cortex (PFC) to the nucleus accumbens plays a critical role in extinction and reinstatement of drug-seeking. The majority of studies examining the trafficking of glutamate receptors have focused on the prefrontal inputs to the nucleus accumbens, rather than changes in the PFC itself. The goal of this work was to examine how disrupted AMPA receptor trafficking in the PFC affects addiction-like behavior. To do this, we utilized mice with an inducible (floxed) deletion of glutamate receptor interacting protein, GRIP, a scaffolding protein necessary for the synaptic localization an aggregation of AMPA receptors. Six weeks prior to any behavioral testing, mice received intracranial viral injections of either Cre recombinase or GFP into the medial prefrontal cortex. Following viral expression and knockdown, all mice were trained on a self-administration procedure. Briefly, mice were taught to perform an operant response to receive sucrose pellets. They next underwent an intravenous catheterization surgery, and after recovery, were allowed to perform the acquired operant response for an infusion of cocaine. We next used a progressive ratio schedule to measure motivation to work for the drug, before the mice entered a period of forced abstinence and the behavior was extinguished. Finally, mice experienced a cue reinstatement trial. Following prefrontal GRIP knockdown, mice exhibited higher breakpoints on a progressive ratio schedule, suggesting that impaired AMPA receptor trafficking in the mPFC increases motivation for drug-seeking behavior. Additionally, prefrontal GRIP KO mice exhibited greater rates of cue-induced reinstatement, indicating that impaired PFC AMPA receptor trafficking increases vulnerability to relapse. Future work aims to examine how disrupting AMPA receptor trafficking in the mPFC affects accumbal physiology. These findings could lead to an improved understanding of the biological alterations associated with addiction and relapse.

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Poster

333. Cocaine and Neurotransmission

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA ROI DA016663-02

Title: Neural biomarkers of risk and reward predict problem stimulant use

Authors: *M. A. BLAIR^{1,2}, J. L. STEWART^{1,2}, A. C. MAY³, M. RESKE⁴, S. F. TAPERT³, M. P. PAULUS^{5,3}

¹Psychology, Grad. Center, City Univ. of New York, New York, NY; ²Psychology, Queens College, City Univ. of New York, Flushing, NY; ³Psychiatry, UC San Diego, La Jolla, CA; ⁴Inst. of Neurosci. and Med. (INM-6) & Inst. for Advanced Stimulation (IAS-6), Julich Res. Ctr., Julich, Germany; ⁵Psychiatry, Laureate Inst. For Brain Res., Tulsa, OK

Abstract: Recreational stimulant (amphetamine and cocaine) use is a growing concern among young adults, with 16% of individuals who experiment eventually developing stimulant dependence. Despite the importance of understanding how to predict which recreational users will become problem users, little is known about the behavioral or neural indices that can forecast the trajectory of occasional to problematic stimulant use. The present longitudinal study utilized follow-up data from a sample of 110 occasional stimulant users (OSU) three years after baseline functional magnetic resonance imaging (fMRI) and clinical interview sessions were obtained. Baseline fMRI recording during a Risky Gains Task (RGT) was analyzed to determine if preexisting neural activation patterns differentiated individuals who became problem stimulant users (PSU) from those who desisted stimulant use (DSU). The RGT was assessed from two distinct perspectives: (1) decision phase analyses evaluated neural and behavioral differences when individuals made a "risky" versus "safe" decision; and (2) outcome phase analyses assessed differences in response to wins versus losses on "risky" trials. For each voxel, a linear mixed effects (LME) analysis was computed to identify significant regions of percent signal change between PSU and DSU and voxelwise clusters were extracted, correcting for familywise error. Results indicate that compared to DSU, PSU exhibited lower activation in frontal executive control, sensory, and emotional processing regions during decision making as well as greater sensitivity to risky rewards and reduced sensitivity to losses. Findings suggest that PSU and DSU can be differentiated by neural biomarkers in several regions of the brain critical for decision making.

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Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: R01 DA006886-19/DA/NIDA NIH HHS/United States

Title: Heterogeneous development of drug abuse: Individual differences and predisposition to addiction

Authors: *N. J. BEACHER¹, J. KULIK¹, J. STAMOS², S. KLEIN¹, D. ESTRIN¹, V. CHEN¹, A. LEONTE¹, A. P. PAWLAK¹, M. O. WEST³ ¹Rutgers The State Univ. of New Jersey, Piscataway, NJ; ²Rutgers Robert Wood Johnson Med. Sch., Piscataway, NJ; ³Psychology, Rutgers Univ., Piscataway, NJ

Abstract: The ability to identify individual predisposition to drug abuse is important for long term prevention of drug addiction. A particularly troubling component of addiction is subsequent relapse to drugs after a period of abstinence. Studies have shown the vast majority of the addicted population is susceptible to relapse triggered by drug-associated cues. During such episodes, 'drug-craving' can be induced by drug-associated cues which can re-invigorate drugassociations in motivational brain regions such as the nucleus accumbens (Nac). Thus, identifying predispositions to cue reactivity is important for the long term prevention of relapse in drug addiction. Differences in cue reactivity have been explored using an autoshaping test which identifies two distinctive behavioral phenotypes; Sign-trackers (ST) and Goal-trackers (GT). This phenomenon is thought to involve incentive salience, where the "self-rewarding" cue becomes associated with a future reward. Historically, GT animals have been largely ignored in drug addiction modeling and have been overshadowed by a common focus on their ST counterparts. Our study questioned whether individual differences in STGT phenotype would predict differences in drug seeking behavior and Nac firing during a 14-day cocaine selfadministration (SA) paradigm. Male and female Long Evans rats identified as ST, GT, or 'Middle' received 14 days' training (6 hours/day) in a tone discrimination paradigm where cocaine availability was contingent on a nose poke response during a 30 sec tone S^D. Single unit Nac responses to the S^D were compared between groups (core/shell, M/F, ST/GT), at different times (early SA sessions v late SA sessions), and during different behavioral events (hit and missed cocaine opportunities). Finally, Ultrasonic Vocalizations (USVs) were recorded to assess affective state with respect to individual baseline USV emission (prior to day 1 of SA). Results may identify a new model for studying individual differences in vulnerability to drug abuse and advance our understanding of cocaine cue-evoked Nac firing patterns that may contribute to relapse.

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Poster

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Title: Mimicking DBS with optogenetics in rats to identify the specific nucleus accumbens neurocircuits underlying relapse to cocaine

Authors: *S. E. SWINFORD-JACKSON, M. E. HOFMANN, R. C. PIERCE Dept. of Psychiatry, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Repeated cycles of abstinence and relapse are a signature of cocaine use disorder. The nucleus accumbens (NAc) is fundamentally implicated in relapse to cocaine. We previously demonstrated that deep brain stimulation (DBS) of the NAc shell suppressed reinstatement of cocaine-seeking in rats; however, the mechanisms of DBS which underlie this effect are not well-understood. For example, DBS affects the activity of all cell-types within the targeted region, but it is unclear whether the impact of DBS on specific populations of neurons drive the attenuation of cocaine-seeking. Optogenetic techniques facilitate manipulation of specific cell types and neural pathways in a temporally-precise manner. Additionally, recently-developed transgenic rat lines which express Cre-recombinase in selective populations of neurons permit investigation into the effect of selectively activating or inhibiting neuronal subtypes on complex behaviors, including rat cocaine self-administration and extinction/reinstatement. Combining an optogenetic approach with transgenic rat lines provides an opportunity to investigate the mechanisms underlying the effect of DBS to suppress reinstatement of cocaine-seeking. This study first validated and characterized the use of optogenetic tools in wild type and transgenic rat lines expressing Cre-recombinase in subtypes of neurons. Ubiquitously-expressed or Credependent adeno-associated viral vectors expressing channelrhodopsin and eYFP were injected into the NAc shell or afferent regions to assess inputs and outputs of the NAc shell. Immunofluorescence was used to indicate viral expression and co-localization with subtypespecific markers. Firing properties of eYFP-positive neurons were recorded during 473nm laser stimulation at varied output intensities, pulse widths, and frequencies. Behavioral analyses are ongoing. This study is an initial step toward our goal of using optogenetics within specific pathways and neuronal subtypes to elucidate the mechanisms of DBS which attenuate reinstatement of cocaine-seeking.

Disclosures: S.E. Swinford-Jackson: None. M.E. Hofmann: None. R.C. Pierce: None.

Poster

333. Cocaine and Neurotransmission

Location: Halls A-C

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Topic: G.08. Drugs of Abuse and Addiction

Support: K99DA040744

Title: Differential regulation of Bdnf and behavior by HDAC and BET inhibition

Authors: *G. C. SARTOR¹, A. M. MALVEZZI¹, H. J. WIEDNER¹, J. M. WASSERMAN¹, C. R. WAHLESTEDT²

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Abstract: Brain Derived Neurotrophic Factor (BDNF) plays a central role in neuronal development, neuroplasticity, and learning and memory. As growing evidence indicates that altered epigenetic regulation of the BDNF gene may contribute to the pathophysiology of multiple brain disorders, epigenetic-based therapies have recently emerged as an attractive approach to modulate BDNF expression. For example, previous studies have shown that histone deacetylase (HDAC) inhibition increases Bdnf expression and enhances learning and memory in rodents. However, the underlying mechanisms by which HDAC inhibition (HDACi) increases Bdnf expression remain unclear. Previously, we found that inhibition of histone acetyl-lysine readers (BET bromodomains) reduces Bdnf mRNA and protein expression and reward-related learning. Because HDAC inhibition increases histone acetylation at the Bdnf promoter and because BET proteins are readers of histone acetylation, we hypothesized that HDACi-induced increases in Bdnf are mediated, in part, by BET proteins. In in vitro and in vivo studies, we found that the Class I/IIb HDAC inhibitor, SAHA, and HDAC3-specific inhibitor, RGFP966, increased Bdnf expression. Further, this HDACi-induced elevation of Bdnf expression was blocked in a dose- and time-dependent manner by the BET inhibitor JQ1. In additional siRNAmediated knockdown studies, we found that the increased Bdnf expression by HDAC2 and HDAC3 knockdown was blocked by knockdown of the BET protein BRD4. Behaviorally, JQ1 blocked the enhanced learning induced by RGFP966 in novel object recognition and cocaine conditioned place preference tests. Ongoing chromatin and electrophysiological studies will further uncover the interaction between HDACs and BETs in the regulation of Bdnf and neuroplasticity. Together, these studies reveal that BET proteins mediate HDACi-induced increase in Bdnf expression and learning and memory.

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Poster

333. Cocaine and Neurotransmission

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Title: Multiple system dysfunction in addiction: Evidence from model-based fMRI

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Abstract: Existing models of addiction describe it as a shift from goal-oriented substanceseeking to reactive and habitual drug-taking. Under this framework, once addiction is formed, activity of the dorso-lateral sensorimotor cortico-striatal circuit (putamen, sensorimotor cortex) overrides activity of the ventral goal-selection cortico-striatal circuit (nucleus accumbens and ventromedial prefrontal cortex). However, this theory does not account for several important findings such as the altered behavioral and neural responses to non-drug-related rewards; and the abnormal neural activities in the ventral cortico-striatal circuit. In this research, we propose that substance-induced reinforcements alter circuit gain and neural dynamics in multiple corticostriatal circuits concurrently. We test the hypothesis with a probabilistic reward-learning fMRI study where 41 individuals with cocaine use disorders (CUD) and 18 healthy controls performed a task involving non-drug rewards. Our fMRI results demonstrate that cocaine addiction is associated with altered responses, such as enhanced activation and prolonged activity in multiple cortico-striatal circuits. Specifically, CUD participants show increased activation in nucleus accumbens and medial prefrontal cortex (ventral goal-oriented circuit) in association with reward values during the onsets of outcomes. In addition, CUD participants showed an increased activation in caudate (dorso-medial associative circuit) which associated to loss values during the onsets of outcomes. Furthermore, as opposed to the decreasing putamen activity (dorso-lateral habitual circuit) after action selection in healthy controls, the same activity in CUD individuals is maintained for a prolonged time. These findings suggest that in addicted individuals, abnormal neural activities can be observed in multiple cortico-striatal circuits, supporting the hypothesis that addiction is characterized by aberrant circuit gain and impaired information processing in multiple systems. Moreover, with non-drug related cues and rewards involved, the results also allow us to generalize this hypothesis to non-drug-related circumstances. This may explain why patients can fall back to relapse so easily even when they are not exposed to the addicted drug.

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Poster

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Leon Levy Foundation

Seaver Family Foundation

Title: Perturbations of the gut microbiome affect drug seeking behaviors

Authors: *D. D. KIRALY¹, E. G. PECK², A. GODINO³, N. L. MERVOSH², E. S. CALIPARI⁴ ¹Psychiatry / Neurosci., ²Icahn Sch. of Med. at Mount Sinai, New York, NY; ³Fishberg Dept. of Neurosci., Icahn Sch. of Med. at Mount Sinai, New York City, NY; ⁴Mount Sinai Sch. of Med., New York, NY

Abstract: The resident bacteria of the gastrointestinal tract, collectively dubbed the gut microbiome, have been shown to have profound effects on both brain and behavior. To date, the majority of research into the effects of the gut microbiome in neuropsychiatric pathology has focused on affective and neurodegenerative disorders. However, we recently demonstrated that altering the gut microbiome has a marked effect on the behavioral and neurobiological responses to cocaine. Animals with depleted gut flora exhibited increased locomotor sensitization and a lower threshold for conditioned place preference for cocaine. Additionally, these mice had altered regulation of the genes encoding brain-derived neurotrophic factor, the D1 dopamine receptor, and other important regulators of behavioral response to cocaine. Here, we utilized a cocaine self-administration paradigm and behavioral economics modeling to assess how shifts in the gut microbiome directly affect motivation to seek and take cocaine. As previously published, we depleted the gut microbiome of rats with a cocktail of broad-spectrum non-absorbable antibiotics via their drinking water for two weeks. These animals were then trained to selfadminister cocaine on a fixed-ratio 1 schedule until they were stably administering for five days. Self-administration acquisition rates did not differ between groups. Animals were then assessed using a behavioral economics threshold task, a within-session method used to assess an animal's motivation to self-administer a reinforcer in the face of increasing cost. Microbiome-depleted rats were insensitive to increases in cost (as measured by effort required) for each milligram of cocaine. This insensitivity to increasing drug cost is analogous to insensitivity to cost seen in human drug abusers. When cue and drug were removed, microbiome-depleted rats exhibited

more rapid extinction of lever pressing for cocaine. However, after allowing for an incubation period after completion of extinction, we find that microbiome-depleted rats exhibited enhanced reinstatement for both cue and cocaine stimuli. Taken together, these findings suggest that changes in the gut microbiome can alter motivation to seek drug, and affect how animals update reward contingencies in a complex manner. Molecular studies are currently underway to determine how underlying changes in gene expression and epigenetic regulation lead to this altered behavioral phenotype.

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Poster

333. Cocaine and Neurotransmission

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Topic: G.08. Drugs of Abuse and Addiction

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NIDA K99-DA042111

Leon Levy Foundation

Seaver Family Foundation

Title: Granulocyte-colony stimulating factor mediates neuronal and behavioral responses to cocaine

Authors: *A. GODINO¹, E. S. CALIPARI¹, E. G. PECK¹, N. L. MERVOSH², J. A. LANDRY^{1,2}, S. J. RUSSO¹, Y. L. HURD^{1,2}, E. J. NESTLER¹, D. D. KIRALY^{1,2,3} ¹Fishberg Dept. of Neurosci., ²Dept. of Psychiatry, ³Seaver Autism Ctr. for Res. and Treament, Icahn Sch. of Med. at Mount Sinai, New York City, NY

Abstract: There is growing evidence implicating dysregulation of the immune system in the pathophysiology of multiple psychiatric disorders. While cocaine is known to affect immune system function, mechanistic links between altered immune function and pathological substance use behaviors remain poorly understood. To better characterize the effect of cocaine on innate immune function, we performed serum profiling of 32 cytokines, chemokines and growth factors after cocaine treatment. This assay identified multiple innate immune mediators that are regulated by chronic self- or experimenter-administered cocaine in mice. However, only granulocyte colony stimulating factor (G-CSF) was both increased by cocaine exposure and also positively correlated with the extent of both locomotor sensitization and levels of cocaine self-

administration. To interrogate the effect of G-CSF on cocaine-induced neuronal activation, we assayed induction of the immediate early gene *c-Fos* following an acute treatment with systemic G-CSF and/or cocaine in multiple brain regions involved in reward processing. G-CSF potentiated the cocaine-induced increase in neuronal activation specifically in the nucleus accumbens (NAc) and medial prefrontal cortex, while having no effect on its own. To assess effects of G-CSF on cocaine-induced behavioral adaptations, animals were pre-treated with systemic injections of G-CSF during locomotor sensitization and conditioned place preference (CPP) paradigms. G-CSF-treated animals exhibited significantly enhanced locomotor sensitization to 7.5mg/kg of cocaine. Similarly, treatment with systemic G-CSF during place conditioning enhanced the formation of cocaine CPP at lower doses (3.75 & 7.5mg/kg). Conversely, we were able to block the formation of CPP at 7.5mg/kg by infusing a G-CSF neutralizing antibody into the NAc. To better understand how G-CSF alters the motivational properties of cocaine, we utilized a behavioral economics threshold procedure in rats selfadministering cocaine. G-CSF-treated animals were less sensitive to gradual increases in cocaine cost, a measure that is indicative of increased motivation to self-administer cocaine. G-CSF treatment also increased total drug intake in both the threshold task and during FR1 selfadministration. Taken together, these experiments define a mechanistic and causal role for G-CSF in both the neuronal and behavioral responses to cocaine. Additionally, we posit that G-CSF is a putative target for development of pharmacotherapeutics to improve treatment outcomes in cocaine-addicted individuals.

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Poster

333. Cocaine and Neurotransmission

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Topic: G.08. Drugs of Abuse and Addiction

Support: Labex-Brain-Bordeaux

Title: Exposure to novelty gates cocaine-evoked plasticity in dopamine neurons

Authors: *G. R. FOIS¹, C. MILIANO², S. CHAUQUET³, X. NOGUES³, J. M. BAUFRETON⁴, S. CAILLE⁵, F. E. GEORGES⁶

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Abstract: A key feature of human and animal behavior is to learn from environmental stimuli to efficiently adapt. The ability to learn about a new environment and to minimize contact with aversive experience is a hallmark of adaptive behavior and recent studies have demonstrated a key role of the ventral tegmental area (VTA) dopamine neurons in this process. Moreover, a critical role in the integration of relevant contextual information is played by the hippocampus, which controls the activity of dopamine neurons in VTA. It is generally recognized that the mesolimbic dopamine pathway originating in the VTA have an important role in the development of addiction. We have recently reported that the ventral subiculum of hippocampus (vSUB) regulate VTA dopamine neurons activity through the Bed nucleus of stria terminalis (BNST), and the potentiation of vSUB-BNST-VTA pathway increase locomotor activity induced by a sub-threshold dose of cocaine given in a novel context. Here we investigate how novelty induced by a switch of context trigger changes in electrophysiological properties of VTA dopamine neurons and the role of novel environment to trigger cocaine-evoked plasticity in dopamine neurons. We combined computational and in vitro/in vivo electrophysiological approaches to uncover the early cellular targets of cocaine in the mesolimbic dopamine system and the consequent adaptations at the circuit and behavioral level. Considering the tight links between stress, arousal and change in social interactions during the exploration of a novel environment, we explore the role of these individual components on cocaine-evoked modifications on integration properties of VTA dopamine neurons. We found that the novelty exposure is necessary for cocaine-induced effects on VTA dopaminergic neurons activity, and that this effect is due to the exploration of a novel environment and is not supported by a stresseffect during novelty exposure. Our study reveals a neuronal circuit encoding behavioural effects of cocaine in rats and the key role of hippocampus to the cocaine-induced effects on VTA dopamine neurons during exposition in a new context.

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Poster

333. Cocaine and Neurotransmission

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Support: CURE Addiction Center of Excellence: Brain Mechanisms of Relapse and Recovery

NIH/NIDA U54DA039002

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Title: "I must avert my eyes": Cocaine patients with a heightened brain response to 33 msec subliminal cocaine cues are more likely to "look away" from cocaine cues in an attentional bias task

Authors: *A. R. CHILDRESS¹, K. JAGANNATHAN¹, P. REGIER¹, J. J. SUH¹, R. EHRMAN^{1,2}, Z. MONGE³, S. DARNLEY¹, T. FRANKLIN¹, R. WETHERILL¹, D. D. LANGLEBEN^{1,2}, M. GAWRYSIAK¹, R. SZUCS-REED¹, K. YOUNG¹, K. KAMPMAN^{1,2}, C. P. O'BRIEN¹

¹Psychiatry, Univ. PENN Perelman Sch. Med., Philadelphia, PA; ²VA Med. Ctr., Philadelphia, PA; ³Duke Univ., Durham, NC

Abstract: Aims: A cardinal feature of addiction is the afflicted individual's profound ambivalence toward the abused drug. Even after experiencing repeated negative (even lifethreatening) consequences of drug pursuit, addicted individuals still experience the strong incentive motivational "pull" of drug reminder cues. This "pull" is reflected both in the high rates of relapse and in the triggering of the brain's motivational circuitry by drug reward cues. Using a behavioral task of attention to cocaine (vs. neutral) cues, we tested whether cocaine patients with a greater brain response to cocaine cues would pay greater attention to the drug cues, reflecting the positive attentional "pull" of the cues - or whether they might actually pay less attention to the cocaine cues ("look away"), reflecting ambivalence for the drug. Methods: In a "fast" event-related BOLD fMRI paradigm, treatment-seeking cocaine inpatients (n=27, ongoing) were exposed to cocaine-related (and comparison) cues of 33 msec duration in a backward-masking paradigm; 48 images of each cue type were presented in quasi-random order. Image preprocessing was conducted within a standard SPM 8 pipeline, with pre-planned contrasts (e.g., drug-neutral; first half of task). Subsequent performance (RT_{neutral} - RT_{cocaine}) in a classic "dot-probe" attentional bias task was used to measure differential attention, toward or *away*, from cocaine (vs. neutral) cues. The mean attentional bias score was used as a regressor (covariate of interest) in the drug-neutral contrast. Statistical parametric maps were thresholded (2 < t < 5) for display.

Results: Strikingly, the brain correlation maps revealed a robust and widespread *inverse* correlation between the brain response to 33 msec subliminal cocaine cues and the attentional bias scores to cocaine cues. The inversely correlated brain regions included multiple nodes of both the subcortical motivational circuitry (caudate, putamen, insula, midbrain tegmentum, medial orbitofrontal cortex) and cortical modulatory regions (inferior frontal gryus, anterior cingulate, dorsolateral prefrontal cortex) regions, as well as the cerebellum.

Conclusions: Intriguingly, cocaine patients with the *strongest* brain response to the 33 msec cues were actually more likely to "look away" from the cocaine cues in the attentional bias task. The "I must avert my eyes" response has been observed in clinical disorders where the tested stimulus contains elements of fear and threat (e.g., phobia). Whether the 'look away' response simply reflects a heightened cue sensitivity - thus a greater risk of relapse -- or a potential coping response, will be tested directly in future outcome analyses.

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Poster

333. Cocaine and Neurotransmission

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA015214

Title: Effect of intra-nucleus accumbens shell infusion of the CB1 receptor antagonist AM251 on reinstatement of cocaine-seeking behavior

Authors: *M. C. KNOUSE, S. E. SWINFORD-JACKSON, M. E. HOFMANN, R. C. PIERCE Dept. of Psychiatry, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Repeated cycles of abstinence and relapse are a defining feature of cocaine use disorder, and there are currently no FDA-approved pharmacotherapies to prevent relapse to cocaine. Several factors can provoke relapse, including a sensitivity to cocaine-related cues, reexposure to cocaine, and stressful life events. Previous studies have demonstrated a role for the cannabinoid type 1 (CB1) receptor in the reinstatement of cocaine-seeking in rodent models. Systemic administration of CB1 receptor antagonists attenuated reinstatement of cocaine-seeking elicited by cocaine priming injections as well as cocaine-associated cues. Administration of the CB1 receptor antagonist AM251 (10 ug/ul) directly into the nucleus accumbens suppressed cocaine-primed reinstatement (Xi et al., 2006). However, the effect of intra-nucleus accumbens administration of AM251 on reinstatement evoked by other stimuli, including cocaine-associated cues, has not been examined. We tested the hypothesis that an intra-nucleus accumbens shell infusion of AM251 would attenuate both cocaine-induced and cue-induced reinstatement of cocaine-seeking behavior. Rats were trained to lever press for cocaine (0.254 mg/infusion) for 21 days on an FR1-5 schedule. Responding on the active lever resulted in an infusion of cocaine paired with activation of a cue light above the lever; inactive lever presses had no consequences. Rats then underwent extinction training where responses on the active lever produced infusions of saline but no light cues were delivered. Cocaine-seeking behavior was reinstated by noncontingent injection of cocaine (10 mg/kg, i.p.) or delivery of the cocaine-paired light cue in separate groups of rats. Fifteen minutes before each cocaine-primed reinstatement session, rats were pretreated with an intra-nucleus accumbens shell infusion (1 ul/side) of vehicle (25% hydroxypropyl- β -cyclodextrin), 5 µg/µL of AM251, or 10 µg/µL of AM251 in a within-subjects design; rats evaluated for cue-primed reinstatement received only vehicle and the 10 μ g/ μ L dose of AM251 in a within-subjects design. Preliminary data suggest that 10 µg/µL of AM251 attenuates cocaine- and cue-primed reinstatement of cocaine-seeking; additional experiments are

ongoing. These data suggest CB1 receptors in the nucleus accumbens shell may be involved in both cocaine-primed and cue-induced reinstatement. Our results replicate and extend prior findings which support the notion that CB1 receptor antagonists may be a potential pharmacotherapeutic for cocaine craving and relapse.

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Poster

333. Cocaine and Neurotransmission

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant 1R01DA029122-03

Title: Ca_v1.2 channels mediate extinction of cocaine-associated memories via its interaction with signaling mechanisms downstream of dopamine D1R activation

Authors: *C. E. BURGDORF^{1,2}, D. FISCHER², A. M. RAJADHYAKSHA^{1,2} ¹Feil Family Brain and Mind Res. Inst., ²Pediatric Neurology, Pediatrics, Weill Cornell Med., New York, NY

Abstract: Cocaine addiction is characterized by a high rate of relapse in response to cocaineassociated cues or contexts despite interventions such as behavioral therapy. Such persistent drug-seeking behavior results in part from established drug-associated contextual memories that are often resistant to extinction learning. Similar to other forms of experience-dependent learning, extinction learning has been proposed to utilize calcium-dependent signaling pathways. We have evidence that extinction of cocaine conditioned place preference (CPP), a commonly employed contextual behavioral model in addiction research, requires Ca_v1.2 within the dorsal hippocampus. In addition, using molecular studies with mice expressing cell-type specific knockout of Cav1.2, we have found that changes in synaptic proteins associated with extinction behavior are also dependent on Cav1.2 channels in dopamine D1 receptor (D1R)-expressing cells. Region-specific studies are investigating whether activity within D1R cells of the hippocampus are required for extinction of cocaine CPP. Activation of D1Rs initiate several signaling cascades, including PKA via interactions with AKAP150, molecules that we find are increased following extinction of cocaine CPP. In addition, PKA and AKAP appear to also regulate the effects of Cav1.2 channel activity, both at the synapse and via changes in gene expression within the nucleus. Ongoing studies are investigating specific Cav1.2-dependent changes in gene expression within D1R-expressing cells of the dorsal hippocampus following

extinction of cocaine CPP. These studies aim to identify the role of $Ca_v 1.2$ in mediating extinction of cocaine-related behaviors.

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Poster

333. Cocaine and Neurotransmission

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The Hartwell Foundation

Weill Cornell Autism Research Program

Title: Cav1.2 channels underlie stress-induced relapse of cocaine-seeking

Authors: *C. C. BAVLEY, R. FETCHO, B. HALL, D. K. FISCHER, C. BURGDORF, C. LISTON, A. M. RAJADHYAKSHA Pediatric Neurol., Weill Cornell Med., New York, NY

Abstract: Cocaine addiction is a large public health concern, and while current treatment methods help reduce drug-taking, they are largely ineffective at preventing relapse. Current evidence supports the diathesis-stress model of substance abuse, which postulates that genetic predisposition alters the response to environmental factors, such as exposure to stressful life events, in order to increase risk for substance abuse and relapse. The gene CACNA1C, which codes for the L-type voltage-gated Ca2+ channel Cav1.2, has been implicated in a multitude of neuropsychiatric disorders, including bipolar disorder, major depressive disorder, and schizophrenia. These disorders show high comorbidity with substance abuse disorders, suggesting that they may share similar etiologies. Additionally, previous studies have identified a role of Cav1.2 in mediating the effects of stress on the brain. However, no studies have examined a role of Cav1.2 in models of stress-induced relapse of cocaine addiction. Preliminary data indicate that heterozygous loss of Cav1.2 or focal knockdown of Cav1.2 within the PL reduce cocaine conditioned place preference (CPP) following exposure to an acute stressor. Current experiments are aimed at identifying the neural circuitry involving the PL that mediates stress-induced reinstatement of cocaine CPP and the role of Cav1.2 channels therein.

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Poster

333. Cocaine and Neurotransmission

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA040837

Title: Impact of intra-nucleus accumbens MS-275, a class I HDAC inhibitor, on the histone post-translational landscape as well as cocaine reinstatement

Authors: ***A. S. THOMAS**¹, B. FANT¹, S. E. SWINFORD-JACKSON¹, N. V. BHANU², B. A. GARCIA², R. C. PIERCE¹

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Abstract: Accumulating evidence suggests that cocaine-induced epigenetic modifications, such as histone post translational modifications (PTMs), contribute to addiction and relapse to cocaine. As such, pharmacotherapies targeting PTMs are interesting new candidates for the treatment of cocaine use disorder. For example, certain histone deacetylase (HDAC) inhibitors have been shown to attenuate cocaine-taking and relapse-like behavior in rodent models (Romieu et al. 2008; Romieu et al. 2011). Although pan-HDAC inhibitors have been investigated with mixed results, more specific inhibitors have yet to be extensively evaluated for efficacy in attenuating cocaine-related behaviors. Class I HDACs are a promising inhibitor target given that these enzymes are the most abundant of their kind in the brain and are more catalytically active than other classes of HDACs. Previous studies demonstrated that intra-nucleus accumbens (NAc) infusion of the class I HDAC inhibitor MS-275 attenuated cocaine-induced locomotor sensitization. When paired with repeated administration of non-contingent cocaine, chronic intra-NAc infusion of MS-275 altered both acetylation and methylation patterns in the NAc, suggesting that MS-275 may broadly alter histone PTMs. We hypothesized that MS-275, cocaine, and their combination would differentially alter histone PTMs in the NAc shell, with potential behavioral consequences. Rats received bilateral infusions (2 µl/side) of MS-275 (500 µM) or vehicle (1% DMSO) into the NAc shell. One hour following intracranial infusion, rats were pretreated with saline (1 ml/kg, i.p.) and placed into open field chambers where locomotor activity was recorded for two hours. This procedure was repeated for two additional days except that rats were pretreated with either cocaine (10mg/kg, i.p.) or saline (1ml/kg, i.p.) immediately prior to placement in the locomotor chambers. Tissue punches of the NAc shell were collected immediately following the third locomotor activity session. Histones were extracted from the NAc shell and analyzed via mass spectrometry to identify differences in histone PTMs across treatment groups. Future studies will investigate the effect of intra-NAc shell infusion of MS-275

on rodent models of cocaine-taking and relapse. Identifying epigenetic targets differentially modified by cocaine, HDAC inhibitors, and their interaction may provide insight for future therapeutic development with even finer targeting.

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Poster

333. Cocaine and Neurotransmission

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Title: Caveolin-1 involvement in cocaine-induced locomotor sensitization

Authors: *K. R. TONN, J. C. DWORSKY, N. M. STEENROD, M. J. THOMAS, P. G. MERMELSTEIN Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Caveolin-1 (Cav1) is an integral membrane protein that creates functional microdomains of neuronal proteins within lipid rafts. Cav1 regulates a variety of signaling pathways, including mGluR-activated G protein cascades, and is involved in membrane trafficking of proteins such as estrogen and dopamine receptors. Recent studies have shown that altering Cav1 expression influences neuronal plasticity and related behaviors in contexts ranging from learning and memory to chronic injury. Given this relationship between Cav1 and experience-dependent plasticity, we hypothesized that Cav1 expression would also be involved in drug-induced changes in neuronal signaling. We utilized a locomotor sensitization paradigm to test this hypothesis. Wild-type C57Bl6 mice received five days of repeated cocaine or saline exposure, followed by a week of abstinence and a subsequent challenge dose of either cocaine or saline. Animals receiving repeated cocaine displayed behavioral sensitization and greater expression of Cav1 mRNA in the nucleus accumbens when compared to saline-treated controls. Changes were specific to the Cav1 isoform, as no differences were observed in Cav2 or Cav3 mRNA within the nucleus accumbens following cocaine treatment. Additional studies are underway to determine the necessity of Cav1 for the development of cocaine-induced locomotor sensitization. Preliminary results in Cav1 knock-out mice show blunted responses to cocaine and impaired sensitization. Further work will investigate the molecular mechanisms underlying the relationship between Cav1 expression and cocaine-induced signaling.

Disclosures: K.R. Tonn: None. J.C. Dworsky: None. N.M. Steenrod: None. M.J. Thomas: None. P.G. Mermelstein: None.

Poster

333. Cocaine and Neurotransmission

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 333.18/SS49

Topic: G.08. Drugs of Abuse and Addiction

Title: Escalating, non-contingent cocaine exposure induces persisting changes in synaptic transmission and LTP in the mouse ventral hippocampus

Authors: C. PRESTON¹, *J. J. WAGNER²

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Abstract: We investigated the actions of a non-contingent cocaine dosing protocol on locomotor sensitization (LMS) and conditioned place preference (CPP). The effects of either cocaine or saline injections were assessed on measures of synaptic transmission and long-term potentiation (LTP) in the ventral hippocampus (vH). Locomotor activity was monitored in C57BL/6J mice that received either saline or cocaine (10 mg/kg i.p.). CPP was established using a modified conditioning protocol similar to the one described by Itzhak & Anderson (2012) that involved cocaine-treated mice receiving an escalating (Esc) dose protocol (4, 8, 16, 24, 16, 24, 32, 32 mg/kg i.p. once daily) on their least preferred side, whereas saline (Sal) mice were treated on each side of the two compartment apparatus. This protocol produced significant CPP and LMS 1 day and 1 week after cocaine conditioning, respectively. Littermates that did not undergo conditioning were handled intermittently to generate a "naïve" control group to establish the baseline LTP magnitude. Slices were prepared from the vH and field EPSPs were recorded in the CA1 region 4 weeks after the final injection day. Following the induction of LTP (100Hz/1sec*3), normalized fEPSP values were increased (1.57 +/- 0.03) in behaviorally naïve mice 60 minutes post-induction. Mice in the Sal group had a significant (p<0.05) increase in LTP (1.69 + - 0.03) as compared to naïve mice whereas the Esc group displayed an elevated but not significant increase in LTP (1.64 +/- 0.04). In order to reduce stress effects related to the behavioral protocol, separate groups of saline- and cocaine-treated mice were pretreated with the κ -opioid antagonist NorBNI (10 mg/kg i.p.) the day prior to initial CPP testing (pretreatment did not alter either LMS or CPP). Compared to naïve animals, the Sal/NorBNI group LTP was no longer significantly elevated (1.62 +/- 0.04). When compared to the Sal/NorBNI group, the Esc/NorBNI group LTP was significantly (p<0.05) decreased (1.52 +/- 0.03). This significant decrease in LTP between the Sal/NorBNI and Esc/NorBNI groups indicates the action of cocaine in the absence of the concomitant effect of stress induced by the behavioral protocol (i.p. injections/novel environment). Interestingly, the Esc/NorBNI treated animals also exhibited a

significant leftward shift in the stimulus sensitivity of their initial baseline fEPSP responses. Together, these findings are consistent with the hypothesis that an enhancement of neurotransmission contributes to a partial occlusion of LTP in the vH of cocaine-exposed mice that persists 4 weeks after the final drug exposure.

Disclosures: C. Preston: None. J.J. Wagner: None.

Poster

333. Cocaine and Neurotransmission

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Topic: G.08. Drugs of Abuse and Addiction

Support: 1R25MH092912-01

G12 MD007600

NSF DBI-1337284

Title: Intranasal oxytocin reduces cocaine conditioned locomotion and anxiety-like behaviors: Possible modulation of the endocannabinoid system within the mesolimbic system

Authors: *G. C. MOLINA-CASTRO, A. N. FIGUEROA-GONZALEZ, A. L. LOYOLA-VÉLEZ, A. V. MARTI-MENDOZA, E. TORRES-HERNÁNDEZ, C. S. MALDONADO-VLAAR

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Abstract: Oxytocin (OT) is a neuropeptide secreted by the hypothalamic paraventricular nucleus (PVN) and commonly associated with social behaviors, stress responses and drug-addiction. Previous studies have shown that OT has anxiolytic properties associated with cues in a cocaine-conditioning paradigm, but the underlying mechanism remains unknown. This study aims to characterize possible mechanistic interactions between OT and the endocannabinoid system mediating cocaine conditioning and anxiety response, in particular the cannabinoid receptor type 1 (CB1). Rats were exposed to activity chambers after receiving systemic intraperitoneal injections of cocaine (10 mg/kg) or saline 0.9%. during five consecutive days. On the testing session (D7), rats received intranasal infusions of OT (1 $\mu g/\mu L$) or vehicle (saline 0.9%) 30 minutes prior being exposed to the cue-associated environment. Our results showed that OT pretreatment reduced both cocaine-paired conditioned locomotion and anxiety-like behaviors. Preliminary immunohistochemical analysis showed a colocalization of OT receptors and CB1 receptors within the nucleus accumbens (NAc), pre-frontal cortex (PFC) and hippocampus in all treated animals. Further pharmacological role of endocannabinoids and glial involvement is under study. The proposed mechanism includes a cross-talk between OT and endocannabinoid

system within mesolimbic system that might be responsible for the anxiety modulation triggered by cocaine exposure. This data suggests intranasal OT as a novel therapeutic approach of cocaine addiction.

Disclosures: G.C. Molina-Castro: None. A.N. Figueroa-Gonzalez: None. A.L. Loyola-Vélez: None. A.V. Marti-Mendoza: None. E. Torres-Hernández: None. C.S. Maldonado-Vlaar: None.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA015214

Title: Kainate receptor expression and function in a rat model of cocaine self-administration

Authors: *M. HOFMANN, B. FANT, S. E. SWINFORD-JACKSON, M. C. KNOUSE, A. S. THOMAS, R. C. PIERCE

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Abstract: Addiction to cocaine remains a major public health concern in the United States due to its high rate of relapse. Chronic exposure to cocaine causes changes to the expression landscape of a variety receptors in the brain including ionotropic glutamate receptors. The clear majority of research on glutamatergic signaling following cocaine exposure has focused on AMPA and NMDA receptors, while our understanding of the role for kainate receptors in models of cocaine addiction remains limited. There are 5 subtypes of kainate receptors, GluK1-5, which form homo- and heteromeric tetramers for functional receptors. Here, we examined the expression of kainate receptor subtypes using qPCR and kainate receptor function using electrophysiology in the nucleus accumbens (NAc) of rats following 21 days of cocaine selfadministration. Rats were separated into two groups: one group self-administered cocaine and one group was a paired yoked-saline group. Initially, rats self-administered cocaine (0.25 mg/infusion i.v.) over a daily 2-hour session on a fixed ratio (FR) 1 schedule of reinforcement until reaching stable responding. Then, rats transitioned to a FR5 schedule of reinforcement. The yoked-saline rats received a saline infusion (59 µL/infusion i.v.) for every cocaine infusion from their paired cocaine self-administering rat. After 21 days of acquisition, brains were removed and used for either qPCR or electrophysiology to assess changes to kainate receptor expression or function, respectively. Using qPCR on NAc punches, we confirmed previous reports demonstrating that GluK2, GluK3, and GluK5 were the predominant subtypes expressed in the NAc. Compared to yoked saline controls, rats that self-administered cocaine demonstrated a

significant increase in the expression of GluK2 and no changes to GluK3 or GluK5 expression. Next, we wanted to assess whether this increase in GluK2 expression translated into changes to kainate receptor function using whole-cell recordings from medium spiny neurons (MSNs) in NAc slices. Application of 10 μ M kainate caused decreases in the holding current and membrane resistance of MSNs indicative of the activation of postsynaptic kainate receptors. However, there was no significant difference in these effects of kainate when comparing MSN recordings from yoked saline rats vs. cocaine self-administration rats. These results suggest the observed increase in GluK2 mRNA from cocaine exposed rats likely did not translate into increased expression of postsynaptic kainate receptors. Future studies will determine the potential role of kainate receptors following extinction and reinstatement in this cocaine self-administration model.

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Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA036582

Title: Role of PT-type cortical neurons in locomotor sensitization, conditioned place preference, and reinstatement of drug-seeking to cocaine

Authors: *A. F. GARCIA^{1,2}, I. G. WEBB¹, S. M. FERGUSON^{1,2,3}

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Abstract: Corticostriatal projections play an important role in addictive behaviors, especially in the processing of drug-associated cues. While most studies examine the prefrontal cortex as a homogenous structure, there are distinct cortical cell types that project to striatum. The intratelencephalic-type (IT-type) neurons project to both striatal hemispheres and contralateral cortex whereas pyramidal tract-type (PT-type) neurons project primarily to ipsilateral striatum and the pyramidal tract. These cortical projections to striatum arise from layer V pyramidal cells, and differ in several ways including dendritic arborization pattern, cell morphology, conduction velocity, presence of h-currents, and projection pattern. Notably, it has also been found that IT-type cells preferentially project to direct pathway striatal neurons and PT-type cells preferentially project to indirect pathway striatal neurons. The role these subtypes of cortical neurons play in addiction-related behaviors has not been examined. In order to begin to address this, we used a combinatorial viral vector strategy (retrograde CAV-Cre in pyramidal tract and a Cre-dependent
Gi/o-coupled DREADD into the anterior cingulate) in male Sprague Dawley rats to selectively target DREADD expression to PT neurons. The effect of transient inhibition of these neurons was examined in cocaine-induced locomotor sensitization, a conditioned place preference to cocaine and reinstatement of drug-seeking following cocaine self-administration. We found that DREADD-mediated inhibition of PT neurons significantly enhanced locomotor sensitization. Furthermore, inhibition of PT neurons enhanced the rewarding properties of cocaine, as animals significantly preferred a chamber associated with PT inhibition plus cocaine compared to just cocaine. Interestingly, this effect of PT inhibition was specific to cocaine, as it had no effect on a hedonic food reward-induced CPP, nor did it produce a place preference by itself. Finally, we found that PT inhibition is sufficient to modulate the effects of cocaine when they are concurrent, but not sufficient to alter pre-existing associations.

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Poster

333. Cocaine and Neurotransmission

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Intramural Research Program (ZIA-DA000069)

Title: Behavioral effects of dietary zinc manipulation on zinc physiology and cocaine locomotor sensitization

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Abstract: Zinc imbalances are associated with many psychiatric and brain disorders, including depression, autism, ADHD, Alzheimer's Disease, and addiction. As an essential element for sustaining life, zinc homeostasis is vital for proper physiological function of cellular processes throughout the body. However, its role in the brain is poorly understood. Brain zinc exists in two forms: about 90% of it is static and found on metalloproteins, while the remaining labile zinc acts on a variety of biological processes and is predominantly found in zinc-containing glutamatergic neurons, which package zinc and glutamate into the same vesicles and co-release them into the synapse. While in the synapse, zinc is known to bind and modulate the activity of a

variety of receptors (including NMDA, AMPA, and GABA receptors). Zinc can also transverse synapses and regulate transcriptional processes in post-synaptic cells. Here, we examine how a dietary manipulation of zinc alters peripheral physiological measurements of zinc (in blood, hair, and urine) and how that subsequently alters brain zinc. Furthermore, animals with varying zinc diets show differential responses to cocaine, which appear to be linked to brain zinc levels and changes in synaptic plasticity. Our work further expands on the role of zinc-mediated signaling pathways that contribute to changes in synaptic plasticity and its potential relevance to addiction.

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Poster

333. Cocaine and Neurotransmission

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Topic: G.08. Drugs of Abuse and Addiction

Support: Mellon Foundation Award to A.L. Riley

a-PVP, S(-)a-PVP and R(+)a-PVP generously provided by K.C. Rice

Title: Conditioned taste avoidance and conditioned place preference induced by racemic and enantiomer forms of α -pyrrolidinopentiophenone

Authors: *K. H. NELSON¹, B. J. HEMPEL¹, M. M. CLASEN¹, C. J. WOLOSHCHUK¹, K. C. RICE², A. L. RILEY¹ ¹Psychology, American Univ., Washington, DC; ²Chem. Biol Res. Br., NIH, NIDA & NIAAA, Rockville, MD

Abstract: *Background:* The synthetic cathinone, α -pyrrolidinopentiophenone (α -PVP), has been reported to have both rewarding and aversive effects. Given that the balance of these effects mediates abuse vulnerability, it is important to assess the factors that might impact it. One factor is the chemical composition of the drug itself. Specifically, work on α -PVP has been with the racemate, and with other synthetic cathinones there are differences between their enantiomers, e.g., differences in potency, general activity and mechanism of action. To address if the aversive and rewarding effects of the enantiomers of α -PVP differ, the present studies examined the ability of racemic α -PVP and its S(-) and R(+) enantiomers to induce conditioned taste avoidance and conditioned taste preferences, respectively.

Methods: In Experiment 1, adult male Sprague-Dawley rats were exposed to a novel saccharin solution, injected with either 0, 0.3, 1.0 and 3.0 mg/kg racemic α -PVP (IP) and placed on one

side of a place conditioning apparatus. The next day, they were injected with vehicle, given access to water and placed on the other side. Following four cycles, saccharin avoidance and place preferences were assessed. Based on the dose that induced avoidance and preferences (3 mg/kg), Experiment 2 was run under identical procedures assessing the aversive and rewarding effects of either racemic α -PVP, S(-) α -PVP or R(+) α -PVP.

Results: In Experiment 1, α -PVP induced dose-dependent taste avoidance as well as significant increases in time spent on the drug-paired side (although this effect was not dependent on dose). In Experiment 2, both the racemate and the S(-) enantiomer produced significant and comparable taste avoidance, whereas the R(+) enantiomer never significantly differed from vehicle. There was no evidence of place preference conditioning at this single dose of α -PVP.

Conclusions: The effects of the S(-) enantiomer of α -PVP paralleled those of the racemate in taste avoidance (as well as hyperthermia: data not described) suggesting that this enantiomer mediates the effects of α -PVP (similar to that seen with MDPV). The failure to see any effects of the various forms of α -PVP in place preference conditioning is likely due to the limited number of subjects tested at this single dose, given that prior work has reported preferences with the racemate. The rewarding effects should be studied further given the reported abuse potential of α -PVP to determine the relative contribution of the enantiomers to its overall affective properties and use and abuse.

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Poster

333. Cocaine and Neurotransmission

Location: Halls A-C

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Topic: G.08. Drugs of Abuse and Addiction

Title: Evaluating the role of mGluR5 in post-cocaine working memory deficits and persistent cocaine-seeking

Authors: *C. M. GOBIN, M. SCHWENDT Psychology, Univ. of Florida, Gainesville, FL

Abstract: Chronic cocaine abuse produces motivational and cognitive deficits that might be rooted in abnormal function of circuitry that includes the medial prefrontal cortex (mPFC). While diminished activity of the PFC during post-cocaine abstinence has been linked to deficits in working memory and cognitive flexibility, over activation of glutamatergic output from the mPFC promotes relapse to drug-seeking. Systemic administration of metabotropic glutamate receptor 5 (mGluR5) antagonists attenuate relapse, but prolonged blockade of mGluR5 might exacerbate post-cocaine PFC dysfunction. Conversely, stimulating mGluR5, while potentially

beneficial for reversing PFC dysfunctions, may increase the risk of relapse. Previously, we showed that chronic cocaine self-administration results in load-dependent impairments in working memory in adult male Sprague-Dawley rats, as assessed with an operant delayed-matchto-sample (DMS) task. Furthermore, we showed that daily systemic administration of the mGluR5 antagonist MTEP (3mg/kg) attenuated cue+context-induced cocaine seeking (after 45-90 d of abstinence) and also impaired working memory performance in the DMS task. Here we wanted to evaluate the effects of prolonged allosteric activation of mGluR5 on post-cocaine working memory performance. After chronic cocaine self-administration, rats trained in the DMS task at short (0-12s) delays followed by testing at the full (0-24s) set of delays for a block of five days. Next, rats received daily systemic injections of a vehicle solution or the mGluR5 positive allosteric modulator CDPPB (30mg/kg) over a block of five days followed by a washout period of five days. Finally, rats underwent a cue+context-induced relapse test with either CDPPB (30mg/kg) or vehicle administered beforehand. mPFC tissue was collected immediately after the test and will be analyzed by quantitative cytochrome oxidase histochemistry to evaluate persistent metabolic activity and by immunoblotting to measure changes in mGluR5-dependent signaling. We predict that (1) CDPPB reverses working memory deficits in rats with a history of cocaine We also predict that (2) metabolic activity within the mPFC will correlate with the magnitude of post-cocaine working memory deficits and (3) activation of mGluR5 signaling will correlate with the 'intensity' of drug-seeking during relapse. Future studies will explore the effects of localized, intra-mPFC interventions targeting mGluR5 for their dual ability to restore post-cocaine cognitive deficits, while reducing cocaine-seeking.

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Poster

333. Cocaine and Neurotransmission

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Topic: G.08. Drugs of Abuse and Addiction

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Title: Cocaine self-administration and extinction induces adaptations in NMDA receptormediated currents in the nucleus accumbens core

Authors: M. T. SEPULVEDA-ORENGO¹, K. L. HEALEY², *K. J. REISSNER³ ¹Dept. Psychology and Neurosci., ²Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; ³Dept. of Psychology and Neurosci., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Our lab has previously shown that cocaine self-administration and extinction leads to adaptations in astrocytes from the nucleus accumbens (NAc) core, including decreased surface

area, volume, and synaptic colocalization (Scofield et al, 2016). Because astrocytes are an important source of the NMDA co-agonist D-serine, we hypothesized that the reduction in synaptic colocalization would lead to impaired astrocyte modulation of NMDA receptor function. We previously showed that the stimulus-response relationship of isolated NMDA receptor currents is reduced in medium spiny neurons (MSNs) from cocaine versus saline experienced animals, an effect which is reversed by D-serine augmentation (SFN abstract, 2016). To extend these findings, we have used whole cell patch-clamp recordings to measure the current-voltage relationship of NMDA receptor-mediated currents, as well as the response to Dserine perfusion of NMDA receptor-mediated currents, in MSNs from cocaine versus saline extinguished rats. We have also assessed the contribution of specific NMDA receptor subunits to the cocaine-mediated adaptations in NMDA receptor currents. Preliminary data suggest that NMDA receptor currents are decreased following cocaine self-administration and extinction at both positive and negative holding potentials, an effect which is reversed by D-serine augmentation. In addition, ifenprodil application reduces NMDA receptor currents most effectively in MSNs from cocaine-experienced rats treated with D-serine augmentation, suggesting that restoration of NMDA receptor function by D-serine augmentation occurs at least in part through increased contributions of NR2B receptor subunits. These findings support the existing evidence suggesting that modulation of synaptic transmission by astrocytes in the nucleus accumbens is impaired following cocaine self-administration, an effect which can be ameliorated by systemic administration of D-serine.

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Poster

333. Cocaine and Neurotransmission

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Topic: G.08. Drugs of Abuse and Addiction

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Title: Cocaine-induced histone methylation on Egr3 and Nab2 promoters

Authors: B. EVANS¹, R. CHANDRA³, M. MCGLINCY¹, A. CHOW¹, K. K. COVER¹, M. ENGELN³, T. C. FRANCIS⁴, *M. LOBO² ²Anat. and Neurobio., ¹Univ. of Maryland Sch. of Med., Baltimore, MD; ³Anat. and Neurobio., Univ. of Maryland Baltimore, Baltimore, MD; ⁴Dept. of Anat. and Neurobio., Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: Epigenetic modifications plays an important role in transcriptional changes in the nucleus accumbens (NAc) in cocaine addiction. However, there is little information about the

role of histone demethylation in the NAc, after exposure to drugs of abuse. The NAc is a critical brain region, which mediates motivation for drugs of abuse. The NAc is composed of two types of medium spiny neurons (MSNs), which are differentiated by their enrichment of dopamine D1 vs. D2 receptors. We previously demonstrated that the transcription factor, Egr3, is upregulated in D1-MSNs and down-regulated in D2-MSNs after repeated cocaine exposure. It is postulated that Egr3 regulates its co-repressor; Nab2 and they both act together as a feedback mechanism to repress Egr3 transcription. Consistent with this, we observed a reduction of Nab2 in D1-MSNs and an increase of Nab2 in D2-MSNs after repeated cocaine. In order to further investigate the molecular regulation of Egr3 and Nab2 we examined the histone lysine demethylase (Kdm1a) enzyme as well as its demethylation targets H3K4me and H3K9me in NAc after repeated cocaine. We observed an increase in Kdm1a mRNA in D1-MSNs and a reduction in D2-MSNs after repeated cocaine. To further assess if Kdm1a might alter methylation on Egr3 and Nab2 promoters after cocaine we performed chromatin immunoprecipitation (ChIP) for histone marks, H3K4me3 and H3K9me2. Repeated cocaine caused changes in H3K4me3 and H3K9me2 on promoters of Egr3 and Nab2. We further found that Kdm1a is enriched on the Egr3 and Nab2 promoters in the NAc after repeated cocaine. To determine if Kdm1a is responsible for bidirectional induction of Egr3 and Nab2 in D1-MSNs vs. D2-MSNs we are developing a CRISPR-Cas9 approach to target specific methylation sites on the Egr3 and Nab2 promoters. In this approach we will alter methylation on the Egr3 and Nab2 promoters by using light-inducible heterodimerizing proteins CRY2-KDM1A and dCas9-CIB1, along with Egr3 and Nab2 gRNAs. This approach will allow us to understand the precise epigenetic mechanisms regulating expression of Egr3 and Nab2 in MSN subtypes during cocaine-mediated behaviors. Overall our studies will provide new information into the effects of cocaine on histone demethylation and its regulation of Egr3 and Nab2 transcription in MSN subtypes.

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Poster

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Title: Mitochondrial fission in nucleus accumbens D1 neuron subtypes mediates cellular and behavioral plasticity to cocaine

Authors: *R. CHANDRA¹, M. ENGELN¹, M. PATTON¹, J. A. MARTIN², C. WERNER², L. M. RIGGS¹, T. C. FRANCIS¹, S. DAS¹, K. GIRVEN¹, P. KONKALMATT³, A. M.

GANCARZ², S. A. GOLDEN⁴, S. INIGUEZ⁵, S. J. RUSSO⁴, G. TURECKI⁶, B. MATHUR¹, C. MEAGHAN¹, D. M. DIETZ², M. K. LOBO¹

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Abstract: Altered brain energy homeostasis is a key adaptation occurring in the cocaineaddicted brain. However, the underlying mechanisms that govern these homeostatic adaptations are not clearly defined. One such mechanism, which is a fundamental component of energy homeostasis and has not been addressed in cocaine abuse are mitochondrial dynamics; which can include mitochondrial biogenesis, fission, and fusion. To provide insight into this we assess mitochondrial dynamics in the two nucleus accumbens (NAc) projection medium spiny neuron (MSN) subtypes, those enriched in dopamine D1 vs. D2 receptors in mice that self-administer cocaine. Using a Cre inducible adeno-associated virus (AAV)-double inverted floxed open reading frame (DIO)-mito-dsRed combined with D1-Cre and D2-Cre mouse lines we label mitochondria in MSN subtypes. Cocaine self-administration (SA) caused an increase in the frequency of smaller mitochondria in D1-MSN dendrites, implicating enhanced mitochondrial fission in D1-MSNs. Consistent with our data we observe an increase in mRNA of Dynaminrelated protein 1 (Drp1), a mediator of outer mitochondrial membrane fission, in NAc of rodents that self-administer cocaine and in postmortem NAc of cocaine dependent individuals. Using the RiboTag approach, we observe an upregulation of ribosome-associated mRNA of Drp1in D1-MSNs and a decrease in D2-MSNs after repeated cocaine. Additionally, the activated form of Drp1 protein is increased in NAc of mice after repeated cocaine injections. We next used a small molecule inhibitor of mitochondrial fission, Mdivi-1, which blocks the activated form of Drp1. Mdivi-1 treatment blunted cocaine conditioned place preference, reduced cocaine locomotor sensitization, and blunted cocaine seeking behavior after cocaine SA. Consistent with these findings, adeno-associated virus (AAV) based Cre-inducible knockdown of Drp1 in D1-MSNs caused reduced seeking behavior after cocaine SA. Finally, we generated Cre-inducible Drp1constitutively active (CA) and Drp1-wild-type (WT) AAVs. Overexpression of Drp1-CA in D1-MSNs increased seeking behavior after cocaine abstinence. In addition, our study demonstrated that overexpression of Drp1-CA in D1-MSNs increased the frequency of smaller mitochondria in D1-MSN dendrites. Since these findings occur in D1-MSN dendrites we are currently investigating how blocking fission or altering Drp1 levels can alter synaptic and structural plasticity. Our findings demonstrate a novel role for altered mitochondrial fission in NAc in cocaine abuse and implicate that blockade of mitochondrial fission has potential therapeutic treatment for cocaine addiction.

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Poster

333. Cocaine and Neurotransmission

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 333.28/SS59

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA F31-DA036989

T32-DA007288

R01-DA033342A

P50-DA015369

Title: Cocaine self-administration causes a persistent reduction of Kv7 channel mediate intrinsic inhibition in the prefrontal cortex

Authors: J. PARRILLA-CARRERO, P. GOSWAMEE, W. BUCHTA, *A. C. RIEGEL Neurosciences, Med. Univ. of South Carolina (MUSC), Charleston, SC

Abstract: Conditioned stimuli associated with self-administration (SA) of cocaine, but not natural rewards like sucrose, elicit persistent craving and drug seeking. Although this response appears to require the dopamine mediated recruitment of nucleus accumbens core-projecting glutamate cells in the prefrontal cortex (PFC), the underlying mechanism remains unclear. Here, we tested the hypothesis that the cue-paired recruitment of dopamine signaling during cocaine-SA persistently disrupts Kv7 channel mediated intrinsic inhibition in PFC glutamate cells. To investigate this, we recorded from glutamatergic pyramidal cells in (L5) of the prelimbic PFC in the current and voltage clamp configuration in slices from naïve rats or rats with a history of conditioned cues (light + tone) presented in conjunction with chronic cocaine-SA, sucrose-SA, yoked-cocaine, or yoked-saline. At 24-hours after completion of operant SA training (14d), most cells in tissue from rats receiving yoked-saline or yoked-cocaine during non-contingent cue presentation resembled responses in naïve rat slices and displayed robust spike-frequency adaptation (SFA). However, cells in slices from contingent cued sucrose-SA or cocaine-SA rats typically lacked SFA, suggesting learning-mediated changes in intrinsic inhibition. Following extinction training (14d), SFA normalized in sucrose-SA control slices, but remained reduced in cocaine-SA slices. Re-exposure of cocaine-SA+extinction rats to drug predictive cues further reduced SFA (75% cells) in PFC slices, without altering SFA in yoked-saline controls. In slices from similarly treated transgenic cFos-GFP rats with a history of cocaine-SA, expression of the GFP-transgene correlated with decreased SFA and reduced Kv7 channel currents. Bath applied

dopamine (10 uM) robustly reduced SFA and Kv7 channel currents in controls, but produced no significant reduction in cocaine-SA tissue suggesting a functional desensitization of Kv7 channels. The Kv7 channel stabilizer retigabine restored SFA when applied to slices from cocaine-SA rats and dose dependently attenuated cue-induced reinstatement when injected in vivo into the PL-PFC. These findings shed light on the functional significance of Kv7 channel mediated intrinsic inhibition in mediating cocaine seeking and may offer a drug target by which cocaine seeking behavior can be treated.

Disclosures: J. Parrilla-Carrero: None. **P. Goswamee:** None. **W. Buchta:** None. **A.C. Riegel:** None.

Poster

333. Cocaine and Neurotransmission

Location: Halls A-C

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R00DA031767

Title: Hypothalamic hypocretin/orexin within ventral midbrain of rat

Authors: *S. J. SIMMONS, R. M. MARTORANA, F. H. TRAN, T. A. GENTILE, J. W. MUSCHAMP

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Abstract: Numerous brain circuits underlie rewarding and reinforcing properties of abused drugs. Once conceived as a relatively homogenous production center of dopamine (DA), anatomy and function of ventral tegmental area (VTA) of Tsai has been re-visited in the recent decade. While VTA houses DA-producing neurons, evidence from the late-2000s revealed a neurochemically- and functionally-distinct nucleus in caudal VTA termed the rostromedial tegmentum (RMTg). Comprised dominantly of GABA-producing neurons, the RMTg receives dense input from lateral habenula and provides inhibition to DA-producing neurons of rostral division of ventral midbrain (i.e. to VTA). Habenular input to RMTg functionally brakes reward/signals aversion, and although clear roles of habenular input to RMTg have been revealed, numerous structures including hypothalamus target RMTg. Hypocretin/orexin (hcrt/ox) peptides are produced within hypothalamus and their transmission underlies wakefulness, arousal and motivation to retrieve salient and palatable reinforcers including high-fat food and drugs of abuse. The present study sought to topographically organize hcrt/ox afferents within ventral midbrain and to provide functional assessment of hcrt/ox transmission within distinct ventral midbrain structures in models of substance use disorder. Rats underwent stereotaxic cannulation surgery to target VTA and RMTg, and fluorescent microspheres were microinjected

to label projection neurons via retrograde transport. Hypothalamus-containing tissue was thereafter immunolabeled for hcrt/ox and sub-regional proportions of hcrt/ox afferents within each target structure were measured. The VTA was found to receive slightly greater input (10.9%) relative to RMTg (8.4%) in cases examined, and no pattern of anterior-posterior topography within hcrt/ox field was observed. Ongoing study is evaluating function of hcrt/ox within ventral midbrain in models of substance use disorder as our exploratory mapping study suggests hcrt/ox may additionally signal to GABA-containing RMTg.

Disclosures: S.J. Simmons: None. R.M. Martorana: None. F.H. Tran: None. T.A. Gentile: None. J.W. Muschamp: None.

Poster

333. Cocaine and Neurotransmission

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Topic: B.12. Glial Mechanisms

Support: NIH Grant MH095044

Title: Neurochemical, metabolomic, and behavioral changes arising from genetic elimination of the putative ceftriaxone target MBLAC1

Authors: *C. RETZLAFF¹, C. L. SNARRENBERG², M. J. GROSS⁷, M. K. HAHN⁷, J. WRIGHT³, D. P. MORTLOCK⁴, S. CODREANU⁵, J. A. MCLEAN⁶, R. D. BLAKELY⁸ ¹Neurosci., Vanderbilt Univ., Jupiter, FL; ²Program in Neurosci., ³Pharmacol., ⁴Mol. Physiol. & Biophysics, Vanderbilt Univ., Nashville, TN; ⁵Chem., Vanderbilt Univ., Nashville, TN; ⁶Chem., Vanderbilt Univ., Nashville, TN; ⁷Brain Inst., ⁸6.Department of Biomed. Science, Charles E. Schmidt Col. of Medicine, Florida Atlantic Univer, Florida Atlantic Univ., Jupiter, FL

Abstract: The etiology of multiple brain disorders stems from a dysfunction in dopaminergic and/or glutamatergic signaling, with phenotypes ranging from movement perturbations to drug dependence to cognitive dysfunction. Our lab conducted a forward genetic screen in *C.elegans* looking to uncover novel regulators of dopamine (DA) signaling, resulting in the discovery of the glial expressed gene *swip-10*. Loss of function mutations of *swip-10* result in a hyperdopaminergic phenotype associated with hyper-excitability of DA neurons which evidence indicates is driven by elevated glutamate (Glu) signaling. In a BLAST search for proteins homologous to SWIP-10, we identified the unstudied protein MBLAC1 (Metallo- β -lactamase domain containing protein 1) as the likely SWIP-10 ortholog. Sequence identity between SWIP-10 and MBLAC1 derives largely from a shared metallo- β -lactamase domain that, in prokaryotes, supports hydrolysis and inactivation of β -lactam antibiotic ceftriaxone (Cef), a drug that has shown

to afford neuroprotection in rodent models of Glu dysfunction as well as to alter glial-dependent control of Glu levels. Because of the potential of MBLAC1 as a Cef target, we used the CRIPSR/Cas9 system to introduce mutations in mouse Mblac1 coding sequences, validating loss of MBLAC1 protein expression by immunoblotting. Here, we report on our initial characterization of the resultant KO line. We are taking a three-pronged approach to uncover phenotypes associated with loss of MBLAC1 expression. First, we are using LC-MS/MS to pursue an untargeted metabolomics approach that can identify small molecules in serum distinguishing WT and Mblac1 KO mice. Analysis of these changes indicates enrichment of pathways that feature sulfur containing metabolites including taurine and taurocholic acid. Second, given the connection of SWIP-10 to C. elegans Glu signaling, we examined expression of glial Glu transporters. Based on Western blot analysis, we observed no significant difference in total GLT1 protein expression in the cortex, whereas a reduction was detected in expression of the Cys/Glu exchanger (XC-) subunit xCT. Lastly, we are exploring behavioral changes arising in Mblac1 KO mice. Initial evaluation of growth rates, reproduction and locomotor/sensory alterations reveals no gross phenotypes. Preliminary studies of drug responses indicate no differences in locomotor response to amphetamine, and we are currently studying the effects of acute and chronic cocaine. Future studies will examine biochemical and behavioral responses to Cef. Supported by NIH Award MH095044 (RDB)

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Poster

334. Brain Circuits Affected by Cocaine

Location: Halls A-C

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Program#/Poster#: 334.01/SS62

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R01DA037257

NIGMS GM09545902

Title: Trim3 in the nucleus accumbens regulates cue-induced cocaine seeking following extended-access cocaine self-administration

Authors: *C. T. WERNER¹, J. A. MARTIN¹, A. LEPACK², Z.-J. WANG¹, A. F. STEWART¹, R. VISWANATHAN¹, A. CACCAMISE¹, R. L. NEVE³, I. S. MAZE², D. M. DIETZ¹ ¹Dept. of Pharm and Tox; Res. Inst. on Addictions; Program in Neurosci., State Univ. of New York at Buffalo, Buffalo, NY; ²Dept. of Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY; ³Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Drug addiction is a chronic brain disease that develops gradually with compulsive use of psychoactive substances. Cocaine is one of such drugs that progressively alters brain regions associated with reward and motivation, including the nucleus accumbens (NAc). The changes in synaptic and epigenetic plasticity mediate cocaine craving evoked by drug-associated cues, which intensifies during abstinence in human addicts and animal models of addiction, and this incubation of craving may underlie persistent relapse susceptibility. The ubiquitin-proteasome system (UPS), which is responsible for proteasomal-dependent protein degradation, has been shown to be involved in cocaine-induced plasticity. Substrates are polyubiquitinated for degradation via E3 ubiquitin ligases. There are over 600 E3s, which are currently being examined for their therapeutic potential in many neuronal disorders due to their high specificity. However, the role of E3s in regulating cocaine-dependent plasticity and relapse behaviors has not been examined. Here, we examined tripartite motif-containing protein 3 (trim3), which regulates the polyubiquitination of substrates involved in drug-induced adaptations. Following extended-access cocaine self-administration, we found dynamic changes in trim3 expression in NAc subcellular fractions. On withdrawal day (WD)1, trim3 levels were decreased in crude synaptosomal fractions from cocaine-treated rats compared with saline controls. Moreover, the levels of the synaptic regulators Shank and γ -actin, which are substrates of trim3, were increased. By contrast, trim3 was decreased in the nuclear fractions on WD30 in cocaine-treated rats compared with saline controls and INO80, a trim3 substrate that regulates transcription, was increased. We used viral-mediated gene transfer in the NAc to demonstrate that trim3 mediates cue-induced cocaine seeking during withdrawal.

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Poster

334. Brain Circuits Affected by Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R01DA037257

NIGMS GM09545902

Title: Examination of E3 ubiquitin ligase Smurf1 following abstinence from cocaine self-administration

Authors: *R. VISWANATHAN, C. T. WERNER, Z.-J. WANG, J. A. MARTIN, D. M. DIETZ Dept. of Pharm and Tox; Res. Inst. on Addictions; Program in Neurosci., State Univ. of New York At Buffalo, Buffalo, NY

Abstract: Drug addiction is a disease characterized by episodes of relapse despite periods of abstinence. It is thought that cellular and molecular events in discrete brain areas of the reward pathway, including the nucleus accumbens (NAc), underlie these long-lasting behavioral changes. Recently, we identified members of the Transforming growth factor (TGF)- β pathway as essential mediators of drug addiction-like plasticity and behavior. However, how TGF-β signaling is regulated in drug addiction has not yet been examined. The TGF- β superfamily of proteins regulates a wide variety of cellular responses, including gene expression and cellular plasticity. Some of these proteins are tagged by E3 ubiquitin ligases for degradation through the ubiquitin-proteasome system (UPS). The UPS regulates the expression of a variety of proteins that play significant roles in synaptic plasticity, learning, memory and behavior and has been shown to be involved in cocaine-induced plasticity. After a 7-day abstinence period following short-access cocaine self-administration in rats, we found that the E3 ubiquitin ligase Smurf1 mediates the expression of the TGF- β family member SMAD1 in the NAc. These data suggest that regulation of SMAD1 via E3 ubiquitin ligases may be involved in drug-dependent cellular and behavioral plasticity. Our future experiments aim to determine the functional role of Smurf1 in mediating drug addiction-like behaviors in an effort to determine if these pathways hold potential therapeutic value in the treatment of substance abuse disorder.

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Poster

334. Brain Circuits Affected by Cocaine

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Program#/Poster#: 334.03/SS64

Topic: G.08. Drugs of Abuse and Addiction

Title: Cocaine regulates extracellular vesicle release in mouse midbrain

Authors: *Y. NAKAMURA, S.-Y. A. TSAI, T.-P. SU Cell. Pathobiology Section, Integrative Neurosci. Res. Br., NIDA IRP, Baltimore, MD

Abstract: Cocaine is an agonist of the sigma 1 receptor (Sig1R) which is a receptor chaperone expressed in the ER membrane. We previously showed, by using NG108 cells, that cocaine evokes the extracellular vesicle (EV) release via the Sig1R-ADP ribosylation factor 6 (ARF6) pathway (Nakamura et al., SfN 2015 and 2016). The present study examined the release of EV in mouse midbrain region after an injection of cocaine (15 mg/kg; i.p.). We first confirmed a

method for the brain EV isolation through a series of differential centrifugations to isolate EVs from the extracellular space of mouse brain (Perez-Gonzalez et al., JBC, 2012), and then examined the EV level by quantification of the EV markers including integrin β 1, Alix, and ARF6. The mitocondrial markers cytochrome-c (Cyto.-c) and the dopamine (DA) neuronal marker tyrosine hydroxylase (TH) were also examined. The EV markers could be seen in the EV sample collected from the mouse midbrain. However, the EV didn't contain Cyto.-c. Those data confirmed that the EV samples, but not cellular debris, could be collected from mouse midbrain. In addition, EVs derived from midbrain, but not cortex or hippocampus, contained a lot of TH. Thus, those data suggest that DA neurons in mouse midbrain region can release TH-containing EVs into extracellular space. Furthermore, 30 min, but not 60 min, after an i.p. injection of cocaine significantly increased the amount of extracellular TH in the form of EV in mouse midbrain. Taken together, our results suggested that the cocaine regulates the EV release from mouse DA neurons via previously the proposed Sig1R-ARF6 pathway. (supported by IRP NIDA NIH)

Disclosures: Y. Nakamura: None. S.A. Tsai: None. T. Su: None.

Poster

334. Brain Circuits Affected by Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Intramural Research Program

Title: Sigma1 receptor modulates cocaine-induced endocannabinoid signaling in the mouse ventral tegmental area

Authors: *D. I. DRYANOVSKI¹, Y. NAKAMURA², T.-P. SU², C. R. LUPICA¹ ¹Electrophysiology Res. Section, ²Cell. Pathobiology Section, Natl. Inst. On Drug Abuse, NIH, Baltimore, MD

Abstract: Endogenous cannabinoids (eCB) are signaling lipids released from neurons to retrogradely activate cannabinoid CB1 receptors (CB1R) located on axon terminals of presynaptic cells to limit neurotransmitter release. The eCB associated with activity dependent modulation of synapses is 2-arachidonoylglycerol (2-AG). We have shown that cocaine can stimulate 2-AG synthesis in the VTA, resulting in CB1R activation on GABAergic axons targeting dopamine (DA) neurons. Moreover, this disinhibition of dopamine neurons by cocaine-induced 2-AG release is functionally relevant since it elevates DA release in target areas, perhaps contributing to the rewarding and addictive properties of cocaine (Wang et al., Cell Rep., 2015). 2-AG synthesis is triggered by activation of G α /11-coupled metabotropic receptors through

activation of phospholipase C- β (PLC β), which hydrolyzes membrane phospholipids to form 1,2dacylglycerol (DAG) and inositol triphosphate (IP3). DAG is converted to 2-AG via diacylglycerol lipase- α (DGL α), whereas IP3 can elevate cytosolic Ca²⁺ by stimulating its release from endoplasmic reticulum (ER). The sigma1 receptor (σ 1R) resides on the mitochondria-ER interface membrane, serving as a protein chaperone, and conferring a conformation of the IP3 receptor that sustains Ca²⁺ signaling. Under basal conditions, σ 1R is bound to ADP-ribosylation factor 6 (ARF6), however, cocaine can bind to σ 1R and release ARF6. Since the σ 1R is involved in Ca²⁺ signaling and the production of 2-AG by cocaine is Ca²⁺ dependent, we examined a possible interaction between these effectors. We measured cocaine-induced 2-AG activity in VTA DA neurons by monitoring its activation of CB1Rs on GABAergic axon terminals and the inhibition of GABA_B-receptor mediated IPSCs. We find that both σ 1R antagonists and ARF6 inhibitors prevent the cocaine-induced inhibition of IPSCs mediated by 2-AG, implicating this system in eCB signaling in the VTA.

Disclosures: D.I. Dryanovski: None. Y. Nakamura: None. T. Su: None. C.R. Lupica: None.

Poster

334. Brain Circuits Affected by Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA R01 DA039865

NIDA R21 DA037636

Title: Circadian transcription factor NPAS2 and metabolic redox sensor SIRT1 interact in the mouse nucleus accumbens (NAc) to regulate cocaine reward-related behavior

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Abstract: Cocaine addiction is a widely prevalent substance use disorder in the United States. With a lack of successful therapeutic options, it is important to investigate the cellular and molecular level changes following cocaine use, and how these changes establish and/or reinforce addiction. As its mechanism of action, cocaine increases mesolimbic dopaminergic signaling via inhibition of dopamine transporter. This increased activity is energy taxing for the cell and can cause both severe oxidative stress and altered mitochondrial function. Interestingly, metabolic changes associated with cocaine use may directly regulate the circadian molecular clock and its output genes through associated metabolic redox sensors. More specifically, the circadian transcription factors CLOCK/NPAS2 and the NAD+ dependent deacetylase, SIRT1, have all been shown to directly respond to changes in levels of the mitochondrial coenzyme NAD+. Previous work in the lab has shown CLOCK and NPAS2 differentially regulate cocaine reward; e.g. mutations in the *Clock* gene increase cocaine preference and self-administration, while mutations in Npas2 yields an opposite phenotype. Moreover, our data suggest NPAS2 regulates reward through its enriched expression in the nucleus accumbens (NAc). Interestingly, SIRT1 modulators have also been shown to regulate cocaine preference, in that SIRT1 agonists increase cocaine preference and vice versa. In addition to NPAS2 and SIRT1 modulation altering cocaine reward, chronic cocaine exposure has been shown to preferentially alter expression of these proteins in the NAc. Given these observations, we investigated how changes in cellular metabolic state may feed into the circadian molecular clock and alter regulation of cocaine reward, and whether an interaction between NPAS2 and SIRT1 in the NAc mediates this regulation. Through co-immunoprecipitation studies, our preliminary findings suggest that NPAS2 and SIRT1 do interact in a shared complex in the NAc, and chronic cocaine may alter this interaction. Furthermore, utilizing high-performance liquid chromatography to assess NAD+ concentration, we observed a diurnal variation of NAD+ levels in the striatum that is disrupted following chronic cocaine exposure. Finally, in mice with a NAc specific viral-mediated knockdown of NPAS2, we determined NPAS2 to be necessary for the increase in cocaine preference seen with a SIRT1 agonist. Ultimately, our findings highlight a mechanism by which chronic cocaine's metabolic changes can directly alter circadian molecular clock function, and how this interaction, mediated by NPAS2 and SIRT1, may afford regulation of cocaine reward-related behavior.

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Poster

334. Brain Circuits Affected by Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: Sussex University: Strategic Development Funds

Title: Regional differences in striatal neuronal ensemble excitability following cocaine and extinction memory retrieval in Fos-GFP mice

Authors: *J. J. ZIMINSKI¹, M. C. SIEBURG², G. MARGETTS-SMITH³, H. S. CROMBAG⁴, E. KOYA¹

²Sch. of Psychology, ¹Univ. of Sussex, Brighton, United Kingdom; ³Univ. of Sussex, Brighton and Hove, United Kingdom; ⁴Univ. Sussex, Brighton, United Kingdom

Abstract: Learned associations between drugs of abuse and the drug administration environment play an important role in addiction. In rodents, exposure to a drug-associated environment elicits conditioned psychomotor activation, which may be weakened following extinction learning. While widespread drug-induced changes in neuronal excitability have been observed, little is known about specific changes within neuronal ensembles activated during the recall of drugenvironment associations. Using a cocaine conditioned locomotion procedure, the present study assessed the excitability of neuronal ensembles in the nucleus accumbens core and shell (NAc^{core} and NAc^{shell}), and dorsal striatum (DS) following cocaine conditioning and extinction in Fos-GFP mice that express green fluorescent protein (GFP) in activated, GFP+, neurons. During conditioning, mice received repeated cocaine injections (20 mg/kg) paired with a locomotor activity chamber (Paired) or home cage (Unpaired). 7-13 days later both groups were re-exposed to the activity chamber under drug-free conditions, and Paired, but not Unpaired, mice exhibited conditioned locomotion. In a separate group of mice, conditioned locomotion was extinguished by repeatedly exposing mice to the activity chamber under drug-free conditions. Following the expression and extinction of conditioned locomotion, GFP+ neurons in the NAc^{core} (but not NAc^{shell} and DS) displayed greater firing capacity compared to surrounding GFP- neurons. This difference in excitability was due to a generalized decrease in GFP- excitability following conditioned locomotion, and a selective increase in GFP+ excitability following its extinction. These results suggest a role for both widespread and ensemble-specific changes in neuronal excitability following recall of drug-environment associations.

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Poster

334. Brain Circuits Affected by Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: R01DA034097 to NPB

NS089633 to NPB

F31DA041214 to RJO

Title: Linking developmentally-regulated genes with drug addiction in the nucleus accumbens of mice overexpressing the RNA-binding protein HuD

Authors: *N. PERRONE-BIZZOZERO, R. J. OLIVER, A. S. GARDINER Neurosciences, Univ. of New Mexico HSC, Albuquerque, NM

Abstract: The neuronal RNA-binding protein (RBP) HuD plays an important role in the posttranscriptional control of gene expression during neuronal development, regeneration, and synaptic plasticity. However, the potential role of HuD in drug addiction is poorly understood. Recent studies in our lab demonstrate that HuD overexpressor (HuD_{OE}) mice display increased cocaine conditioned place preference (CPP) compared to wild type (WT) littermates. In addition, CPP trained HuD_{OE} mice have increased expression of HuD and its validated addiction-related gene targets Camk2a and Bdnf in the nucleus accumbens (NAc), potentially driving this enhanced place preference behavior. To further understand the role of this RBP in drug addiction, we performed RNA-immuno-precipitation and sequencing studies (RIP-seq) of HuDbound RNAs in the striatum of HuD_{OE} mice, using an antibody against myc-tagged HuD. About 1700 coding transcripts showed significant >2 fold enrichment (p<0.05) in the HuD IP vs. WT IP and were defined as putative HuD targets. Importantly, 85.5% of these contained consensus motifs for HuD binding in their 3'UTRs. Comparison of HuD targets and genes in the Knowledgebase of Addiction-related genes database (KARG, http://karg.cbi.pku.edu.cn) revealed that HuD targets represent 17.5% of all the genes in KARG. HuD targets were also significantly enriched in neurogenesis and Wnt signaling pathways, and included many neuronal development-associated genes such as the transcription factors Satb1, Sabtb2, Mef2a, Mef2c, Foxp1, Neurod6, Creb1, Mecp2, Sox5, Sox11 as well as Bdnf, Wnt5a, Jag1, Fz3b, Gs3kb, Dcx, Ndel1, Nlgn3, Nrg1, Nrn1, Nrxn2, Rtn3, Rock2, Stmn3, Sema4d, etc. Given our previous work showing a role for HuD in neurogenesis (Wang et al, PNAS 2015) and our findings that many HuD targets play a role in axonal and dendritic development, we began examining the effect of HuD overexpression on neuronal morphology within the NAc. We found that HuD_{OE} animals have an increased frequency of immature thin spines, with a decrease in intermediate stubby spines but no change in mature mushroom spines within the NAc. This alteration in structural plasticity is similar to changes found in animals trained in CPP, possibly suggesting that these HuD_{OE} animals may be primed for increased drug-reward as determined by their enhanced place preference behavior. Overall, these results are consistent with the notion that some of the plasticity-associated changes during drug addiction not only recapitulate early neuronal development but also recruit similar molecular pathways such as those enriched in HuD targets.

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Poster

334. Brain Circuits Affected by Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: R01DA034097 to NPB

F31DA041214 to RJO

UNM-HSC Research Allocation Committee (RAC) funds to MDO and NPB

Title: HuD regulation of circRNAs expression and localization during cocaine seeking behaviors

Authors: *M. DELL'ORCO, R. J. OLIVER, N. MELLIOS, N. PERRONE-BIZZOZERO Neurosciences, Univ. of New Mexico HSC, Albuquerque, NM

Abstract: Substance use disorder (SUD) is a chronic disease characterized by compulsive drug seeking and use. SUD is thought to be mediated by aberrant expression of proteins regulating synaptic plasticity in neurons such as RNA-binding proteins (RBPs). Several RBPs have been previously associated with addiction including neuronal HuD. Specifically, HuD regulates the expression of many genes listed in the Knowledgebase of Addiction related genes (KARG) database such as c-FOS, BDNF, GAP43, and CAMK2A. At the molecular level HuD binds to cis-acting elements in RNA molecules governing their processing, subcellular localization, translation and degradation. One such group of RNA molecules that could be regulated by HuD are circular RNAs (circRNAs), a novel category of non-coding RNAs that are derived from the back-splicing and covalent joining of exons. Due to their abundant expression in the brain and their localization at synapses, circRNAs could be of importance to neuronal development and neuroplasticity. Notably, circRNAs contain numerous consensus regulatory sequences for RBPs. Our bioinformatics analyses revealed that 29% of mouse circRNAs bear consensus HuD binding motifs. Thus, we aimed to explore whether HuD interaction with circRNAs may be involved in the control of synaptic plasticity during drug addiction. A subset of circRNAs showed altered expression in the nucleus accumbens (NAc) during cocaine-conditioned place preference (CPP). Within this set, we focused on circRNAs related to genes known to be involved in neuroplasticity. For example, circLmo7 and circHomer1, which are downregulated by CPP, and circTulp4 and circSabt2, which are upregulated. Using RNA immunoprecipitation assays (RIP) followed by qRT-PCR we validated the interaction of HuD with circLmo7, circHomer1 and circTulp4. To assess whether HuD binding can alter target circRNAs localization, we also evaluated circRNAs levels in synaptoneurosomes and found that circHomer1 was significantly enriched in this compartment in HuD overexpressing mice (HuD_{OE}) compared to wild-type (WT) mice. Furthermore, we found that the levels of both cirHomer1 and Homer1 mRNA, which is also a known target of HuD, were increased in the NAc of HuD_{OE}. These results are consistent with previous studies showing changes in Homer1 mRNA and protein levels after cocaine administration. Since HuD levels are increased in CPP and HuD_{OE} show increased cocaine CPP, investigating the role of HuD-circRNAs interactions in cocaine-induced neuroplasticity and behavior may uncover new regulatory processes and molecular targets involved in drug addiction.

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334. Brain Circuits Affected by Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant P50 DA015369 (PWK)

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Title: Projections from the nucleus accumbens to the ventral mesencephalon collateralize in the ventral pallidum

Authors: *T. PARDO, J. A. HEINSBROEK, P. W. KALIVAS Neurosci., Med. Univ. of South Carolina, Charleston, SC

Abstract: D1-receptor expressing medium spiny neurons (D1-MSN) in the Nucleus Accumbens (NAc) send projections to the Ventral Pallidum (VP) and the Ventral Tegmental Area, as well as the Substantia Nigra (VTA/SN). In the neighboring dorsal striatum, D1-MSNs passing through the Globus Pallidus are known to form branching collaterals in response to hyperactivity of the striatum. As of now, it is unknown whether D1-MSN in the NAc that project to the VTA/SN also collateralize in the VP, and whether other D1-MSN pathways exists that exclusively innervate the VP or VTA/SN. Since accumbens D1-MSNs drive motivated behavior such as social interaction, food consumption, and addictive behavior (drug use and relapse to drug seeking) it is important to dissect the precise anatomy of D1-MSN projections to VP and VTA/SN. Ultimately, investigating the functional relevance of these different pathways may yield novel treatments for addiction and other psychiatric disorders. We hypothesized that the NAc contains D1-MSN pathways that terminally project to the VP and that a subset of VTA projecting neurons collateralize in the VP. To map the projections from D1-MSN in the accumbens, wild-type C57BL/6 and D1-tdTomato reporter mice received unilateral injections of Fluorogold (FG), via iontophoresis, and retrograde latex microspheres (retrobeads), via pump microinjection, into the VP and VTA respectively. After 7 to 10 days of retrograde transport, mice were perfused, and their brains were sliced and immunohistochemically stained for FG, tdTomato, and/or NeuN. Analyses comprised verification of the tracer placement using histochemical stains, and the counting of accumbens neurons that were retrogradely labeled with only FG, only retrobeads, or both. Preliminary results from cell counts suggest that most D1-MSN that project to the VP collateralize into the VTA/SN. Future experiments will assess the role of D1 projections to the VP and VTA/SN in drug seeking behavior.

Disclosures: T. Pardo: None. J.A. Heinsbroek: None. P.W. Kalivas: None.

334. Brain Circuits Affected by Cocaine

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Support: DA012513

DA015369

Title: Cell type specific regulation of cocaine seeking in the ventral pallidum

Authors: *J. A. HEINSBROEK, A.-C. BOBADILLA, D. N. NEUHOFER, P. W. KALIVAS Med. Univ. of South Carolina, Charleston, SC

Abstract: The ventral pallidum (VP) comprises a critical structure in the neural circuit that drives relapse to cocaine seeking in response to drug cues. It receives inputs from the nucleus accumbens, and various other limbic structures, and drives motivated behavior by means of projections to the ventral tegmental area, subthalamic nucleus, habenula and mediodorsal thalamus. GABAergic inputs from nucleus accumbens dopamine D1- and dopamine D2receptor expressing medium spiny neurons converge onto VP neurons, but carry opposing motivational information. How this information is integrated by the VP to encode the motivation to seek and take cocaine remains unknown, but the VP contains both GABAergic and glutamatergic neurons, and these populations may drive different motivational states during relapse. We investigated the efferent and afferent connectivity of VP GABAergic and glutamatergic neurons and found overlapping but distinct innervation and projection patterns. To dissect the functional role of these neurons, we expressed designer receptors exclusively activated by designer drugs (DREADDs, activated by clozapine-N-oxide) in the ventral pallidum of mice to bidirectionally regulate neuronal activity during drug seeking and drug taking tests. Global inhibition of all VP neurons using the Gi coupled DREADD hM4D reduced cue induced reinstatement. In contrast, global activation the VP using the Gq coupled DREADD hM3D strongly augmented cue-induced cocaine seeking, and triggered reinstatement in extinguished mice. Interestingly, selective chemogenetic manipulation of VP glutamatergic neurons using Vglut-IRES-Cre mice revealed effects opposite to global manipulations: Stimulating glutamatergic neurons prevented reinstatement, and reduced breakpoint responding for cocaine during a progressive ratio test. Furthermore, inhibiting glutamatergic neurons increased breakpoint, induced reinstatement in extinguished mice, and induced a trend towards enhanced cue-induced reinstatement. Preliminary results in VGAT-IRES-Cre mice suggest that stimulating VP GABAergic neurons increased breakpoint responding for cocaine and induced reinstatement in extinguished mice. Collectively, these results indicate differential roles for GABAergic and glutamatergic VP neurons in cocaine seeking and cocaine taking behaviors. Ongoing

experiments are set out to record from GABAergic and glutamatergic VP neurons and dissect the specific contribution of their projections to drug seeking.

Disclosures: J.A. Heinsbroek: None. A. Bobadilla: None. D.N. Neuhofer: None. P.W. Kalivas: None.

Poster

334. Brain Circuits Affected by Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA009621

NIH Grant DA038110

Title: Nucleus accumbens core group I mGluR signaling after the incubation of methamphetamine craving

Authors: *C. MURRAY, M. MILOVANOVIC, J. A. LOWETH, M. E. WOLF Neurosci., Rosalind Franklin Univ. of Med. and Scien, North Chicago, IL

Abstract: In a rodent model of addiction, cue-induced drug craving intensifies or "incubates" during withdrawal from extended-access drug self-administration. After the incubation of cocaine craving, we have shown that synapses in the nucleus accumbens (NAc) core are strengthened by the accumulation of calcium-permeable AMPA receptors (CP-AMPARs) while group I mGluR-mediated synaptic depression shifts from an mGluR5-dependent mechanism that is expressed presynaptically to an mGluR1-dependent mechanism that is expressed postsynaptically by the removal of CP-AMPARs. After prolonged withdrawal from cocaine selfadministration (withdrawal day 48), we detected a decrease in mGluR5 surface expression and a decrease in its association with Homer scaffolding proteins. These alterations may be related to the loss of mGluR5-medated synaptic depression. In the NAc core of methamphetamine incubated rats, CP-AMPARs similarly accumulate and are removed by mGluR1; however, the status of mGluR5 has not been addressed. To this end, rats self-administered saline or methamphetamine (6 h/d for 10 d) and were killed for biochemical studies after 3, 21, or 48 days of withdrawal. Our biochemical findings reveal no change in surface mGluR5 levels (determined with biotinylation) or in the physical association between mGluR5 and Homer proteins (determined using co-immunoprecipitation) at any of the three withdrawal times, diverging from prior cocaine results that demonstrated decreases in both of these measures. Electrophysiological recordings of NAc medium spiny neurons from methamphetamine-incubated animals will test

mGluR5 function to extend these findings. Support: DA009621, DA038110

Disclosures: C. Murray: None. M. Milovanovic: None. J.A. Loweth: None. M.E. Wolf: None.

Poster

334. Brain Circuits Affected by Cocaine

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Support: NIDA Grant DA015835

NIDA Grant DA040414

Title: Dopamine neuromodulation and the regulation of protein translation in cultured nucleus accumbens neurons

Authors: *M. T. STEFANIK¹, C. MURRAY², M. E. WOLF¹

¹Neurosci., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL; ²Neurosci., Rosalind Franklin Univ. of Med. and Scien, North Chicago, IL

Abstract: Protein synthesis is critical for the generation and maintenance of long-lasting synaptic modifications that lead to changes in behavior. Recent work from our lab suggests that, during withdrawal from extended-access cocaine self-administration, adaptations in medium spiny neurons (MSNs) of the nucleus accumbens (NAc) leading to enhanced drug seeking are maintained by dysregulated local translation. In other brain regions, dopaminergic signaling plays a key role in protein synthesis- dependent synaptic plasticity. However, while the NAc receives dopamine (DA) input that is influenced by drugs and DA neurotransmission is involved in both the acute and long-term effects of drugs of abuse, the relationship between DA receptor signaling and protein synthesis in MSNs is not understood. To explore this, we used a co-culture system in which PFC neurons obtained from P1 offspring of homozygous enhanced cyan fluorescent protein (ECFP)-expressing mice were co-cultured with NAc MSNs obtained from P1 Sprague-Dawley rats. PFC neurons restore excitatory inputs to MSNs but can be distinguished by fluorescence. Direct detection of de novo protein synthesis in MSNs was achieved with fluorescent non-canonical amino acid tagging (FUNCAT). Fluorescence immunocytochemistry was used to quantify expression of specific proteins of interest in these cells. Incubation of cultures with the D1R agonist SKF81297 or the D2R agonist quinpirole failed to influence protein translation in MSNs. We re-examined DA receptor effects after increasing excitatory synaptic activity in the cultures using the GABA-A receptor antagonist bicuculline (2 hr). The

D1R antagonist SCH23390, but not the D2R antagonist eticlopride, reduced bicuculline-induced protein synthesis, despite a lack of TH+ neurons in the co-cultures. One potential explanation is that bicuculline-stimulated translation is mediated by glutamate acting at D1R/NMDAR heteromers, which have been reported in other brain regions. Immunofluorescent labeling for D1Rs and the obligatory NMDAR subunit GluN1 suggested the existence of a small population of D1R/NMDAR heteromers on the co-cultured MSNs. We examined signaling of these heteromers by stimulating translation with bicuculline and co-incubating with the NMDAR antagonist APV or APV + SCH23390. Bicuculline- stimulated translation was reversed by either treatment, suggesting that blockade of either receptor in the heteromer may disrupt translation. These results further the understanding of mechanisms controlling local protein synthesis in the NAc and provide evidence for functional cross-talk between D1 and NMDARs in cultured NAc neurons.

Disclosures: M.T. Stefanik: None. C. Murray: None. M.E. Wolf: None.

Poster

334. Brain Circuits Affected by Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: DA009621

F32 DA036963

Title: Biochemical and electrophysiological studies of NMDA receptors in the rat nucleus accumbens during incubation of methamphetamine craving

Authors: *A. M. WUNSCH, D. T. CHRISTIAN, M. MILOVANOVIC, M. E. WOLF Neurosci., Rosalind Franklin Univ., North Chicago, IL

Abstract: One of the largest problems associated with methamphetamine (METH) addiction is the high rate of relapse, even following long periods of abstinence. While recent studies on incubation of METH craving (a model of cue-induced METH relapse) have focused primarily on behavioral pharmacology or neural circuitry, much less is known about the synaptic mechanisms associated with the incubation of METH craving. Our lab has demonstrated that, during withdrawal from METH, calcium-permeable AMPA receptors accumulate in medium spiny neurons of the nucleus accumbens core within 7 days of withdrawal from METH. However, much less is known about NMDA receptor contributions to incubation of METH craving. Our lab recently found that incubation of cocaine craving is accompanied by time-dependent changes in the subunit composition of NMDA receptors in NAc, involving GluN2B and GluN3 subunits. The main goal of this project is to determine whether NMDA receptor subunit composition and function is altered during incubation of methamphetamine craving. We used biochemical techniques to determine whether levels of GluN1, GluN2A, GluN2B, GluN3A, or GluN3B were altered during METH withdrawal. Rats underwent extended access METH self-administration (6 h/day for 10 days), and tissue from nucleus accumbens core was harvested for surface biotinylation on withdrawal day 21, a time at which incubation of METH craving is maximal. We found no significant changes in either the total or surface expression of any NMDA receptor subunit at this time point. Since functional NMDA receptors are located primarily at the synapse, future studies will assess whether incubation of METH craving is accompanied by changes in expression of NMDA receptor subunits in a synaptic fraction. Lastly, we will use electrophysiological methods to determine whether NMDA receptor function is altered during incubation of METH craving. This work will better define the synaptic mechanisms that contribute to cue-induced relapse following METH self-administration, which may help to identify therapeutic targets for preventing METH relapse.

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Poster

334. Brain Circuits Affected by Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant K99DA038110

Title: Effects of cocaine and chronic stress exposure on BLA neuronal activity

Authors: *J. A. LOWETH¹, S. MUNSHI², A. CACCAMISE¹, J. A. ROSENKRANZ², M. E. WOLF¹

¹Neurosci., ²Cell. & Mol. Pharmacol., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: A major challenge for treating cocaine addiction is the propensity for abstinent users to relapse. Two important triggers for relapse are cues associated with prior drug use and stressful life events. To study their interaction in promoting relapse during abstinence, we used the incubation model of craving and relapse in which cue-induced drug seeking progressively intensifies ("incubates") during withdrawal from extended-access cocaine self-administration. We have recently shown that exposure to repeated but not acute restraint stress during the first two weeks of withdrawal accelerates the initial rate of incubation of cue-induced cocaine craving, although craving plateaus at the same level observed in controls. These data indicate

that chronic stress exposure during early withdrawal may result in increased vulnerability to cueinduced relapse during this period. Previous studies have shown that chronic stress exposure enhances excitatory drive to the basolateral amygdala (BLA), a region critical for behavioral responses to stress. Given that glutamate projections from the BLA to the NAc are critical for incubation of cocaine craving, we hypothesized that cocaine withdrawal and chronic stress exposure produce a synergistic increase in BLA neuronal activity which accelerates incubated craving. To assess this, we have conducted in vivo extracellular recordings from anesthetized rats to compare neuronal activity in the BLA of rats that self-administer saline or cocaine (6h/day for 10 days) and then either undergo repeated restraint stress or control conditions during the first two weeks of withdrawal. Recording were conducted between weeks 2 and 3 of withdrawal from extended-access cocaine or saline self-administration (0-5 days after the last restraint stress or control session). Our data indicate that cocaine exposure alone enhances neuronal activity within the BLA and that repeated stress exposure during withdrawal from cocaine self-administration may further enhance BLA neuronal excitability, which could contribute to the acceleration of incubation of cocaine craving observed in these animals. These studies may identify potential mechanisms by which chronic stress enhances cue-induced relapse vulnerability in abstinent cocaine addicts.

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Poster

334. Brain Circuits Affected by Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA DA009621

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NIDA DA036963

Title: Extended access cocaine self-administration leads to increased GluN3-containing NMDA receptor function in the rat nucleus accumbens

Authors: *D. T. CHRISTIAN, K. Y. TSENG, M. E. WOLF Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: Cue-induced cocaine craving intensifies or incubates during withdrawal from extended-access cocaine self-administration. After ~1 month of withdrawal, levels of GluA2-lacking, Ca2+-permeable AMPARs (CP-AMPARs) increase in the nucleus accumbens (NAc)

and thereafter their activity is required for expression of incubated craving. NMDARs play a major role in Ca2+ signaling, but little is known about whether they are altered during incubation of cocaine craving. However, incorporation of GluN3- and GluN2B-containing NMDARs accompanies increases in CP-AMPAR levels in the ventral tegmental area elicited by a single cocaine injection (Yuan et al., 2013). To determine if NMDAR transmission in the NAc is altered following extended-access cocaine self-administration, we utilized whole-cell patch clamp electrophysiology in NAc medium spiny neurons (MSN) from rats that self-administered saline or cocaine. We measured evoked NMDAR-mediated synaptic responses across various membrane holding potentials (-80 to +40; 40mV steps) and then characterized these responses using antagonists selective for GluN2B- or GluN3-containing receptors. As expected from prior studies, MSN from saline rats exhibited NMDAR currents sensitive to GluN2B- but not GluN3selective antagonists. During the first week of withdrawal, we observed an increase in NMDAR currents at positive potentials in cocaine rats compared to saline controls that was attributable to NMDARs containing GluN2B but not GluN3. Between 2-3 weeks of withdrawal (still prior to detection of elevated CP-AMPAR levels), we observed increased NMDAR currents at both negative (-80 to -40mV) and positive (+40mV) holding potentials. The same results were obtained during a later period of withdrawal (>39 days) when cocaine craving is maximal. Pharmacological studies conducted in late withdrawal indicated that increased NMDAR currents reflected incorporation of NMDARs that contain both GluN2B and GluN3, as well as NMDARs containing GluN2B but not GluN3. Studies are in progress to determine if these sequential changes in NMDAR subunit composition are required for the elevation of CP-AMPAR levels and the incubation of cocaine craving.

Disclosures: D.T. Christian: None. K.Y. Tseng: None. M.E. Wolf: None.

Poster

334. Brain Circuits Affected by Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: ANR-15-CE37-0010

Title: Interoception and relapse to cocaine addiction: Lo,g-term disruptions of the amygdalainsula pathway

Authors: *P. BELUJON, A. C. M. SALIN, M. SOLINAS Lab. de Neurosciences Expérimentales et Cliniques, Univ. Poitiers, Poitiers, France

Abstract: The insular cortex integrates and transforms interoceptive stimuli into a general emotional awareness. In drug addiction, peripheral effects combined with the hedonic experience

associated with the drug are integrated by the IC. This bodily experience is a key component in the memorization of drug effects within a specific context. Thus, during abstinence, drug cues could reactivate the memory of the body response and emotion associated with drug use by activation of the IC, initiating the physical sensation of craving reinforcing drug seeking behavior. Functional imaging studies in abstinent users have shown an increased synchronization of the IC and the amygdala, involved in the process of emotional responses. This suggests disruption of functionality in this circuitry that could increase vulnerability to cues associated to drug use. Our hypothesis is that chronic drug exposure induces long-lasting disruption within the amygdala-IC pathway. In particular, exposure to drug cues would activate the amygdala, and through its projection to the IC, would reactivate the representations of the interoceptive effects of the drug leading to intense craving, increasing the risk of relapse. The aim of the present study was to examine the cocaine-induced persistent changes in i) spontaneous activity of the basolateral amygdala (BLA) and the IC and ii) the synaptic plasticity in the BLAOIC pathway. Using in vivo extracellular recordings in anesthetized rats one month after ten 6h-session of cocaine self-administration, we found an increase in the bursting activity and firing rate of BLA putative projection neurons. Although no significant changes were observed in the spontaneous activity of IC neurons, we found that synaptic plasticity in the BLA-evoked activity of IC neurons was disrupted in cocaine abstinent rats. In particular, we found that chronic cocaine leads to an increased long-term potentiation in the IC after high frequency stimulation of the BLA, with a loss of depotentiation after low-frequency stimulation. Therefore, in cocaine rats, the LTP is abnormally persistent and fails to be down-modulated. These data suggest that chronic cocaine use induced disruption of the BLA-IC information flow, which could lead to an increase in drug-seeking behavior.

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Poster

334. Brain Circuits Affected by Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Title: Cocaine increases permeability of the blood-brain-barrier in the hippocampus: Implications for drug use and abuse

Authors: *M. M. CLASEN, D. N. KEARNS, T. L. DAVIDSON, A. L. RILEY American Univ., Washington, DC

Abstract: Rationale:

Davidson and his colleagues (2014) have recently demonstrated that consumption of a Western diet (WD) high in saturated fats, carbohydrates and sugars increases blood-brain-barrier (BBB)

permeability that results in the infusion of cytokines and microglia that selectively damages the hippocampus (see also Kanoski et al., 2010). Such damage also reduces hippocampal-dependent inhibitory control, as measured by the Pavlovian serial feature negative (sFN) task, thought to further drive WD intake, spurring a vicious cycle eventually resulting in obesity. The present studies applied this same rationale to assess if cocaine use influences BBB integrity and inhibitory functioning in a manner similar to that induced by the consumption of a WD. Methods:

Experiment 1: Sprague-Dawley rats (n = 24) were injected intraperitoneally with cocaine (20 mg/kg) or saline for 18 consecutive days at which point they were anesthetized and injected with the molecular dye sodium fluorescein (NaFl). Brains were immediately removed, and the expression of NaFl was evaluated in the hippocampus, striatum, and cortex to assess changes in BBB permeability.

Experiment 2: Sprague-Dawley rats (n = 24) were trained on a serial feature negative (sFN) discrimination task in which a tone signaled a food pellet and a light followed by a tone signaled the absence of food (all stimuli were 5 sec in duration). Concurrent with this training (and in the same session), subjects were given a simple discrimination in which a clicker or white noise (counterbalanced) was followed by food (CS+) or no food (CS-). Following acquisition of discriminative control, subjects were maintained on the training conditions for 18 days. Following each daily session, they were given an intraperitoneal injection of cocaine (20 mg/kg) or equivolume saline and changes in sFN performance were evaluated. Results:

Cocaine increased BBB permeability as evidenced by increased levels of NaFl in the hippocampus (Experiment 1) and decreased discriminative performance in the sFN task indicative of a loss of inhibitory control of responding (Experiment 2). Conclusions:

Cocaine exposure resulted in a loss of integrity of the BBB (increased permeability) and a decrease in inhibitory control in the sFN discrimination. Such effects may provide a mechanism for escalated drug intake in drug-abusing populations. Specifically, the drug-induced loss of BBB integrity may result in selective damage to the hippocampus that, in turn, results in a loss of inhibitory control of drug intake that causes more damage, less control and further escalation, i.e., a vicious cycle similar to that posed for excessive eating and the resulting obesity.

Disclosures: M.M. Clasen: None. D.N. Kearns: None. T.L. Davidson: None. A.L. Riley: None.

Poster

334. Brain Circuits Affected by Cocaine

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University of Minnesota Doctoral Dissertation Fellowship (AEI)

Title: Cell type- and receptor type-specific mechanisms of cocaine-induced synaptic AMPAR depotentiation in the nucleus accumbens

Authors: *A. E. INGEBRETSON, M. J. THOMAS

Neurosci., Univ. of Minnesota Dept. of Neurosci., Minneapolis, MN

Abstract: The nucleus accumbens (NAc) is a key mesocorticolimbic structure in which drugs of abuse exert rewarding and reinforcing effects. Composed principally of medium spiny neurons (MSNs), the NAc receives converging glutamatergic inputs from cortical and limbic afferents that drive MSN activity and synaptic plasticity. In rodent models of addiction, changes in glutamatergic synaptic strength at NAc MSNs play a central role in drug-seeking behavior. Previous studies from our lab have shown that repeated exposure to cocaine potentiates AMPA receptor (AMPAR)-mediated signaling in the mouse NAc shell during a period of abstinence (10-14 days) from drug exposure. This increase in synaptic strength is reversed ("depotentiated") by a single re-exposure to cocaine or bout of stress, suggesting that this switch in NAc synaptic excitability might act as a trigger for relapse, promoting renewal of drug-seeking. However, the cellular mechanisms underlying this plasticity have not been well characterized. The present study used an ex vivo cocaine challenge model of synaptic AMPAR depotentiation to examine the neuromodulatory roles of dopamine and endocannabinoids (eCBs) in promoting cocaineinduced plasticity. Application of dopamine receptor agonists promoted synaptic depotentiation, while dopamine antagonists prevented the induction of depotentiation, suggesting a critical role for dopamine in gating cocaine-induced AMPAR plasticity at glutamatergic inputs to the NAc. Additionally, cannabinoid type 1 receptors (CB1s), receptors located pre-synaptically that bind to eCBs released from MSNs and decrease the probability of glutamate release, mediated cocaine-induced synaptic depotentiation, demonstrating that both eCBs and dopamine participate in modulating excitatory synaptic strength in the NAc. Current studies are further investigating the contribution of specific dopamine receptor subtypes located on distinct cell subyptes in the NAc. MSNs of the NAc are segregated into two cell subpopulations based on expression of the dopamine receptor type 1 (D1-MSN) or dopamine receptor type 2 (D2-MSN). These subpopulations have divergent projection targets and exert opposite effects on reward-related behavior. We are currently examining cocaine-induced AMPAR depotentiation in specific cell populations of the NAc to investigate the relationship between specific dopamine receptor and CB1 signaling in promoting plasticity. Our findings demonstrate that re-exposure to cocaine coopts multiple signaling pathways in the NAc shell to induce alterations in synaptic strength, identifying mechanisms that may be targeted for potential pharmacotherapies.

Disclosures: A.E. Ingebretson: None. M.J. Thomas: None.

334. Brain Circuits Affected by Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant DA-033370

Title: Lateral habenular norepinephrine contributes to anxiogenic behaviors in male rats

Authors: *E. M. PURVIS, A. KLEIN, L. COLLINS, A. GUILLEN, B. JAMES, K. LEE, M. MAYES, L. ZHOU, A. ETTENBERG Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: Recent research has identified the lateral habenula (LHb) as a brain region playing an important role in the production of stressful and anxiogenic states. Of particular interest for the current project have been the norepinephrine (NE) projections to the LHb emanating from the locus coeruleus (LC). NE has long been known to be involved in arousal, stress and anxiety. The current research was therefore devised to assess the effects of reducing NE release within the LHb on rodent anxiogenic behavior. Male rats were implanted with bilateral guide cannula aimed at the LHb and subsequently treated with intracranial infusions of the selective α_2 adrenergic auto-receptor agonist dexmedetomidine (DEX) prior to behavioral testing. Doses of DEX (0, 0.5, 1.0 µg/side) were delivered in volumes of 0.25 µL over 120-s infusion durations and ambulatory and anxiogenic responses were assessed using tests of spontaneous locomotion, open field behavior, and acoustic startle response. Results demonstrated that DEX administration significantly reduced the overall locomotor behavior of subjects at both doses, indicating that infusion of even small doses of this α_2 agonist into the LHb can have profound effects on the subjects' general levels of alertness and activity. DEX was also found to attenuate anxiety as evidenced by reduced latencies to enter and more time spent in the center regions of the open field as well as a reduction in the magnitude of an acoustic startle response (ASR) to a 110 dB stimulus. Taken together, the results of the locomotor, open field, and startle-response tests support the assertion that reductions in NE release within the LHb produce statistically reliable reductions in both arousal and anxiety. This work was funded by NIDA grant DA-033370 awarded to AE.

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334. Brain Circuits Affected by Cocaine

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Support: NIDA Grant DA-033370

Title: Activation of serotonin _{1B} receptors in the lateral habenula attenuates the negative/anxiogenic effects of cocaine

Authors: *A. KLEIN, E. M. PURVIS, K. AYALA, L. COLLINS, A. GUILLEN, B. JAMES, J. KRUG, K. LEE, M. MAYES, L. ZHOU, A. ETTENBERG Psychological and Brain Sci., UC Santa Barbara, Santa Barbara, CA

Abstract: In addition to its initial rewarding effects, cocaine produces a significant "crash" characterized by a state of dysphoria and anxiety. Any full account of a subject's motivation to seek cocaine must therefore consider the relative balance between these positive and negative effects of the drug. While the neurobiology of the reinforcing aspects of cocaine has been well established, less is known about the systems responsible for its negative effects. Cocaine alters many neurotransmitter systems, including serotonin (5-HT), which has been linked to states of anxiety and depression in both human and animal subjects. The Lateral Habenula (LHb) has been recently implicated in mediating aversive/anxiogenic states and expresses a variety of 5-HT receptors. Thus, the present study was therefore devised to test the hypothesis that serotonergic signaling in the LHb contributes to the anxiogenic effects of cocaine. Male albino rats were trained to traverse a straight alley and enter a goal box for an infusion of IV cocaine (1.0mg/kg) on each of 16 single daily trials. The dual positive and negative effects of cocaine were reflected in the animals' development of a unique approach/avoidance conflict about entering the drugpaired environment as it learns the dual effects of the drug. Pretreatment with bilateral intra-LHb infusions of the selective 5-HT_{1B} auto-receptor agonist CP94,253 (0.0µg, 0.1, or 0.25µg/side in 0.5/µl) reduced the frequency of these approach-avoidance "retreat" behaviors while leaving the positive incentive properties of the drug intact (i.e., start latencies were unchanged). This effect of the autoreceptor agonist was then reversed by co-administration with the selective 5-HT_{1B} antagonist, NAS-181. Together these data suggest a role for 5-HT signaling within the LHb in mediating the aversive effects of cocaine.

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Title: BDNF controls the ER stress response protein expression after repeated cocaine administration in the rat dorsal striatum

Authors: J. KIM, J. YANG, I. RYU, M. GHANG, S. SON, E. HAN, *E. CHOE Pusan Natl. Univ., Pusan, Korea, Republic of

Abstract: Brain-derived neurotrophic factor (BDNF) is a key molecule involved in the regulation of glutamatergic neurotransmission in response to chronic stimulation of psychostimulants. This study demonstrated that BDNF in the dorsal striatum regulates the endoplasmic reticulum (ER) stress response after repeated exposure to cocaine. The results showed that unilateral intracaudate infusion of BDNF (0.40, 0.75, or 1.50 μ g/ μ L) decreased the repeated cocaine-induced increase in the expression of immunoglobulin heavy chain binding protein (BiP) sensing unfolded or misfolded proteins in a dose-dependent manner. Unilateral intracaudate infusion of BDNF (0.75 μ g/ μ L) also decreased the phosphorylation of c-Jun N-terminal kinase (JNK), which had been initially elevated by seven consecutive daily intraperitoneal injections of cocaine (20 mg/kg/day). These decreases were reversed by unilateral intracaudate infusion of the specific tropomyosin receptor kinase B (TrkB) antagonist, cyclotraxin B (1 ng/ μ L). These findings suggest that BDNF regulates the unfolded protein response via TrkB-linked JNK inactivation in the dorsal striatum after repeated cocaine administration, thus contributing to the restoration of the ER functions.

Disclosures: J. Kim: None. J. Yang: None. I. Ryu: None. M. Ghang: None. S. Son: None. E. Han: None. E. Choe: None.

Poster

334. Brain Circuits Affected by Cocaine

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 334.22/TT17

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH grant DA035958

NIH grant AA02919

Title: Effects of cocaine on spontaneous dopamine release varies across striatal regions

Authors: *J. T. YORGASON, C. FINUF, S. STEFFENSEN Brigham Young Univ., Provo, UT

Abstract: The mesostriatal dopamine system originates in the substantia nigra and ventral tegmental area, and projects throughout the striatum including the caudate, putamen, nucleus accumbens core and shell regions. Dopamine release in these regions underlies drug reinforcement learning, and adaptations to psychostimulants in these regions is thought to be responsible for habitual drug seeking behavior. We have recently shown that local striatal cholinergic interneurons can drive dopamine release independent of dopamine cell firing. This occurs through excitatory cholinergic activity onto nicotinic receptors on dopamine terminals. In the nucleus accumbens core, cocaine increases dopamine transient frequency without increasing cholinergic firing. Previous reports have suggested that cholinergic interneurons by region in their sensitivity to dopaminergic drugs. However, it is unknown if this regional heterogeneity contributes to the effects of cocaine on dopamine transmission. Therefore, we examined cocaine's effects on cholinergic-dependent dopamine transients throughout the striatum. In general, cocaine increased dopamine transient frequency and amplitude. However, cocaineinduced increases in dopamine transients differed by region, with greatest increases observed in the accumbens shell. Future studies will compare dopamine transient responses to cocaine in cocaine-naïve and experienced mice across these brain regions to determine what neural adaptions are occurring in prolonged use states.

Disclosures: J.T. Yorgason: None. C. Finuf: None. S. Steffensen: None.

Poster

334. Brain Circuits Affected by Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

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Title: Altered resting state functional connectivity of the lateral and medial hypothalamus in cocaine dependence

Authors: *S. ZHANG¹, C.-S. R. LI²

¹Yale Univ. Sch. of Med., New Haven, CT; ²Dept Psychiatry, Yale Univ., New Haven, CT

Abstract: Background: The role of dopamine in cocaine misuse has been extensively documented and this literature involves a wide swath of brain regions including the hypothalamus of the mesocorticolimbic circuit. Preclinical work from earlier lesion studies to recent multidisciplinary investigations has suggested that the hypothalamus could be broadly divided into lateral hypothalamus (LH) and medial hypothalamus (MH) each differentially involved in waking/feeding and resting/satiety. However, little is known of the altered resting state functional connectivity (rsFC) of the LH and MH in individuals with cocaine dependence (CD).

Methods: Resting state fMRI data (3T, 10 minutes, eye closed) of 70 CD and 70 age/gender matched healthy controls (HC) were analyzed. We performed linear regressions for individual subjects between the averaged time course of each of the LH and MH seeds and the time courses of each individual voxel of the whole brain. The correlation coefficients were converted to z scores by Fisher's z transform, and a two-sample t-test was applied to the "z maps" to identify altered rsFC of the LH and MH in CD as compared to HC.

Results: Compared to HC, CD showed increased LH functional connectivity with dorsolateral prefrontal cortex (dlPFC) and decreased functional connectivity with the precuneus. Further, CD showed increased MH connectivity with the inferior parietal lobule (IPL) and decreased connectivity with the vmPFC, temporal gyrus, fusiform gyrus, and ventral striatum. These connectivity differences were correlated to clinical measures. For instance, the connectivity strength between LH and dlPFC was positively correlated with total amount of cocaine use in the past month (p = 0.004, r = 0.35) and number of days of cocaine use in the past month (p = 0.03, r = 0.27). The connectivity strength between MH and vmPFC was negatively correlated with number of years of cocaine use (p = 0.03, r = -0.27). The connectivity strength between MH and VS was negatively correlated with cocaine craving as assessed by the Tiffany Cocaine Craving Questionnaire (CCQ) (p = 0.008, r = -0.33).

Conclusions: The findings demonstrated altered functional connectivity patterns in CD for LH and MH and may provide new insight of our understanding of circuit level deficits in cocaine dependence.

Disclosures: S. Zhang: None. C.R. Li: None.

Poster

334. Brain Circuits Affected by Cocaine

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Program#/Poster#: 334.24/TT19

Topic: G.08. Drugs of Abuse and Addiction

Support: U01DA041174-01

R01DA041528-01

Title: Thalamocortical connectivity and intrinsic thalamic connectivity associated with recency of cocaine use

Authors: *A. S. HUANG, R. Z. GOLDSTEIN Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Drug addiction is a complex disorder that is characterized by excessive drug use despite adverse consequences. Current models highlight the importance of the midbrain reward system, and the prefrontal cortex cognitive control network in underlying core symptoms of addiction. Although the thalamus has an important role linking these two networks, its involvement in drug addiction is not well understood. Recent investigations of thalamic function indicate that it has an important role in integrating information across networks (Hwang et al., 2017), suggesting that it has a broader role in cognition. In this study, we collected resting state functional magnetic resonance images from 19 recent cocaine users, 26 abstinent cocaine users and 31 healthy controls. We analyzed functional integration and functional connectivity of the thalamus with three different resting state analysis techniques. Thalamocortical connectivity was analyzed using seed-based correlations from different thalamic regions based on Behrens et al., 2003 parcellation of the thalamus. Intrinsic thalamic connectivity was examined with regional homogeneity analysis (ReHo) and the fractional amplitude of low frequency fluctuations (fALFF). Compared to healthy controls, in the cocaine addicted individuals we expect the mediodorsal region of the thalamus to show increased connectivity with orbitofrontal and medial frontal regions and decreased connectivity with dorsolateral prefrontal regions. We also expect mediodorsal regions of the thalamus to show increased connectivity with striatal regions. Based on literature with other drugs of abuse (Denier et al., 2015), we expect the thalamus to show increased ReHo and fALFF values in recent cocaine users compared to abstinent cocaine users, and for cocaine users to show greater ReHo and fALFF values compared to healthy controls. Our findings will show the effects of recent cocaine use on the thalamocortical system and may highlight new roles for the thalamus in cocaine addiction.

Disclosures: A.S. Huang: None. R.Z. Goldstein: None.

Poster

334. Brain Circuits Affected by Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA grant DA028968

Title: Neuronal sub-type specific translational changes following long-access cocaine selfadministration in mice

Authors: *E. C. ANDRADE, J. ALVAREZ, E. F. SCHMIDT, N. HEINTZ Lab. of Mol. Biol., The Rockefeller Univ., New York, NY

Abstract: Studies aimed at understanding the molecular basis of drug addiction have focused largely on the mesolimbic dopamine system and related structures. From these studies, long-lasting alterations in transcription are a clear consequence of repeated exposure to drugs of abuse. What remain elusive are the specific contributions of neuronal subtypes within these regions to addiction. To better understand the contributions of different cell-types, bacterial artificial chromosome (BAC) transgenic mice which drive the expression of EGFP-tagged ribosomal protein L10a in defined cell populations (known as bacTRAP transgenic lines) were trained to self-administer cocaine. Cell-types of interest include those found in the mesolimbic dopamine system, specifically D1 and D2 medium spiny neurons in dorsal striatum and nucleus accumbens. Following 2.5 weeks of extended cocaine self-administration, polysomal mRNAs were isolated by affinity purification and analyzed by RNA-seq to identify translational profiles of each cell type. Results from these studies provide insight into the neurocircuitry most affected by cocaine use as well as genes and pathways most affected.

Disclosures: E.C. Andrade: None. J. Alvarez: None. E.F. Schmidt: None. N. Heintz: None.

Poster

334. Brain Circuits Affected by Cocaine

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 334.26/TT21

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA

Title: Dynamic remodeling of MSN connectivity upon cocaine self-administration

Authors: *M. SALERY, E. S. CALIPARI, A. GODINO, E. G. PECK, E. J. NESTLER Fishberg Dept. of Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Drug addiction is a chronic neuropsychiatric disorder characterized by pathological motivation to seek and take drugs, persistent drug consumption despite adverse consequences and ultimately high risk of relapse even long after cessation of drug taking. These drug-induced

behavioral alterations are thought to rely on maladaptive changes in brain circuits involved in reward-associative learning. Extensive work has described drug-induced changes at the molecular, cellular and circuit levels and has implicated stable synaptic adaptations along with neuronal network reorganization in the long-lasting effects of drugs of abuse. However, longterm neuronal substrates specific to drug-seeking and their causal role in driving relapse behaviors remain poorly understood. In order to identify neuronal processes involved in such pathological behaviors, we are analyzing the effect of reexposure to cocaine-associated cues on the synaptic architecture in reward circuits with a focus on the nucleus accumbens (NAc). The NAc is predominantly composed of GABAergic medium spiny neurons (MSNs) which play a key role in integrating molecular signals underlying reward learning and addiction. To first assess how exposure to drug-paired cues affects MSN connectivity, we are measuring the morphology and density of dendritic spines within this neuronal population during reinstatement of drug seeking. Further characterization of MSN connectivity involves the identification and functional manipulation of input-specific synaptic morphological changes as well as the cellular processes engaged within MSN spines long after drug use. Together this work will deepen our understanding of the neuronal underpinnings specific to reinstatement, setting the ground for putative therapeutical strategies targeting relapse to drug taking.

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Poster

334. Brain Circuits Affected by Cocaine

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 334.27/TT22

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA

Title: Novel approaches to study the role of endogenous Δ FosB in the action of cocaine and morphine within the nucleus accumbens

Authors: *C. K. LARDNER¹, P. J. HAMILTON¹, H. M. CATES², D. M. WALKER¹, E. A. RIBEIRO¹, J. FENG³, E. J. NESTLER¹

¹Fishberg Dept. of Neurosci. and Friedman Brain Inst., Icahn Sch. of Med. at Mount Sinai, New York, NY; ²Dept. of Pharmacol., Weill Cornell Med. Sch., New York, NY; ³Dept. of Biol. Sci., Florida State Univ., Tallahassee, FL

Abstract: The onset and persistence of addiction is, in part, mediated by mechanisms of transcription. However, between classes of abusive drugs, little is known about the overlap of common transcriptional mechanisms and how these converge and diverge to effect an addictive

phenotype. One well-characterized transcriptional regulator shared between different classes of abusive substances is Δ FosB, a Fos family transcription factor. Δ FosB is a splice variant of FosB, arising from a truncation which lends it unusual stability due to the loss of N-terminal degron domains. Consequently, Δ FosB accumulates intracellularly following repeated induction of the *Fosb* gene, which has been shown to occur after chronic exposure to many substances, including cocaine and morphine. A stimulant and an opiate, respectively, the pathogenesis of cocaine and morphine abuse both involve the induction of Δ FosB. However, it is unknown to what degree Δ FosB coordinates their unique effects within the nucleus accumbens. We are utilizing two tools to address this gap in knowledge. First, the scope and extent of Δ FosB's transcriptional activity genome-wide has thus far been elusive due to the lack of antibodies suitable for chromatin-immunoprecipitation (ChIP)-seq. To address this, we generated a knockin mouse in which Δ FosB is expressed with a hemagglutinin (HA) tag fused at its N-terminus, which allowed for anti-HA targeting of Δ FosB using ChIP-seq following chronic cocaine or morphine administration. Next, to elucidate how D1- and D2-type medium spiny neurons coordinate the action of cocaine and morphine, we developed novel CRISPR/Cas9 tools to deliver nuclease-dead Cas9 fused to epigenetic modifying moieties to the Fosb locus. We observed how D1-/D2-type MSN-specific manipulation of Δ FosB influences cocaine- and morphine-mediated behavioral and morphological outcomes. Understanding the transcriptional scope and cell type-specific roles of Δ FosB in the effects of cocaine and morphine will not only clarify their mechanisms of action and reveal new avenues for therapeutic development, but also aid in understanding how diverse pharmacological substances converge at the level of transcription to commonly as well as divergently influence the function of the brain's reward system.

Disclosures: C.K. Lardner: None. P.J. Hamilton: None. H.M. Cates: None. D.M. Walker: None. E.A. Ribeiro: None. J. Feng: None. E.J. Nestler: None.

Poster

334. Brain Circuits Affected by Cocaine

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 334.28/TT23

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA

Title: Mechanisms of epigenetic priming in the nucleus accumbens underpin cocaine addiction

Authors: *P. MEWS¹, H. KRONMAN², E. S. CALIPARI³, E. J. NESTLER⁴ ¹Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY; ²Dept of Neurosci. and Friedman Brain Institute,, Icahn Sch. of Med. At Mount Sinai, Brooklyn, NY; ³Mount Sinai Sch. of Med., New York, NY; ⁴Icahn Sch. Med. At Mount Sinai, New York, NY Abstract: Drug addiction is a debilitating neuropsychiatric disorder with severe health, financial and societal consequences. Recent increases in drug abuse and the lack of efficacy of conventional pharmacotherapy drive the need for advanced mechanistic insight. Drugs of abuse, in addition to their acute effects, cause persistent aberrations in gene expression in the nucleus accumbens (NAc), the key brain region of reward learning. We found that chronic cocaine exposure permanently alters the inducibility of key neuronal genes to future stimuli in the NAc, referred to as gene priming and desensitization. It is hypothesized that drug-induced changes in the epigenetic landscape underlie such latent transcriptional dysregulation in states of addiction. However, epigenetic priming of transcription is virtually unexplored in the context of psychiatric disease and molecular mechanisms remain opaque. Further, the brain is comprised of a number of heterogenous cell-types, making the cell-type specific identification of these changes critical. In the NAc two functionally exclusive types of medium spiny neurons (MSNs), the D1 and D2 dopamine receptor expressing subtypes, comprise 90% of the total neuronal population. Thus, we defined the differential D1 and D2 MSN transcriptional priming caused by chronic cocaine. These novel subtype-specific analyses lay the foundation for mechanistic studies of epigenetic regulation in drug addiction. Next, we investigated the cocaine-induced aberrations in chromatin structure that drive epigenetic priming in the NAc. Using ATAC-seq, we assessed chromatin accessibility in D1 and D2 MSNs genome-wide and differentiate immediate from persistent changes caused by chronic cocaine. An understanding of the epigenetic processes involved will ultimately allow us to identify novel pharmaceutical targets for epigenetic therapies of psychiatric diseases.

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Poster

334. Brain Circuits Affected by Cocaine

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Topic: G.08. Drugs of Abuse and Addiction

Support: T32DA007135

P01DA008227

Title: History of cocaine self-administration alters transcriptome-wide responses to cocaine reexposure throughout the brain's reward circuitry

Authors: *D. M. WALKER¹, E. S. CALIPARI², H. M. CATES⁴, E. LOH³, I. PURUSHOTHAMAN³, A. GODINO³, P. MEWS¹, E. J. NESTLER⁵ ¹Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY; ²Neurosci., ³Icahn Sch. of Med. at Mount Sinai, New York, NY; ⁴Pharmacol., Weill Cornell Med. Col., New York, NY; ⁵Icahn Sch. Med. At Mount Sinai, New York, NY

Abstract: Cocaine addiction is a chronic, relapsing disorder involving maladaptive plasticity in brain reward circuits associated with changes in gene expression. The behavioral responses to self-administration withdrawal and re-exposure have been well-defined in rodent models. However, the gene expression changes underlying the observed circuit wide and behavioral dysregulation remains elusive. We hypothesized that chronic cocaine "re-programs" the transcriptome, resulting in sensitization and desensitization of molecular targets upon reexposure to cocaine. To test this hypothesis, we assigned male mice to one of six groups: saline or cocaine self-administration (SA) + 24hr withdrawal (WD) (S24/C24); saline/cocaine SA + 30d WD + acute saline/cocaine + 1hr (SS/SC/CS/CC). Then we conducted RNA-sequencing on 6 interconnected reward associated brain regions (PFC, NAc, DS, BLA, vHIP, VTA) to determine how a history of cocaine SA influences transcriptome-wide responses at baseline and after cocaine re-exposure. We focused on patterns of gene expression that were altered in response to specific stimuli when compared to the same baseline (S24). Genes that were uniquely altered by acute cocaine after cocaine SA+WD (CC) displayed region-specific regulation, with the greatest numbers seen in NAc, DS, and BLA. Further analysis revealed that the transcription factor, Hnf4a, is a predicted upstream regulator in all three regions and associated with genes that control RNA posttranscriptional processes and neurological diseases. In contrast, genes uniquely altered by cocaine SA + acute saline (CS), possibly a reflection of genes exhibiting "craving," were most affected in vHIP and PFC and associated with neuronal branching. Regulation of subsets of these primed, desensitized, and incubating genes correlates with the SA behavior displayed by individual mice. These data provide further insight into the relationship between these correlated brain regions. Further, this is the most comprehensive picture to date of transcriptome-wide regulation by cocaine SA and WD throughout the brain's reward circuitry, and it will guide future studies of the molecular basis of cocaine addiction.

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Poster

334. Brain Circuits Affected by Cocaine

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 334.30/TT25

Topic: E.03. Basal Ganglia

Support: MRC CASE Studentship

Title: Conditional deletion of the GABAA α 4 subunit in D1 but not D2 expressing Medium Spiny Neurons affects cocaine-induced locomotor activity and associated neural ensembles

Authors: *J. ROBERTSON, T. MACPHERSON, D. N. STEPHENS, S. L. KING Univ. of Sussex, Brighton, United Kingdom

Abstract: GABA_A receptor α 4 subunits partner with δ subunits to form extrasynaptic receptors (α 4-GABA_ARs) which mediate tonic inhibition of neurons. The α 4 subunit is expressed on Medium Spiny Neurons (MSNs) of the striatum, particularly the Nucleus Accumbens (NAc). Deletion of α 4-GABA_ARs makes MSNs more excitable and we have previously demonstrated that α 4-GABA_ARs are involved in conditioned behaviours such as cocaine-conditioned place preference and conditioned reinforcement.

We have extended these studies to behavioural sensitisation. As well as testing α 4-GABA_AR constitutive knockout (α 4-KO) mice, we assessed the role of α 4-GABA_ARs on Dopamine D1R and D2R-containing neurons specifically in mediating cocaine induced locomotor activity and behavioural sensitisation. We generated two conditional knock-out lines by breeding 'floxed' α 4 mice with mice expressing Cre-recombinase under either the Drd1 or Drd2 promoter to conditionally delete *gabra4* in D1 or D2 receptor containing neurons (D1- α 4-KO and D2- α 4-KO mice respectively).

D1- α 4-KO mice and D2- α 4-KO mice were compared to their 'floxed' α 4 littermates (D1- α 4-WT and D2- α 4-WT, respectively (n=16 per group). Genotypes were divided into two groups with half receiving daily IP injections of either cocaine (10mg/kg) or saline (10ml/kg) for 10 days. On day 11 all mice were given a saline injection to examine whether cocaine treated mice exhibited conditioned activity. Locomotor activity was recorded for 60 minutes post injection. No genotype showed any differences in baseline locomotor activity and administration of cocaine increased locomotion in all genotypes. However in Session 1 D1- α 4-KO mice showed greater cocaine-potentiated activity compared to D1- α 4-WT's. Interestingly cocaine treated D1- α 4-KO's also showed a greater level of conditioned activity on the saline test day. No phenotypic differences were seen with α 4 KO mice (compared to WTs) or the D2- α 4-KO mice. Our results indicate that deletion of α 4 in D1 neurons results in an increased acute response to cocaine and greater conditioned activity but does not affect end-point behavioural sensitisation.

behaviours. Experimental groups were divided with half administered cocaine (20mg/kg) and half saline, and their brains perfused after 90 minutes. We observed increased cFos in the NAc core and shell following acute cocaine and this was further increased in sensitised animals. α 4 KO and D1- α 4-KO's displayed greater cocaine induced cFos in the NAc core following acute cocaine and greater cocaine induced cFos in the NAc core *and* Shell post sensitisation.

Disclosures: J. Robertson: None. **T. MacPherson:** None. **D.N. Stephens:** None. **S.L. King:** None.

Poster

335. Cortical and Allocortical Mechanisms of Attention

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 335.01/TT26

Topic: H.01. Animal Cognition and Behavior

Title: Effect of social isolation during adolescence on behavior in adult mice

Authors: *M. NOBACK, N. WHITE, G. ZHANG, J. BARROW, G. CARR Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Social isolation (SI) during adolescence is a risk factor for several psychiatric disorders, including schizophrenia, anxiety, and depression, but the mechanisms by which it contributes to the development of these disorders is unknown. In mammals, adolescence is a critical period in which social interaction with peers is essential for normal brain development. SI is associated with increased neuroinflammation, and altered brain structure and neurochemistry. These SI-mediated effects may underlie SI-associated risk, but the pathologically relevant effects of SI are not clear. We have established a model of SI during adolescence (postnatal day 21-35) in mice that evokes significant behavioral changes. Specifically, male mice exposed to SI during adolescence are hyperactive in a novel open field and display an abnormal exploration pattern suggesting altered attentional processes. Moving the SI mice to group housing (postnatal day 35 on) does not alter their phenotype, suggesting the behavioral changes result from altered neurodevelopmental during a critical period. One possible cause of the abnormal development in SI mice is the lack of opportunity to engage in social play during the postnatal day 21-35 period when normal play behavior peaks. Here we show the effects of intermittent social play behavior (1 hour/day) on the SI phenotype and highlight potential prophylactic measures for SI-induced abnormalities. Along with identifying mechanisms relevant for prevention, these studies identify potential substrates amenable to treatment in adulthood.

Disclosures: M. Noback: None. N. White: None. G. Zhang: None. J. Barrow: None. G. Carr: None.

Poster

335. Cortical and Allocortical Mechanisms of Attention

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 335.02/TT27

Topic: H.01. Animal Cognition and Behavior

Support: Wellcome Trust 093104

Autonomous Province of Trento ("Grandi Progetti 2012," "ATTEND")

Title: Layer dependent attentional modulation of broad and narrow spiking cells in primate V1

Authors: A. THIELE¹, D. FERRO³, M. BOYD², *S. PANZERI³

¹Newcastle Univ., Newcastle upon Tyne, United Kingdom; ²Newcastle Univ., Newcastle Upon Tyne, United Kingdom; ³ISTITUTO ITALIANO DI TECNOLOGIA, Genova, Italy

Abstract: Attention is critical to high level cognition and it improves perceptual abilities. Many studies have delineated how attention affects neuronal firing rates, rate variability, and neuronal correlations, but a detailed understanding how this differs between cortical layers and different cell types is only just emerging[1, 2].

We investigated how attention affects neuronal firing rates, rate variability, and neuronal correlations in broad and narrow spiking cells[3] in macaque V1, while animals performed a top down cued spatial attention task. Recordings were performed with 16 contact laminar silicone probes (150 um intercontact distance) inserted normal to the V1 surface. Layer location was assignment based on current source density and latency analysis, with the earliest current sink and visual responses assigned to layer $4c\alpha$. Layer assignment of sufficiently active cells (>3Hz) was possible for 325/364 broad spiking cells and 224/262 narrow spiking cells. Sustained neuronal activity (following the initial transient after stimulus onset) was higher in supra and infragranular than in granular layers in both cell types. Narrow spiking cells showed a trend towards higher firing rates than broad spiking cells. Attention induced firing rate changes were quantified using Area Under the Receiver Operating Characteristics (AUROC). Braod and narrow spiking cell AUROC distributions did not significantly differ (p=0.72, rank-sum test). AUROCs were largest (p<0.05, rank sum test) in supragranular layers and smallest in granular

layers. Rate variability was assessed using spike count based Fano Factors (FF), and gain variance. Both FFs and gain variance were significantly larger in narrow than broad spiking cells (p=0.004, rank sum test). Across both cell types, FF and gain variance was largest in supragranular layers, intermediate in granular, and smallest in infragranular layers (all pairwise comparisons p<0.05, rank sum test). Attention did not affect FF in either cell type or layer. However, gain variance was reduced by attention in both cell types. The attention induced reduction in gain variance was largest in supragranular layers, and smallest in infragranular layers (latter comparison p=0.029, rank sum test).

[1] A. S. Nandy, J. J. Nassi, and J. H. Reynolds, Neuron 93, 235 (2017).

[2] T. van Kerkoerle, M. W. Self, and P. R. Roelfsema, Nature communications 8, 13804 (2017).

[3] A. Thiele et al., J Neurosci 36, 7601 (2016).

Disclosures: A. Thiele: None. D. Ferro: None. M. Boyd: None. S. Panzeri: None.

Poster

335. Cortical and Allocortical Mechanisms of Attention

Location: Halls A-C

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Program#/Poster#: 335.03/TT28

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01AG050518

Title: Effects of medial prefrontal cortical administration of the orexin-2 receptor antagonist, TCS-Ox2-29, on attentional performance in rats

Authors: *A. TAPP, E. B. MANESS, J. A. BURK Col. of William and Mary, Williamsburg, VA

Abstract: Orexins are excitatory neuropeptides that come in two isoforms, Orexin A and Orexin B, and serve as ligands for the G-protein coupled orexin 1 and orexin 2 receptors (Ox1R and Ox2R, respectively). Changes in orexinergic transmission are thought to contribute to attentional processing. While several studies have examined the role of Ox1Rs in attention, less research has assessed the contribution of Ox2Rs. Moreover, several lines of evidence suggest that the right medial prefrontal cortex is particularly critical for visual attentional performance. Taking all of this into consideration, the goal of the present experiment was to test the effects of Ox2R blockade, via administration of TCS-OX2-29, in the left or right medial prefrontal cortex on visual attention. The results suggest that low dose administration of TCS-OX2-29 into the right, but not into the left, medial prefrontal cortex enhanced attentional performance. We speculate that relatively mild antagonism of Ox2Rs may have increased the sensitivity of these receptors to subsequent orexin transmission, thereby enhancing attentional performance. Ongoing projects in our laboratory are assessing whether these effects are observed when TCS-OX2-29 is infused into other brain regions known to be critical for attentional performance.

Disclosures: A. Tapp: None. E.B. Maness: None. J.A. Burk: None.

Poster

335. Cortical and Allocortical Mechanisms of Attention

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01AG050518

Title: The role of basal forebrain orexin-2-receptors in attentional performance in rattus norvegicus

Authors: *J. A. BURK¹, A. P. VIJ¹, E. B. MANESS¹, J. R. FADEL² ¹Psychology, Col. of William and Mary, Williamsburg, VA; ²Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: Changes in homeostatic function, including sleep patterns and energy balance, are often predictive of age-related neurocognitive disorders such as Alzheimer's and Parkinson's disease. The basal forebrain has been implicated in both attentional processing and responses to homeostatic cues, suggesting a possible link between the significantly impaired attention task performance of Alzheimer's and Parkinson's patients and shifts in their homeostatic regulation. Recent research suggests a possible mechanism: the basal forebrain receives input from hypothalamic neurons that release the excitatory neuropeptides, orexin-A and orexin-B (orexins). Orexins bind with varying affinities to orexin-1- and orexin-2-receptors on cholinergic neurons in the basal forebrain and increase the rate at which they fire action potentials in response to attentional demands and regulation of homeostasis. Degeneration of these cholinergic projections to the cortex is a hallmark of Alzheimer's and Parkinson's, but it is currently unknown if impairment is due solely to loss of these projections, or if the decline can be attributed to loss of particular receptors on these neurons. In order to evaluate the necessity of orexin-2-receptors for attentional performance, TCS-OX2-29, an orexin-2-receptor selective, non-peptide antagonist was administered via bilateral infusion into the basal forebrain of Rattus norvegicus prior to completion of an attention task with a distracter present. The findings suggest a dose and block dependent decline in correct rejections at the 40 nm TCS-OX2-29 infusion into the basal forebrain on the sustained attention task with distracter compared to saline administration. An exploratory analysis revealed improvements in relative hits at 2 nm and 20 nm TCS-OX2-29 infusions into the basal forebrain. Combined, these findings suggest that the orexin-2-receptor is involved in attentional performance, and that blocking the orexin-2-receptor with TCS-OX2-29 may, at high doses, result in a decline in correct rejections, while lower doses of 2 nm and 20 nm may be involved in sensitization of the orexin-2-receptor, making it more likely to respond to binding of orexin A and orexin B.

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Poster

335. Cortical and Allocortical Mechanisms of Attention

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Title: The role of noradrenaline in spatial attention

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Abstract: Visual spatial attention facilitates the processing of information in a specific location of space. According to Posner and Peterson (1990), it is divided into three functional components, subserved by specific neural networks and neuromodulators. Alerting serves to maintain high sensitivity to incoming stimuli, orienting serves to select an information in space and executive control serves to resolve conflict due to limited capacity of the attention system. In this model the locus cœruleus-noradrenaline system (LC-NA) is proposed to specifically modulate the alerting process. However, accumulating evidence suggests a broader role of the LC-NA system in spatial attention (Aston-Jones & Cohen 2005). The objective of the present study was to reexamine its role in spatial attention.

We tested the effect of injections of a NA-reuptake inhibitor (Atomoxetine, ATX), which enhances NA transmission, in seven monkeys performing a task derived from the Posner spatial cueing paradigm (Fan et al. 2002). Monkeys had to saccade to a target in one side of the screen (10° eccentricity). For 80 % of the trials, a cue accurately predicted the upcoming target location ('valid cue'). For the remaining 20%, the cue was either absent ('no cue') or presented on the opposite side of target location ('invalid cue') or two cues were simultaneously presented ('neutral cue'). In addition, a distractor could appear simultaneously with the target onset, randomly either in the same oreaction timesr in the opposite hemifield of the target. Our results showed that ATX increased the number of initiated trials and/or the number of correct trials. Following ATX injections, reaction times in valid trials were reduced leading to an improvement of attentional orienting. The other attentional processes, i.e. alerting and executive control, were also affected by ATX injections but results across monkeys were more mitigated. In conclusion, enhancing NA transmission mainly modulated the orienting process within a highly predictive context. These results suggest that NA influences different facets of spatial attention and that this effect might depend on the context.

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Poster

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ANR BLAN-SVSE4-023-01

Title: Neural coding of social gaze in the amygdala

Authors: *S. GILARDEAU, M. JAZAYERI, S. PINÈDE, S. WIRTH, J.-R. DUHAMEL Inst. Des Sci. Cognitives, Bron Cedex, France

Abstract: Primates have a highly structured and developed social life. To achieve such complexity, appropriate communication between individuals is required. Much of this communication is achieved through the exchange of visual information. Indeed the eyes provide information about an individual to its conspecifics depending on its status, disposition and emotional state, especially thanks to the different gaze interactions they engage in. Despite the importance of social gaze in the behavioral repertoire of primates (Ballesta and Duhamel, 2015, Dal Monte et al, 2016), the neural substrates that support this behavior are still little known. The macaque amygdala has been shown to contain neurons that respond selectively to different part of faces, bodies and, notably to eye gaze and direct eye contact while viewing videos of conspecifics (Mosher et al. 2014). In the present study, we investigated the neural correlates of gaze behavior during live interactions between two macaque monkeys, sitting face to face, both while they engaged in spontaneous visual interactions and as they performed a structured social decision task.

We report that monkeys actively seek to interact with one another through gaze, notably by showing longer fixations during mutual than averted gaze and more frequent social gaze initiation by dominant than subordinate monkeys. Single unit recordings in the amygdala revealed different types of neuronal responses. Neurons responded to the eye region of the partner monkey, and some of these responded selectively to mutual gaze. Interestingly neurons could respond with short (<60ms) or long (>150ms) latency, suggesting two possible routes for visual input to the amygdala. We further observed cells responding to a more complex type of gaze interaction, namely joint attention. Such activity was characterized by increases in firing rate when the two monkeys jointly gazed at the workspace, and by a striking enhancement of the neuronal responses when the recorded animal was following the partner's gaze shift to that location. To conclude, our results confirm the role of the primate amygdala in social gaze and extend its involvement to joint attention mechanisms. References:

Ballesta, S. and Duhamel, J.-R. (2015) Rudimentary empathy in macaques' social decisionmaking. *PNAS* 112, 15516-15521.

Dal Monte et al. (2016) Live interaction distinctively shapes social gaze dynamics in rhesus macaque. *J Neurophysiol* 116: 1626 1643.

Mosher CP et al. (2014) Neurons in the monkey amygdala detect eye contact during naturalistic social interactions. *Curr Biol* 24: 2459-2464.

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Poster

335. Cortical and Allocortical Mechanisms of Attention

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Title: The claustrum enables resilience to distraction through gain control of cortical sensory processing

Authors: *G. ATLAN¹, A. TEREM², N. PERETZ-RIVLIN¹, G. POZNER², K. SEHRAWAT¹, B. J. GONZALES², G.-I. TASAKA¹, Y. GOLL², R. REFAELI^{1,2}, O. ZVIRAN², B. LIM³, M. GROYSMAN¹, I. GOSHEN¹, A. MIZRAHI^{1,2}, I. NELKEN^{1,2}, A. CITRI^{1,2,4} ¹Edmond and Lily Safra Ctr. For Brain Sci., ²Inst. of Life Sci., Hebrew Univ. of Jerusalem, Jerusalem, Israel; ³Biol. Sci., UCSD, La Jolla, CA; ⁴Canadian Inst. for Advanced Res., Toronto, ON, Canada

Abstract: The claustrum is a thin neuronal structure, located deep in the mammalian brain. Enclosed between the insular cortex and the striatum, it has been shown to be reciprocally interconnected with sensory cortices, frontal regions, and subcortical nuclei. We have recently proposed that by regulating cortical gain, the claustrum may suppress task-irrelevant sensory

stimuli and support attention (Goll et al. 2015). However, the delicate anatomy of the claustrum has greatly limited functional studies. We identified a genetic marker that is locally selective for claustrum neurons. A transgenic mouse line allows the utilization of CRE-dependent viral vectors to drive specific expression in a subpopulation of claustral neurons. Using these as starter cells, we conducted brain-wide anterograde tracing and rabies-based retrograde tracing experiments, and verified that these cells display the characteristic reciprocal connectivity of the claustrum (Atlan et al. 2016). To address our hypothesis regarding the involvement of the claustrum in attention, we assessed the impact of claustral activation on sensory responses in the auditory cortex of anesthetized mice. Specifically, we played an array of logarithmically spaced pure tones (3-70 kHz), and optogenetically activated claustrum neurons or processes while performing loose-patch recordings or bulk calcium photometry, respectively. We observed that the firing of the majority of affected single cells was reduced selectively at their preferred frequency. Population calcium activity, however, revealed that claustral activation drives divisive normalization at the circuit level; demonstrating that the claustrum is capable of broadly desensitizing cortical responses, while maintaining their baseline tuning. Finally, we established that the claustrum is important for performing attentional tasks by silencing its activity during operant and naturalistic behaviors. In both assays, mice displayed increased sensitivity to auditory distraction during claustral suppression. Our work serves as the first functional perturbation of the claustrum, demonstrating that it can regulate cortical excitability, and impact resilience to distraction. Therefore, the claustrum could be a novel target for medical treatment of disorders in which perception and attention are impaired, such as autism, schizophrenia, and ADHD.

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Poster

335. Cortical and Allocortical Mechanisms of Attention

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Support: Alfred P. Sloan Foundation

Title: Serotonergic efficiency underlies causal effect of 5-HTP on attention allocation

Authors: *H. B. WEINBERG-WOLF¹, N. FAGAN², O. DAL MONTE³, M. TRINGIDES⁴, G. ANDERSON⁴, S. W. CHANG⁴

¹Psychology, Yale Univ., New Haven, CT; ²Yale Univ., New Haven, NY; ³Dept. of Psychology, ⁴Yale Univ., New Haven, CT

Abstract: Serotonergic transmission, measured via cerebrospinal fluid (CSF) concentrations of the serotonin metabolite 5-hydroxyindoleacetic Acid (5-HIAA), is linked to psychiatric disorders, particularly depression (Sharp and Cowen, 2011). Impaired serotonergic transmission is also linked to poor impulse control, impaired social functioning, extreme aggression, and even mortality in human and non-human primates (Higley & Linnoila, 1997; Westergaard, 1999). Diversity in serotonergic transmission is often independent from central concentrations of serotonergic compounds. In fact, when scientists modulate central serotonin to examine its effects on behaviors associated with depression, they often report inconsistent results. It is likely that this is in part due to individuals' diversity in central serotonergic transmission efficiency, a characteristic usually overlooked in these studies.

Previously (Weinberg-Wolf et. al, 2016, SfN abstract), we reported that systemically administering the serotonin precursor I-5-hydroxytryptophan (5-HTP) results in robust bidirectional modulation of attention in rhesus macaques, whereby 5-HTP increased looking duration in animals with low baseline attention (Attend+ group) but decreased looking duration in animals with high baseline attention (Attend- group).

Here, we elucidate the biochemical mechanisms underlying this causal effect of serotonin on attentional allocation by examining central serotonergic efficiency. While central concentrations of 5-HTP and serotonin at baseline positively predicted the direction in which, and magnitude by which, 5-HTP modulated attention, CSF analyses also confirmed that 5-HTP increased central concentrations of 5-HTP and serotonin in *both* groups. Previous literature indicates that the ratio of 5-HIAA to serotonin is the best representation of serotonergic efficiency (Roy and Linnoila, 1988). We found that the 5-HIAA to serotonin ratio indeed predicted both looking duration at baseline and also the percent change from baseline in looking duration following 5-HTP administrations. Attend+ animals exhibited lower baseline 5-HIAA to serotonin ratios, and thus poorer serotonergic efficiency, looked for shorter periods of time at baseline and exhibited more positive changes in looking duration due to 5-HTP than their Attend- counterparts. Our results suggest that differences in central serotonergic efficiency underlie diversity in how serotonergic manipulations causally influence attention. These findings may inform treatment plans for serotonergic interventions in disorders like depression and anxiety.

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Poster

335. Cortical and Allocortical Mechanisms of Attention

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant AG046580

Title: Attentional control in young and aged rats following chemogenetic inhibition of prefrontal projection neurons

Authors: *S. JOSHI, M. DUGGAN, J. STRUPP, V. PARIKH Psychology and Neurosci., Temple Univ., Philadelphia, PA

Abstract: The prefrontal cortex (PFC) governs top-down control of attention and is known to be vulnerable in aging. However, increased recruitment of PFC in aging is hypothesized to reflect an adaptive reorganization process that might compensate for declining bottom-up sensory processing and ensuing attentional capacitates. Therefore, we hypothesized that reduced activity of PFC efferents would exert differential effects on attentional capacities between young and aged subjects, with the latter exhibiting a more robust decline in performance. Wistar rats (young: 3 months and aged: 22 months) were trained in an operant attention task that consists of a random sequence of signal (central panel light of different durations; 500 ms, 50ms, 25ms) and non-signal trials, divided into 3 blocks. We utilized a chemogenetic approach involving DREADD (Designer Receptors Exclusively Activated by Designer Drugs) to silence projection neurons in the PFC. Animals trained to criterion (\geq 70% correct responses), received bilateral infusions of AAV vector expressing hM4D(Gi) under the control CaMKIIa promoter into the medial PFC. Subjects were kept on task for 4-weeks post-surgery after which their performance was evaluated under an attention-taxing condition, a behavioral testing session consisting of the presentation of visual distractors (flashing house light) in the 2nd block. Clozapine-N-oxide (CNO; 1mg/kg i.p.) was administered to suppress the activity of prefrontal projection neurons by activating engineered Gi-coupled receptors 1-hr prior to this testing session. For control conditions, animals received an injection of vehicle. As expected, the ability to discern signal from non-signal events was reduced during the distractor presentation, while this deficit completely recovered in the post-distractor block in vehicle-injected young rats. CNO injection worsened the performance of young animals during the pre-distractor (CNO: 0.27±0.06; vehicle: 0.45±0.05) and post-distractor (CNO: 0.35±0.09; vehicle: 0.63±0.06) blocks, respectively. In general, aged rats performed less accurately on signal trials with shorter signal durations. Contrary to our predictions, CNO-mediated Gi inhibition in the PFC did not affect attentional performance in these animals. Lack of sensitivity to the suppression of PFC output neurons in aging might indicate that restricted modulation of PFC efferents is not sufficient to compensate for age-related decline in attentional capacities. It is possible that other cortical regions involving the frontoparietal networks are also involved in this adaptive process.

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Poster

335. Cortical and Allocortical Mechanisms of Attention

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Topic: H.01. Animal Cognition and Behavior

Title: Neuronal activity in primate area FST during covert spatial attention

Authors: *A. R. BOGADHI, L. N. KATZ, A. BOLLIMUNTA, R. J. KRAUZLIS Lab. of Sensorimotor Res., Natl. Eye Inst., Bethesda, MD

Abstract: Inactivation of the superior Colliculus (SC) leads to attention deficits in covert spatial attention tasks, but the circuits through which SC inactivation leads to attention deficits are not known. Using fMRI in a block design, we recently identified area FST in superior temporal sulcus as one of the areas that is significantly affected by SC inactivation in a covert spatial attention task. Because of the limitations of the fMRI study, we do not know which aspects of the task modulate neuronal activity in area FST or how they might be affected by SC inactivation. Here we report neuronal recordings (single and multi-unit combined and hereafter referred to as units) from area FST in a covert spatial attention task. The task consisted of 4 stimulus conditions: Baseline (B), Foveal Attention (FA), Single Peripheral Stimulus (SPA) and Peripheral Attention (PA) blocks. Each condition was blocked and presented in a randomly interleaved order. In B trials, the relevant stimulus was a central fixation point that dimmed at randomized times. FA trials were similar to B trials but added 2 peripheral motion-change stimuli as irrelevant distracters. In PA trials, the fixation point did not dim and the 2 peripheral motion-change stimuli were relevant. SPA trials were similar to PA trials except there was only one peripheral motion-change stimulus. The task of the monkey was to maintain central fixation and report the relevant stimulus change (fixation dimming in B & FA blocks, peripheral motion change in SPA & PA blocks) by releasing a lever to get a juice reward. We have recorded 312 units in area FST of 2 monkeys using single electrodes and 24-contact vprobes. Most units (n = 186) were responsive to the peripheral motion stimulus in PA trials and showed a significant onset response or significant delay period activity to the peripheral stimulus. Units in FST (n = 146) also showed a significant attention-related modulation (comparing PA and FA conditions) with a median modulation index of 0.1, and more than onethird of the units (n = 124) were significantly modulated by the peripheral motion-change. Our preliminary results suggest that units in area FST not only show a significant attentionrelated modulation but also report the peripheral change event. Further experiments will address how the above-mentioned modulations in FST units are affected by SC inactivation in a covert spatial attention task.

Disclosures: A.R. Bogadhi: None. L.N. Katz: None. A. Bollimunta: None. R.J. Krauzlis: None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: Johns Hopkins University Catalyst Award

Title: Exogenous and endogenous control of visuospatial attention in freely behaving mice

Authors: *W.-K. YOU¹, S. P. MYSORE^{1,2}

¹Neurosci., The Johns Hopkins Univ., Baltimore, MD; ²Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Spatial attention, the ability to select and preferentially process the most important stimulus location in the environment at any instant, is critical for adaptive behavior. To uncover the neural circuit mechanisms underlying spatial attention control, we wish to exploit the power offered by the mouse system for genetically based approaches for the measurement and perturbation of cell-type specific neural activity. As a first step in this endeavor, we develop parameterized paradigms for visuospatial attention in mice using a touchscreen-based operant set-up. Here, we study, explicitly, exogenous as well as endogenous control of visuospatial attention in unrestrained mice using, respectively, a flanker task and a spatial expectation task. Our results reveal systematic changes in behavioral performance and perceptual sensitivity driven either by stimulus salience (exogenous influence) or learned expectation (endogenous influence), consistent with results from similar primate studies. Taken together, we demonstrate that mice exhibit behavioral signatures of visuospatial attention similar to that of humans and monkeys. We propose that mice can serve as a viable model for studying the neural mechanisms of spatial attention.

Disclosures: W. You: None. S.P. Mysore: None.

Poster

335. Cortical and Allocortical Mechanisms of Attention

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Topic: H.01. Animal Cognition and Behavior

Support: 1 R01 EY027718, SPM

Title: Signatures of competition in an inhibitory nucleus in the midbrain attention network

Authors: *H. M. SCHRYVER, S. P. MYSORE

Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Attention involves selecting the most behaviorally relevant ("highest priority") stimulus in the environment to guide behavior. The brain mechanisms underlying this function rely on circuitry in the midbrain, specifically the superior colliculus (SC, in mammals, or optic tectum, OT, in birds). Past work has established a causal role for the SC in selecting a target amongst competing distractors. This specific role for the SC in stimulus selection has been shown to be mediated by competitive response suppression provided by a nearby inhibitory nucleus, the Imc (isthmi pars magnocellularis). However, the functional properties of the inhibitory Imc, specifically, how it encodes stimulus competition, are not well understood. Here, with electrophysiological experiments in the barn owl, we will examine signatures of competition in the Imc. Specifically, by contrasting them with those in the OT, we will determine the degree to which the Imc actively performs the computations underlying selection. In addition, with paired recordings in the OT and Imc, we will directly compare the temporal dynamics of competition to determine where competition is first resolved in this circuit. Results from this work will inform us of how this midbrain circuitry that is required for stimulus selection operates, mechanistically.

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Poster

335. Cortical and Allocortical Mechanisms of Attention

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Topic: H.01. Animal Cognition and Behavior

Support: R01 EY027718, SPM

Title: Neural mechanisms underlying categorization for stimulus selection

Authors: *N. R. MAHAJAN, S. P. MYSORE Johns Hopkins Univ., Baltimore, MD

Abstract: The selection of the most behaviorally relevant stimulus among multiple competing stimuli is critical for the control of gaze and spatial attention. The superior colliculus (SC), a

midbrain sensorimotor hub, is known to play a key role in this selection. Previous work in the barn owl has revealed that the optic tectum (OT, avian analog of the SC) explicitly categorizes competing stimuli into two categories, "highest priority" and "others". In addition, a GABAergic satellite nucleus to the OT, called the isthmi pars magnocellularis (Imc), generates the competitive inhibition that is necessary for this categorization. However, the functional pattern of inhibition from the Imc to the OT is not known. Anatomically, the Imc is known to receive focal input from the topographic OT space map, and to send inhibition back broadly to all regions of the OT space map except the region from which it receives input, i.e., a "donut-like" pattern of connectivity. In addition to directly inhibiting the OT, the Imc also inhibits it indirectly by suppressing a powerful cholinergic amplifier of OT representations called the Ipc. Because the pattern of inhibition from the Imc to the Ipc is not clear, it is unknown whether the net inhibition from the Imc onto the OT, through the direct as well as indirect pathways, is actually donut-like. Computationally, such a donut-like pattern of inhibition could substantially enhance categorical selection among competing stimuli in the OT. Here, we will directly examine the spatial pattern of net functional inhibition from the Imc to the OT in the barn owl using in vivo extracellular recordings, iontophoretic inactivation and computational modeling. Results can reveal the neural circuit implementation of key computations underlying stimulus selection.

Disclosures: N.R. Mahajan: None. S.P. Mysore: None.

Poster

335. Cortical and Allocortical Mechanisms of Attention

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Topic: H.01. Animal Cognition and Behavior

Support: NIMH IRP

Title: Different patterns of neuronal infection in the rodent thalamus and temporal cortex revealed by retrograde transport of pseudorabies virus injected into the dorsal and ventral prefrontal cortex

Authors: *M. PERKINS, K. F. MESSANVI, Y. CHUDASAMA Natl. Inst. of Mental Hlth., NIH, Bethesda, MD

Abstract: The mammalian prefrontal cortex plays a critical role in complex executive behaviors by enabling several cognitive operations such as attention, working memory, response control and decision-making. Lesions to different regions of the rodent PFC cause specific cognitive impairments. For example, ablating the dorsal part of the PFC (dPFC), which comprises the dorsal anterior cingulate cortex, and the dorsal extent of the prelimbic cortex affects aspects of attention and working memory. In contrast, the ventral part of the PFC (vPFC) which includes

the infralimbic cortex, orbitofrontal cortex, and the rostral and ventral extent of the prelimbic cortex, is implicated in behavioral control and flexibility (Chudasama, 2011, Beh Neurosci. 125:327-343). This differentiation in behavior prompted us to map the organization of direct and indirect inputs to the dPFC and vPFC, and establish how these anatomical pathways might influence different cognitive functions.

We injected a GFP-expressing transsynaptic Pseudorabies virus (PRV-152) and an mRFPexpressing Pseudorabies virus (PRV-614) directly into the dPFC and vPFC respectively using stereotaxic procedures. First-order projections were determined at 48hrs post-inoculation. Retrogradely-infected neurons were found in the ventral hippocampus and the amygdala following vPFC injection, but not following dPFC injection. In addition, while animals with vPFC injections showed infected neurons in several midline thalamic nuclei including the reuniens and rhomboid, the dPFC-injected animals showed a pattern of infection that was sparse but restricted to the dorsal midline thalamus, namely the paratenial and paraventricular nuclei. In the temporal cortex, neurons projecting to the vPFC were abundant in the perirhinal and entorhinal cortex, while the presence of neurons projecting to the dPFC was moderate and mainly located in the perirhinal cortex.

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Poster

335. Cortical and Allocortical Mechanisms of Attention

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Topic: H.01. Animal Cognition and Behavior

Support: NIMH IRP

Title: Specific projections of the non-specific midline thalamus

Authors: *G. LARYEA, M. B. LEVENTHAL, Y. CHUDASAMA Section on Behavioral Neurosci., NIMH, Bethesda, MD

Abstract: The midline thalamic group of nuclei are generally divided into a dorsal group comprising the paraventricular, paratenial, and intermediodorsal nuclei, and a ventral group which include the rhomboid and reuniens nuclei. This midline thalamic complex is viewed as the non-specific thalamus because the nuclei project broadly to the cortical mantel and receive diffuse input from the brain stem. Unlike the sensory, motor and associative thalamic nuclei which are well defined in terms of their functions, significantly less is known about the functional anatomy of the midline thalamic structures. Recent studies implicate midline thalamus in diverse behavioral functions including learning and cognition (Prasad et al., 2017, Neurosci. 345:77-88), spatial navigation (Hembrook and Mair, 2011. Hippocampus. 21:815-826), and fear-

related memory (Penzo et al., 2015. Nature. 519:455-459). This functional heterogeneity is likely due to the distinct connections of these nuclei with different cortical and subcortical structures. In this study, we explore the anatomical organization of the dorsal and ventral midline thalamic nuclei using a retrograde viral tracer called Pseudorabies virus (PRV). Single or double injections of the PRV with either a GFP tag (PRV-152) or a dTomato Red tag (PRV-614) were injected into dorsal or ventral midline thalamic nuclei of Long Evans rats. After immunofluorescent processing of serial sections, fluorescent imaging was used to identify the distribution of cells labelled with GFP or dTomato Red. Our preliminary evidence indicates that the dorsal midline thalamus receive first order input from preoptic areas, piriform cortex, the most caudal extent of the dentate gyrus, and the caudal dorsal entorhinal field. Unfortunately, our ventral thalamic injections did not accurately target the reuniens and rhomboid nuclei. However, one preliminary case in which the injection was centered on the caudal reuniens revealed the main source of input coming from the medial and lateral septum and the ventral entorhinal field. Further analyses of both first order and second order neuronal projections will enable a better understanding of the organization of midline thalamic circuits and their contribution to behavior.

Disclosures: G. Laryea: None. M.B. Leventhal: None. Y. Chudasama: None.

Poster

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Topic: H.01. Animal Cognition and Behavior

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Title: The neuropeptide galanin in the ventral medial prefrontal cortex modulates impulse control

Authors: *K. MESSANVI, M. PERKINS, J. DU HOFFMANN, Y. CHUDASAMA NIMH / Section on Behavioral Neurosci., NIH, Bethesda, MD

Abstract: The locus coeruleus (LC), the primary source of noradrenaline in the brain, also synthesizes and co-releases several peptides such as galanin, neuropeptide Y and brain-derived neurotrophic factor (BDNF). Galanin is expressed in ~80% of LC neurons in rat brain, and the LC constitutes the only source of galanin to cortex and hippocampus (Hökfelt et al., 1998, Ann. NY Acad. Sci., 863, 252-263). Consequently, galanin has been implicated in cortex- and hippocampus-dependent functions, and is thought to play a role Alzheimer's Disease, addiction, depression, stress and anxiety disorders (Weinshenker and Holmes, 2016, Brain Research 1641, 320-337). Specifically, cortical galanin receptor 1 (Gal-1) is thought to mediate cognitive functions, whereas galanin receptor 2 (Gal-2) is thought to play a role in mood and anxiety.

In this study, we explore whether galanin is involved in cognitive-executive function. Given the role of LC-derived noradrenaline in attentional processing, and the high expression levels of galanin receptors in the ventral prefrontal cortex (vPFC), we examined the contribution of Gal-1 receptor activation to performance in the 5-Choice task using a touchscreen platform. In this task, rats were trained to reach a high level of accuracy (> 80%) and low levels of omission (< 20%). We then implanted bilateral cannulae targeting the vPFC. Next, trained rats received counterbalanced infusions of either 3 nmol or 5 nmol of the Gal-1 receptor agonist M617 or saline. Rats with local infusions of saline or 3 nmol of M617 showed normal levels of performance with respect to target accuracy, behavioral control and speed of response. In contrast, rats infused with 5 nmol of M617 showed a selective increase in the number of premature responses (mean premature responses \pm SEM: 5nmol = 30 \pm 7.5; 3nmol = 20.8 \pm 4.4; Saline = 15 ± 8.4), with no concomitant change in attention, motor or motivational status. These rats were also sensitive to temporal unpredictability of the visual stimulus. Together, these data implicate vPFC Gal-1-mediated neurotransmission in impulse control mechanisms. Future studies will examine the relationship between Gal-1 and Gal-2 receptors to evaluate whether they mediate similar or unique cognitive processes.

Disclosures: K. Messanvi: None. M. Perkins: None. J. du Hoffmann: None. Y. Chudasama: None.

Poster

335. Cortical and Allocortical Mechanisms of Attention

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 335.17/TT42

Topic: H.01. Animal Cognition and Behavior

Support: NIMH Intramural Training Program

Title: Single-neuron correlates of spatial attention and choice in auditory and prefrontal cortex

Authors: *C. R. CAMALIER¹, M. MISHKIN¹, B. B. AVERBECK² ¹Lab. of Neuropsychology, NIH, Bethesda, MD; ²NIMH/NIH, Bethesda, MD

Abstract: Auditory spatial attention describes our effortless ability to listen to sound from one location while suppressing distracting sounds from other locations. Two key areas implicated in this ability are auditory cortex and prefrontal cortex, but a detailed understanding of how sound is spatially filtered has been hampered by the lack of a robust animal model, particularly one that shares key similarities with humans. To address this, we have developed a novel spatial auditory selective attention task for macaques, based closely on human directed listening paradigms. In this task, a macaque monkey is cued to a particular side and must report the presence of a difficult-to-detect auditory target (embedded in noise) only if it appears on the cued side. If it

appears on the uncued side, he must ignore it.

We collected single neuron data from prefrontal cortex (caudal principal sulcus; n=948) and caudal auditory cortex (primarily A1; n=847) in two monkeys during this task. In both areas, direction of cued side significantly affected responses in about 20% of the neurons. A comparison of patterns between PFC and AC indicated that AC was earlier than PFC in signaling the sensory stimulus (ipsi/contra), but PFC was earlier than AC in signaling the decision made (release/ignore). We applied a classification approach on firing rates of the responsive populations to analyze patterns of firing rates related to error (e.g. misses) vs correct trials, This indicated that the first location to exhibit patterns of activity that classified significantly lower than correct was in AC during the masking noise period. Thus, at least some errors are due to an inability to suppress noise at the level of sensory cortex. These results add insight into the cortical dynamics of auditory decision making during effortful listening.

Disclosures: C.R. Camalier: None. M. Mishkin: None. B.B. Averbeck: None.

Poster

335. Cortical and Allocortical Mechanisms of Attention

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 335.18/TT43

Topic: H.01. Animal Cognition and Behavior

Support: NIMH Grant MH083317

Title: Impaired NRG1/ErbB4 signaling decreases the vHPC-mPFC synchrony and causes attention deficit

Authors: *Z. TAN¹, H. ROBINSON¹, Y. LIU¹, F. LIU¹, D. YIN², H. WANG¹, T. LIN¹, G. XING¹, W. XIONG¹, L. MEI¹ ¹Dept. of Neurosci. & Regenerative Med., Augusta Univ., Augusta, GA; ²East China Normal Univ., Shanghai, China

Abstract: Top-down attention which is thought to be controlled by prefrontal cortex (PFC) is essential for daily life. Accumulating evidence have shown that hippocampus is involved in the process of attention. However, the underlying mechanism is rarely investigated. Here we show that the synchrony between ventral hippocampus (vHPC) and medial prefrontal cortex (mPFC) is increased in delta and theta band when mice performing correct trials compared with error trials in a 5-choices serial reaction time task (5CSRTT). Interestedly, the ErbB4 mutant mouse which is a schizophrenia mouse model, also shows impaired vHPC-mPFC synchrony and attention deficit. While specifically block NRG1/ErbB4 signaling or optogenetically inhibit ErbB4+ interneurons excitability in vHPC reduces vHPC-mPFC synchrony, slows down processing speed, and causes attention deficit. The same operations in mPFC have no effect on vHPC-mPFC

synchrony and processing speed but still causes attention deficit, which may through a different mechanism. Thus, NRG1/ErbB4 signaling modulates vHPC-mPFC synchrony, which is critical for top-down attention.

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Poster

335. Cortical and Allocortical Mechanisms of Attention

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH106912

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Title: Selective modulation of dopamine D3 receptors and norepinephrine transporters enhances sustained attention without the risk for abuse

Authors: *C. MARSHALL¹, Z. BRODNIK¹, D. BERNSTEIN¹, O. MORTENSEN¹, M. REITH², J. SHUMSKY¹, N. SNYDER¹, B. WATERHOUSE^{1,3}, R. ESPAÑA¹, S. KORTAGERE¹

¹Drexel Univ., Philadelphia, PA; ²New York Univ. Sch. of Med., New York, NY; ³Rowan Univ., Glassboro, NJ

Abstract: Dopamine (DA) and norepinephrine (NE) have long been studied as modulators of prefrontal cortex (PFC)-mediated cognitive tasks including sustained attention. However, most catecholaminergic agents such as methylphenidate pose a risk of being abused due to their non-selective effects on DA transporters (DAT), thereby limiting their therapeutic effects. In this study, we have developed a novel pharmacological strategy to selectively combine the agonist effects on a DA D3 receptor (D3R) with the inhibitory effects on a NE transporter (NET) to improve sustained attention without promoting DAT effects. Our recently designed SK609 - a selective D3R agonist - was further characterized for monoamine transporter activity. Our results demonstrate that SK609 not only selectively inhibits the NET at an IC₅₀ value of ~500nM but also behaves as a substrate of NET. Systemic dosing of SK609 in naïve rats led to a 300% and 150% increase in NE and DA levels respectively in the PFC as measured by microdialysis. In naïve, male rats trained to perform to criteria in an operant visual stimulus task of sustained attention, SK609 produced a typical inverted-U dose response when tested between 2-20mg/kg doses with a peak effect at 4mg/kg. The peak effect produced by SK609 was blocked by a pre-

treatment with either 0.05mg/kg of raclopride - a D2/D3R antagonist or 0.25mg/kg of prazosin, an alpha-1 adrenergic receptor antagonist, confirming that the improvement was indeed due to both DA and NE modulation in the PFC. In addition, SK609's effect on improving sustained attention was more prominent among low performing animals. The peak dose of 4mg/kg of SK609 did not produce any increase in locomotor effects and was not self- administered suggesting a lack of psychostimulant effects. These results demonstrate that SK609 has the potential to treat PFC-mediated cognitive deficits without abuse liability which distinguishes SK609 from amphetamines and methylphenidate.

Disclosures: C. Marshall: None. Z. Brodnik: None. D. Bernstein: None. O. Mortensen: None. M. Reith: None. J. Shumsky: None. N. Snyder: None. B. Waterhouse: None. R. España: None. S. Kortagere: None.

Poster

335. Cortical and Allocortical Mechanisms of Attention

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 335.20/TT45

Topic: H.01. Animal Cognition and Behavior

Support: University of Mississippi: Sally McDonnell Barksdale Honors College

University of Mississippi: Department of Psychology

Title: The effects of atomoxetine on distribution skew in a two choice reaction time task in the rat

Authors: *Z. V. REDDING, P. CHAWLA, K. E. SABOL Psychology, Univ. of Mississippi, University, MS

Abstract: *RATIONALE* Norepinephrine (NE) is integral to cognitive processes such as attention (Arnsten et al., 2005, J. of Clin Neurosci (67), 7-12). The NE reuptake inhibitor, atomoxetine (ATX), has been used to treat attention-deficit/hyperactivity disorder. When evaluating reaction time (RT) distributions, ATX decreased RT variability (Kratz et al., 2012, Prog in Neuro-Psychopharm & Biol Psych (37), 81-89), or had no effect (Bedard et al., 2015, J. of Child Psychol & Psychiatry (56), 40-48; Nandam et al., 2011, Biol Psychiatry (69), 902-904). Using the ex-Gaussian model, Ni et al (2016, J. of Psychopharm (30), 459-467) found a decrease in distribution skew, which is thought to reflect attentional lapses (Leth-Steensen et al., 2000, Acta Psychologica (104), 167-190). This study explores the effects of ATX on performance in a two-choice reaction time task (2CRTT) in rats. RT is broken down into initiation time (IT) and movement time to isolate effects on attention. IT is the time from stimulus presentation until the rat first reacts to the stimulus. Measures are derived from the shape of the distribution of ITs, a

distinct peak and a rightward skew. The peak (mode) represents sensorimotor processing when rats are attentive. The skew (measured by subtracting mode from mean, devmode) is caused by slow ITs (Sabol et al., 2003, Behav Pharm (7), 489-500). It was hypothesized that ATX would dose-dependently decrease IT devmode, with no effect on IT mode. METHODS Twenty male Sprague-Dawley rats were trained on the 2CRTT for eight weeks. To perform the 2CRTT the rat placed its nose into a central aperture for a randomly varied foreperiod. After the foreperiod, a stimulus light came on above an aperture to the left or right of the rat. The rat then removed its nose from the central aperture (IT) and entered the aperture underneath the illuminated light. A water drop was given for correct responses. The rat began trials by entering the central aperture. Beginning in the ninth week, ATX was administered thirty minutes before testing, twice a week. Doses were assigned via the Latin square procedure and included saline, 0.1, 0.5, and 1.0 mg/kg. Each rat received each drug dose two times. RESULTS There was a main effect of ATX on IT devmode [F(3,57) = 7.411, p < .001]. Post-hoc analyses showed that 0.5 and 1.0 mg/kg were decreased compared to saline. ATX did not affect IT mode. DISCUSSION ATX's effects on lapses in attention were likely due to increased NE availability in cortical areas that control attention during ongoing tasks (Corbetta & Shulman, 2002, Nat Rev Neuro (3), 201-215). The effect of ATX on devmode (lapses in attention) in our model reflects the reduction in RT variability reported in some human studies.

Disclosures: Z.V. Redding: None. P. Chawla: None. K.E. Sabol: None.

Poster

335. Cortical and Allocortical Mechanisms of Attention

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 335.21/TT46

Topic: E.03. Basal Ganglia

Title: The role of the intralaminar thalamic nuclei in attention and orienting

Authors: *G. D. WATSON, H. H. YIN

Dept. of Psychology and Neurosci., Duke Univ., Durham, NC

Abstract: The parafascicular (Pf) and centromedian (CM) nuclei of the intralaminar thalamic complex receive multimodal inputs from multiple brainstem regions. Considered a part of the reticular activating system, these nuclei send diffuse projections throughout the striatum. However, the specific role of these thalamic nuclei in attention, as well as the functional significance of their efferent and afferent projections, is not well understood. To visualize input and output connectivity differences between Pf and CM, canine adenovirus type 2 engineered to express Cre recombinase (CAV2-Cre) was injected into Ai14-tdtomato mice. To examine their contributions toward behavior, Pf and CM were pharmacologically inactivated with muscimol during free field exploration. 3D motion capture was then combined with optogenetic activation

and inhibition to precisely quantity movement. In separate experiments, terminal stimulation of projections from the superior colliculus and locus coeruleus was performed. Finally, attention was assessed during the presentation of stimuli while optogenetically manipulating activity in these nuclei. Our results collectively show that Pf and CM have unique connectivity patterns that underlie different aspects of attention and behavior.

Disclosures: G.D. Watson: None. H.H. Yin: None.

Poster

335. Cortical and Allocortical Mechanisms of Attention

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 335.22/TT47

Topic: H.01. Animal Cognition and Behavior

Title: Effects of 5-HT1B antagonists on behavior in dopamine transporter knockout mice

Authors: *Y. H. SABER¹, F. HALL¹, R. ELHAG², F. RESENDIZ² ¹Pharmacol. and experimental therapeutics, Univesity of Toledo, Pharm. Col. and Pharmace, Toledo, OH; ²the university of toledo, TOLEDO, OH

Abstract: Background. Genetic deletion of the dopamine transporter in mice (DAT KO) induces a syndrome of behavioral effects that are characteristic of ADHD, that include locomotor hyperactivity, and impairments in prepulse inhibition of acoustic startle (PPI) and the cliff avoidance reaction. Moreover, many of these effects can be ameliorated by treatments that are effective in treating ADHD in humans. The most commonly used medications to treat ADHD are psychomotor stimulants. The use of these drugs present several problems that would be alleviated by a non-stimulant alternative. Atomoxetine was produced as an alternative but it also has drawbacks. We have recently shown that DAT KO-induced hyperactivity can be reduced by the 5-HT1B antagonist SB 224289. Here we report further studies with this drug and an exploration of behavioral deficits in male and female mice. Methods. Locomotor activity, PPI and CAR were examined in male and female DAT +/+ and DAT -/- mice. The effects of SB 224289 on the PPI and CAR deficits observed in DAT KO mice were examined, as well as upon DAT KO-induced locomotor hyperactivity. Results. In the current studies, the effects of DAT KO were found to be sex dependent (the effect of sex has not been previously examined), with greater effects observed in female mice. Effects of SB 224289 were found for all 3 tests, although the effects were most robust in the CAR and locomotor tests. Conclusions. In the present studies the effects of DAT KO on behavior were found to be sex-dependent, with greater effects in females. These findings will require confirmation in subsequent studies. Moreover, we have found additional evidence that antagonism of the 5-HT1B receptor may reverse some deficits in DAT KO mice, encouraging further investigation of this potential novel target for the treatment of ADHD.

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Poster

335. Cortical and Allocortical Mechanisms of Attention

Location: Halls A-C

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Program#/Poster#: 335.23/TT48

Topic: H.01. Animal Cognition and Behavior

Support: NEI R01EY022062

Title: Single neuron correlates of ERP changes linked to spatial attentional shifts in behaving monkeys

Authors: *C. LOCKWOOD¹, W. VAUGHN², C. J. DUFFY³

¹Univ. of Rochester, Fairport, NY; ²Univ. of Rochester, Rochester, NY; ³Univ. Of Rochester Med. Ctr., Rochester, NY

Abstract: Cued shifts of spatial attention modulate behavioral responses to visual stimuli, with spatially valid cues yielding faster responses and invalid cues yielding slower responses (Posner, 1980). These behavioral effects have been linked to changes in cognitive ERPs in both humans (Woodman and Luck, 1999) and monkeys (Woodman, et al., 2007). We previously studied cue validity effects on human ERPs, finding spatial attentional task effects on a variety of response components (CNVs, N200s, N2PCs, and P300s). We have now extended those studies using monkey ERPs, LFPs, and single neuron recordings to identify neuronal correlates of the attentional effects seen in human ERPs. We trained two Rhesus monkeys to maintain search coil or IOG monitored centered visual fixation during the exogenous spatial cueing of optic flow discrimination. Left or right side flashed spots preceded left or right optic flow foci-of-expansion (FOEs). The monkeys then pressed a button to indicate the side of the FOE. The cue was valid (same side as the subsequent FOE) in 75% of the trials, invalid (opposite side of the FOE) in 25%. We recorded ERPs from 32 transcranial electrodes permanently implanted in the monkeys' skull. The first 18 months of recording was devoted to obtaining behavioral and ERP data across a variety of stimulus and task parameters. We then implanted a posterior parietal microelectrode recording chamber over one hemisphere, preserving most ipsilateral, and all contralateral, ERP electrodes. Microelectrodes were inserted daily into cortical area MST for LFP and SU recordings in the task. In monkeys, as in humans, validly cued trials yield faster push button RTs than invalidly cued trials. Monkey ERPs recorded during these trials showed the same component structure seen in humans, with very similar timing but larger amplitude and broader bandwidth than seen in human scalp recordings. MST neurons showed FOE selective firing rate changes in response to optic flow onset. These responses were greatly modulated by the cued location of spatial attention, often reversing Lt/Rt FOE selectivity. Spatial attention modulates ERPs and single neuron activity, often altering fundamental neuronal response properties. Our

findings suggest that posterior cortical neurons are under continuous attentional control focusing signal processing on behaviorally relevant stimulus characteristics.

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Poster

335. Cortical and Allocortical Mechanisms of Attention

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 335.24/TT49

Topic: H.01. Animal Cognition and Behavior

Support: BRAIN Initiative Grant

Title: The prevalence of ON and OFF cortical states in macaque visual cortex

Authors: *N. KHARAS¹, S. R. DEBES², A. R. ANDREI³, V. DRAGOI⁴ ¹Neurobio. and Anat., Uthouston Med. School-Md Anderson Cancer Ctr., Houston, TX; ²Neurobio. and Anat., Univ. of Texas At Houston, Houston, TX; ³Neurobio. & Anat., McGovern Med. Sch., Houston, TX; ⁴Dept Neurobiol/Anat, Univ. of Texas at Houston Dept. of Neurobio. and Anat., Houston, TX

Abstract: Fluctuations in neuronal firing are observed to the order of milliseconds. Traditionally, oscillatory synchronous activity was previously characterized as on and off states during rest and sleep whereas desynchronized activity was a hallmark of wakefulness. Recent studies indicate that on and off states are present in wakefulness but the abundance of these states remains unclear. To investigate whether the fluctuations in on and off states in different behavioral states, such as wakefulness and rest, are ubiquitous we recorded single and multi-unit activity along a cortical column in primary visual cortex (V1) and mid-level visual cortex (V4) of Rhesus macaques. We subsequently employed unsupervised machine learning to identify on and off states in neuronal populations and measured the frequency of transitions between the two states to examine how commonly a cortical column switches between two states. The number of transitions per second between states was significantly greater in rest than in wakefulness. The occurrence of on and off states in wakefulness was rarely observed. Furthermore, the trial-bytrial frequency of transitions weakly correlated with global brain state measures of local field potential (LFP) and changes in pupil size. Our findings indicate that on and off states are not ubiquitous during wakefulness, an increase in prevalence of on and off states and transitions between them arise during rest, and local state in a visual cortical column dynamically mirrors the global brain state. Defining the presence of distinct states and the propensity of neuronal populations to transition between states aids in understanding the influence of endogenous activity in cortical processing.

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335. Cortical and Allocortical Mechanisms of Attention

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Topic: F.08. Biological Rhythms and Sleep

Support: Wellcome-DBT India Alliance IA/I/12/1/500529

DST INSPIRE Fellowship

Title: Phase-relationships of High-Threshold bursting Thalamocortical neurons and their role in modulating Alpha oscillations

Authors: *P. RAMAKRISHNA, R. SHARMA, S. NADKARNI Biol. department, IISER Pune, Pune, India

Abstract: A subclass of Thalamocortical neurons called High-Threshold bursting Thalamocortical (HTC) neurons have been implicated as the potential source of Alpha oscillations in the Thalamus. These neurons have inherent channel properties that ensure that each cell fires at approximately 10 Hz even when the cell is disconnected from the rest of the network. It has also been found that acetylcholine and network inputs can modulate this intrinsic rhythm. These HTCs are coupled via gap junctions allowing them to synchronize. Extant data suggests a connectivity profile of these cells wherein at most 4 cells are seen to be directly coupled to a single cell. Robust alpha oscillations as seen in the thalamus keenly constrains the strength of these connections. Based on these observations, we propose a network motif of HTC cells that includes a biophysically realistic description of ion channels that orchestrate the individual rhythms. This allows for a large population of HTC neurons to be entrained to produce the characteristic 10 Hz oscillation. Separately it has been observed in experiments that injecting external current introduces phase differences while maintaining phase locking. Our model, with the predicted network motif accurately reproduces these phase relationships in response to current injection. Furthermore, we propose that ambient acetylcholine levels could modulate the phases of individual cells leading to a modified spectral behavior of this network. A transition from a single peak at 10 Hz to dual peaks at 10 Hz and 20 Hz within a few hundred milliseconds is seen in EEG recordings in behavioral tasks requiring attention. The rate at which the switching of frequency bands take place suggests that intrinsic channel properties of HTCs alone cannot orchestrate the change. Our model provides a biophysical basis for this surprisingly rapid transition without disrupting the intrinsic rhythm of individual cells.

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Poster

336. Cortical Systems and Mechanisms of Disease

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 336.01/TT51

Topic: E.03. Basal Ganglia

Support: Intramural Research Program, NINDS, NIH

Title: Modulation of neural activity in the parafascicular thalamic nucleus alters beta oscillations in the basal ganglia-cortical motor circuits and modifies motor function in hemiparkinsonian rats

Authors: E. BRAZHNIK, N. NOVIKOV, A. J. MCCOY, *J. R. WALTERS Neurophys/Pharmacol Sec, NIH NINDS, Bethesda, MD

Abstract: Excessive synchronization of local field potential (LFP) activity in the 15-35 Hz range in the basal ganglia (BG) of Parkinson's disease (PD) patients and in rodent models of PD is thought to contribute to motor dysfunction in PD. Recently we presented evidence that synchronized activity in BG output (substantia nigra pars reticulata, SNpr) in hemiparkinsonian rats entrains activity in the ventromedial thalamus (VM) and supports transmission of 25-35 Hz oscillatory activity throughout the BG-thalamocortical circuits (Brazhnik et al., J. Neurosci. 2016). The parafascicular thalamic nucleus (Pf) receives inhibitory input from BG and provides output to the striatum and subthalamic nucleus. It has been suggested that activity in the PF also contributes to the expression of exaggerated beta oscillations in the BG and may therefore play a role in supporting PD motor symptoms. However, our recent observations have shown that unlike the VM, exaggerated beta oscillations are not evident in LFP recordings from the PF thalamic nucleus in the hemiparkinsonian awake behaving rat model (Brazhnik et al., SFN Abst. 2015).

This study uses the awake behaving hemiparkisonian rat with 6-OHDA-induced unilateral dopamine cell lesion to gain further insight into the potential role of the Pf nucleus in supporting expression of beta oscillations in the BG-motor circuits. Electrodes were implanted in the SNpr, dorsal striatum and motor cortex (MCx), and a cannula was inserted into the Pf to allow modification of Pf activity via local infusion of muscimol, a GABA-A agonist, or picrotoxin, a GABA-A antagonist. After unilateral dopamine cell lesion, the hemiparkinsonian rats can walk on a circular treadmill in the direction ipsiversive to the unilateral lesion, with their affected paws on the outside of the circular path, and have considerable difficulty walking in the opposite direction, contraversive to the lesion. Microinfusion of muscimol into the Pf substantially reduced beta LFP power in MCx and SNpr and MCx- SNpr coherence (by ~50-58% of baseline at 1h post-infusion) while temporarily restoring contraversive walking (up to 3 h). The effect of picrotoxin infusion into the Pf on MCx and SNpr LFP power and coherence and on contraversive walking in dopamine cell-lesioned and control rats is currently being assessed.

These data support the view that following substantial loss of dopamine, manipulations that induce tonic inhibition of the Pf activity improve motor function.

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Poster

336. Cortical Systems and Mechanisms of Disease

Location: Halls A-C

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Program#/Poster#: 336.02/TT52

Topic: E.03. Basal Ganglia

Support: Intramural Research Program, NINDS, NIH

Title: The cognitive role of theta, beta, and gamma band oscillatory activity in the basal gangliathalamocortical circuit in the awake, behaving hemiparkinsonian rat

Authors: *A. R. WEISS, E. BRAZHNIK, N. NOVIKOV, M. J. PRESTON, J. R. WALTERS Neurophysiological Pharmacol. Section, NINDS, Bethesda, MD

Abstract: The motor symptoms of Parkinson's disease (PD) have been linked to the emergence of exaggerated oscillatory activity in the 13 - 35 Hz beta range in local field potentials (LFP) recorded in the subthalamic nucleus (STN), substantia nigra pars reticulata, and motor cortex of PD patients and animal models. PD patients and parkinsonian animal models are also known to express dopamine-dependent cognitive impairments, implying effects of dopamine loss on prefrontal cortex function. The electrophysiological correlates of these cognitive symptoms are not well understood. The anterior cingulate cortex (ACC) is a cortical area known to be involved in set-switching and executive function. Previous studies in human ACC have shown modulation of 3 - 8 Hz theta band activity in response to expected and unexpected outcome valences (Weiss AR et al., SFN 2015 abstract). Here, we investigate the involvement of basal gangliathalamocortical circuits in dopamine-impaired and healthy rats in response to salient cues predicting the onset of expected or unexpected treadmill-induced walking. This study used awake, behaving 6-OHDA-lesioned hemiparkinsonian rats performing a circular treadmill walking task to compare synchronized activity in the ACC, STN, and ventral medial thalamus (VM). Electrode bundles were implanted in the ACC, STN, and VM of rats with either unilateral dopamine cell lesions or saline-controls. Rats were trained to expect epochs of treadmill walking after a tone, with subsequent epochs manipulating expectancy through tone-towalk and tone-to-no-walk epochs. LFPs and spiking activity were recorded during epochs surrounding auditory stimuli and treadmill walking in control animals and 7, 14, and 21 days after dopamine cell lesion. Beta band activity with a peak of 30 - 35 Hz was shown to be coherent between the ACC, STN, and VM of dopamine-lesioned animals with increasing spectral power, peak frequency, and coherence with increasing alertness from a rest state to

walking. The temporal properties of theta, beta, and gamma oscillatory band event related potentials were modified by expected and unexpected treadmill-walking trials. Both control and dopamine-lesioned groups exhibited a robust theta frequency response to unexpected trials. Phase amplitude coupling is being assessed.

This study suggests that prefrontal cortex functions associated with dopamine-modulated executive function may be altered in PD and parkinsonian conditions. Our results provide further insight into the significance of excessive oscillatory activity in PD and its influence on cognitive systems.

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Poster

336. Cortical Systems and Mechanisms of Disease

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Topic: E.03. Basal Ganglia

Support: KAKENHI(24500379 and 15H05879 to KI, 16H02454 to M.T.)

CREST to E.H. and M.T

Title: Arrangement of multisynaptic inputs from the basal ganglia to the dorsal and ventral premotor cortical areas in macaques: retrograde transneuronal double labeling with fluorescent rabies viral vectors

Authors: *K.-I. INOUE¹, M. FUJIWARA¹, S. UEZONO¹, S. TANABE¹, H. ISHIDA², E. HOSHI², M. TAKADA¹ ¹Primate Res. Institute, Kyoto Univ., Inuyama, Japan; ²Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan

Abstract: Transneuronal labeling with neurotropic viruses is a powerful technique for analyzing the architecture of a neural network playing a particular functional role. Among such viruses, the challenge virus standard (CVS) strain of rabies virus (RV) is quite useful, because it is taken up exclusively by axon terminals and moves in the retrograde direction to cross individual synapses in a time-dependent manner. Here, we report a newly-developed RV vector based on the CVS strain of which genome was manipulated to provide the ability to express foreign genes efficiently. We confirmed that this vector retains the infectious property of the parental CVS strain though its propagation rate is slightly decreased and exhibits brilliant labeling of infected neurons. Then, we employed retrograde transneuronal double labeling with RV vectors expressing GFP and RFP to examine the cellular origins of multisynaptic projections from the

basal ganglia to the dorsal and ventral premotor cortical areas (PMd, PMv). These motor-related areas are known to be involved in distinct aspects of motor control, and such functional specializations may be ascribable to differential neuronal connectivities. For elucidating the organization of multisynaptic inputs responsible for forelimb movements, we injected the two RV vectors separately into the forelimb regions of the PMd, and PMv of macaque monkeys, and compared the distribution patterns within the basal ganglia of neurons retrogradely and transsynaptically labeled from the injection sites. We found that the distributions of GFP- and RFP-labeled neurons were generally segregated with only partial overlap. We also observed that there were some neurons double-labeled with the two vectors, which indicates that these neurons simultaneously innervate both the PMd and the PMv by way of axon collaterals in a multisynaptic fashion. The present results indicate that the newly developed fluorescent RV vectors that enable us to perform multicolored retrograde transneuronal tracing provide powerful tool to advance the understanding of the organization of complex neural networks in the primate brain.

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Poster

336. Cortical Systems and Mechanisms of Disease

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 336.04/TT54

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01 NS089470

Title: Phase-specific closed loop optogenetic stimulation in behaving mice

Authors: *Y.-C. KIM, D. Y. JUNG, S.-W. HAN, N. S. NARAYANAN Neurol., Univ. of Iowa, Iowa City, IA

Abstract: Neuronal synchrony and coherence are observed throughout neuronal networks, but the significance of these signals is unclear as there are no tools to directly probe them. Here, we used a closed loop control to target an optogenetic manipulation of particular phases of on-going oscillations during interval timing. We calculated the phases of ongoing rhythm by Morlet wavelet transformation. We were able to optogenetically stimulate in < 10ms roundtrip time from on-going LFP to light output. Offline analysis with no phase delay showed that stimulation were targeted accurately smaller than 1 radian standard deviation. We tested these tools in two paradigms. First, we have shown that frontal delta oscillations (1-4 Hz) are critical for interval timing. We stimulated in or out of phase in mice and examined stimulation effects on behavior. Second, striatal beta oscillations (12-25 Hz) are markedly enhanced in movement. We stimulated

basal ganglia nuclein in or out of phase with this oscillation and observed effects on movement. These data could be helpful in developing adaptive, phase-response brain stimulation for many applications.

Disclosures: Y. Kim: None. D.Y. Jung: None. S. Han: None. N.S. Narayanan: None.

Poster

336. Cortical Systems and Mechanisms of Disease

Location: Halls A-C

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Topic: H.01. Animal Cognition and Behavior

Support: NIH NRSA F31NS092190

NIH R01 NS089470

Title: Striatal D1-medium spiny neurons in levodopa-induced dyskinesias

Authors: *S. L. ALBERICO¹, Y.-C. KIM², N. S. NARAYANAN³ ²Neurol., ¹Univ. of Iowa, Iowa City, IA; ³Neurol., Univ. of Iowa Roy J and Lucille A Carver Col. of Med., Iowa City, IA

Abstract: A major limitation in treating Parkinson's disease (PD) is the development of L-Dopainduced dyskinesias (LID), which are disruptive involuntary movements that lead to a decreased quality of life in PD patients. Prior research demonstrates that mice lacking the D1-dopamine receptor (D1DR) do not develop LID. Here, we investigate the role of D1DRs and D2DRs in LIDs in the striatum of a 6-OHDA PD mouse model. We injected the D1DR antagonist SCH23390 (.1 mg/kg, ip) and the D2DR antagonist sulpiride (1mg/kg) and investigated changes in neuronal activity. We found that administration of SCH23390 and sulpiride modulated striatal activity but only SCH23390 decreased some subtypes of LIDs. We further studied the role of the D1DR in D1-Cre mice by optogenetically inhibiting or stimulating D1DR expressing neurons in the striatum while simultaneously recording striatal activity. We found that stimulating D1neurons drives dyskinesias. These results may elucidate how D1DR expressing neurons in the striatum dysfunction during LID and further the understanding of the mechanism underlying LID.

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Poster

336. Cortical Systems and Mechanisms of Disease

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 336.06/TT56

Topic: H.01. Animal Cognition and Behavior

Support: NIH R25 NS079173

Title: The effect of alpha-synuclein overexpression on dendritic spines of the prefrontal cortex in a mouse model of Dementia with Lewy Bodies

Authors: *G. M. ALDRIDGE¹, C. S. LALONDE², Q. ZHANG¹, N. S. NARAYANAN² ¹Neurol., Univ. of Iowa, Iowa City, IA; ²Neurol., Univ. of Iowa Roy J and Lucille A Carver Col. of Med., Iowa City, IA

Abstract: Lewy Body dementias, which include Parkinson's disease dementia (PDD) and Dementia with Lewy Bodies (DLB), are characterized by abnormal accumulation of synuclein aggregates in the cortex. Furthermore, overexpression of synuclein in patients with genetic abnormalities is associated with these diseases. It is not known whether synuclein exerts a direct or indirect pathological effect in the prefrontal cortex, where many of the symptoms of these diseases localize. In this study, we utilize an alpha-synuclein overexpression model of Lewy Body Dementias to determine if overexpression of synuclein in the prefrontal cortex alone can alter dendritic spines and neurite anatomy. Dendritic spines are the post-synaptic connections that facilitate communication between neurons, and abnormalities in these structures are associated with most types of dementia. By utilizing an AAV-6 viral vector coding for full length human alpha-synuclein we were able to induce targeted overexpression of this protein bilaterally in the prefrontal cortex of adult mice. After 3 months, the brain was processed either for immunohistochemistry or golgi-cox impregnation for dendritic spine structure. Staining for synuclein revealed overexpression of the protein within medial prefrontal cortex, with a punctate pattern of staining of neurites in this region. Preliminary analysis of dendritic spines in these animals shows a trend towards decreased dendritic spine density in basilar dendrites from layer 2 and 3 pyramidal cells. This finding would be in line with recently published data using transgenic mice and fibrils, which suggests alpha-synuclein overexpression leads to decreased dendritic spine density in cortex (Blumenstock et al., 2017). Our work extends these findings into a viral vector model that allows examination of the local effect of alpha-synuclein overexpression in prefrontal cortex. Further immunochemistry studies will examine colocalization to determine which neurite types (axonal or dendritic) are accumulating alphasynuclein in this model.

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Poster

336. Cortical Systems and Mechanisms of Disease

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 336.07/TT57

Topic: H.01. Animal Cognition and Behavior

Support: R01 NS089470

Title: A human prefrontal-subthalamic circuit for cognitive control

Authors: *R. KELLEY¹, O. FLOUTY⁴, E. EMMONS¹, Y.-C. KIM², J. KINGYON¹, J. R. WESSEL³, H. OYA⁴, J. D. GREENLEE, M.D.⁵, N. S. NARAYANAN⁶ ²Neurol., ³Psychology, ¹Univ. of Iowa, Iowa City, IA; ⁴Neurosurg., Univ. of Iowa Hosp. and Clinics, Iowa City, IA; ⁵Dept Neurosurg, Univ. Iowa, Iowa City, IA; ⁶Neurol., Univ. of Iowa Roy J and Lucille A Carver Col. of Med., Iowa City, IA

Abstract: The subthalamic nucleus (STN) is a key site controlling motor function in humans, and deep-brain stimulation (DBS) of the STN can improve motor function in Parkinson's disease. STN DBS can have cognitive side-effects; however, the reason for this is unclear. Here, we show that in humans the STN is monosynaptically connected with cognitive brain areas such as the prefrontal cortex. We discovered that STN single neurons and field potentials are modulated during cognitive processing, and coherent with 4 Hz prefrontal cognitive control signals. These data predict that low-frequency DBS might compensate for cognitive impairments in PD patients; accordingly, we found that STN DBS at 4 Hz improved cognitive performance. These data provide the first functional evidence of a human hyperdirect pathway in cognitive control.

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Poster

336. Cortical Systems and Mechanisms of Disease

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Topic: H.01. Animal Cognition and Behavior

Support: NIH 1F31 MH110092-01A1

NIH R01 NS089470-01

Alfred P. Sloan Scholarship

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Title: Frontostriatal ensembles learn during interval timing

Authors: *E. EMMONS, B. DE CORTE, N. NARAYANAN Univ. of Iowa, Iowa City, IA

Abstract: Interval timing refers to temporal processing on the scale of seconds to minutes. The prefrontal cortex and striatum are both critical to adequate interval timing and share a prominent projection. However, the manner in which they shape the neural representation of temporal information is unknown. Here we investigated the dynamics of temporal processing in the medial frontal cortex (MFC) and dorsomedial striatum (DMS) in an interval-timing task in rats. This elementary cognitive task involves working memory and attention. First, we trained rats on an interval-timing task with a 12-second delay (FI12). Following training, animals were implanted with microwire electrode arrays unilaterally in the MFC and DMS. After recovery, animals were retrained on the FI12 interval-timing task. Neural activity was recorded from the MFC and DMS during the FI12 task. The next day, a second interval of 3-second delay (FI3) was added to the task such that trials pseudorandomly alternated between FI12 and FI3 delays. Behavior and neural recordings were assessed over three days of the two-interval timing task. Animals learned the FI3 trial within the first session but improved over each successive day. MFC and DMS recordings revealed that neural changes accompanied the learning of the two-interval task. Single-neuron and single-trial analyses revealed shifting representations of neural activity and behavior. Gradual changes in neural firing rate over time, or "ramping" activity patterns, were found in MFC and DMS neurons during interval-timing behavior. These patterns of "ramping" activity changed as the animals learned the two-interval task. This observation suggests that ramping activity in frontostriatal circuits is fundamentally related to temporal processing. These data demonstrate the importance of frontostriatal circuits in basic cognitive tasks and suggest strategies for further elucidation of cognition.

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Poster

336. Cortical Systems and Mechanisms of Disease

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Topic: H.01. Animal Cognition and Behavior

Support: Eurostars Grant E!9683/CIIP-20152001

Retos RTC-2015-3898.1

Title: ORY-2001, a dual LSD1/MAOB inhibitor in development for neurodegenerative diseases, normalizes aggressive behavior in SAMP-8 mice and social avoidance in isolated rats

Authors: D. ROTLLANT¹, M. LUFINO¹, C. MASCARÓ¹, C. GRIÑAN², M. PALLÀS², R. NADAL³, A. ARMARIO⁴, *T. MAES¹

¹R&D, ORYZON GENOMICS S.A., Cornella de Llobregat, Spain; ²Fac. of Pharm. and Food Sci., Univ. of Barcelona, Barcelona, Spain; ³Psychobiology Unit, Sch. of Psychology, Bellaterra, Spain; ⁴Inst. de Neurociències, Univ. Autònoma de Barcelona, Bellaterra, Spain

Abstract: The aging of the population is increasing the incidence of neurodegenerative disease and the corresponding impact on families and on governmental budgets. While cognitive decline is a hallmark of Alzheimer's disease (AD), the behavioral and psychiatric symptoms that often accompany the disease probably pose the heaviest burden on immediate family and caretakers, and ultimately lead to the patients' internalization in special care units. ORY-2001, an orally bioavailable and brain penetrant dual LSD1-MAOB inhibitor in development for the treatment of CNS disease, is currently finalizing a First-in-Human clinical trial to assess safety, tolerability and pharmacokinetics. We previously reported that ORY-2001 restores the cognitive deficit in SAMP-8 mice, a model for accelerated aging and Alzheimer's disease. Preliminary observations revealed that untreated, but not ORY-2001-treated male SAMP-8 mice housed in groups, presented skin lesions initially considered to be a consequence of the accelerated aging phenotype, but ultimately attributed to increased fighting. These early findings induced us to evaluate the effect of ORY-2001 on social behavior. Individually-housed male mice were treated for 5 weeks with vehicle (SAMP-8 and SAMR1) or ORY-2001 at 0.32 or 0.96 mg/kg/d (SAMP-8) from 5 months of age. The effect on behavior was evaluated using the resident intruder (RI) test. Social exploration parameters in the RI test were similar for the SAMP-8 and SAMR1 strain, but clear differences were observed in the aggression parameters. The number of clinch attacks was significantly higher in SAMP-8 than in SAMR1 mice (p<0.01); but reduced to SAMR1 control levels by ORY-2001 treatment at both doses (p<0.001). Gene expression analysis of the prefrontal cortex of stressed versus non stressed vehicle-treated SAMR1 mice and vehicle- or ORY-2001-treated SAMP-8 mice showed that SAMP-8 mice presented a modified stress response capacity that was partially restored by ORY-2001 treatment. ORY-2001 was further studied in the rat isolation rearing model. Isolated adult Sprague Dawly rats were treated at PND61 with vehicle or ORY-2001 (0.16 or 0.48 mg/kg/d) for ~1 month. Isolation did not produce any significant effect on anxiety or locomotor activity tested in the elevated plus maze (EPM). However, social avoidance was greatly increased in isolated rats (p<0.001) in the RI test, and restored to non-isolated rat levels by treatment with ORY-2001 (p<0.05). These data illustrate the potential of ORY-2001 for the treatment of behavior disturbances in a variety of neuropsychiatric disorders in addition to neurodegenerative diseases.

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Mascaró: A. Employment/Salary (full or part-time):; ORYZON GENOMICS S.A.. C. Griñan: None. M. Pallàs: 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.; CR for ORYZON GENOMICS S.A. R. Nadal: 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.; CR for ORYZON GENOMICS S.A. A. Armario: 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.; CR for ORYZON GENOMICS S.A. T. Maes: A. Employment/Salary (full or parttime):; ORYZON GENOMICS S.A., 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.; ADDF. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ORYZON GENOMICS S.A..

Poster

336. Cortical Systems and Mechanisms of Disease

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 336.10/TT60

Topic: E.03. Basal Ganglia

Support: Fulbright scholarship

Brown University Open Graduate Education

Title: Towards a computational account of theta band (4-8 Hz) power modulation in the subthalamic nucleus under response conflict

Authors: *P. MOOLCHAND¹, S. R. JONES¹, M. J. FRANK² ¹Dept. of Neurosci., Brown Univ., Providence, RI; ²Brown Univ., Brown Inst. for Brain Sci., Providence, RI

Abstract: The Subthalamic Nucleus (STN) plays a fundamental role in arresting automated responses during response conflict. It prevents premature activation of the output of the Basal Ganglia (BG) to buy the Executive Control (EC) system in prefrontal cortex (PFC) time to resolve the conflict and elicit more appropriate behaviors. Experiments in primates have shown that theta band (4-8 Hz) power in the Local Field Potential (LFP) and spike rates in the STN increase commensurate with the level of conflict. Moreover, recent lines of evidence suggest that the STN can act as a conflict detector by integrating competing motor signals to prevent

impulsive responses. Adapting prior cellular models of STN and Globus Pallidus externus (GPe), we have built a novel large-scale biophysically constrained and reciprocally coupled subthalamopallidal (STN-GPe) network. We perturb the network with simulated cortical signals representing competing motor actions to understand the electrophysiological basis of the STN signal modulations and how cortico-STN topography impact these computations. Our results show a balance between intrinsic behaviors of the STN-GPe network and specific patterns of cortical drive is necessary for theta band expression in the network. We conjecture that theta dependent increased spiking in the STN network is the key component for "braking" unwanted impulsive responses.

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Poster

336. Cortical Systems and Mechanisms of Disease

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 336.11/DP08/TT61 (Dynamic Poster)

Topic: E.03. Basal Ganglia

Title: Distribution and morphology of transplanted human neural stem cells in rats with penetrating ballistic-like brain injury

Authors: *S. GAJAVELLI¹, S. W. LEE¹, M. S. SPURLOCK¹, Z. HU¹, K. N. RIVERA¹, L. QUESADA¹, *G. R. GAJAVELLI¹, *C. J. ALVAREZ¹, A. MAHAVADI², D. A. SHEAR³, T. G. HAZEL⁴, R. M. BULLOCK¹

¹Miami Proj Cure Paral, Univ. Miami, Miami, FL; ²Neurosurg., Univ. of Miami, Miami, FL; ³Walter Reed Army Inst. of Res., Silver Spring, MD; ⁴Neuralstem, Inc, Germantown, MD

Abstract: Severe penetrating traumatic brain injury (PTBI) is associated with the worst outcomes in terms of high mortality and severe disability among survivors. Loss of cells combined with incomplete endogenous repair mechanisms may be an underlying cause of disability. Currently no treatment strategies are available. Using a fetal human neural stem cell line (hNSC; Neuralstem Inc.) that has been approved by FDA for use in clinical trials we have previously shown durable engraftment in a rat model of penetrating ballistic-like brain injury (PBBI). However, the best transplant location to rebuild lost circuitry is unknown. Here we describe follow-on studies wherein cells were transplanted either in the lesion core or in the penumbra in PBBI animals. 12 weeks post-transplantation we assessed graft survival and lesion volume by histology.

PBBI was induced in anesthetized rats. At one-week post-PBBI, immuno-suppressed rats

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received stereotactic injections of green fluorescent protein (GFP) expressing hNSCs (1 million/rat) into the lesion core (Intralesional; n=10) or lesion penumbra (Perilesional; n=10). An additional group of uninjured rats received hNSC transplant as controls (n=10). 12 weeks post-transplant brain sections were stained to assess (1) axonal damage, (2) lesion volume, and (3) distribution of transplant-derived projections.

Results of lesion volume measurements at 12-weeks post-transplantation revealed significant reductions in lesion volume following both perilesional and intralesional engraftment of hNSCs (p < .05 vs. PBBI+Vehicle). PBBI-injured animals that received perilesional hNSC engraftments showed a significant reduction in lesion volume compared to animals that received intralesional hNSC transplants. Silver staining revealed significant damage to the intratelenchephalic and corticostriatal white matter. GFP+ hNSC survival was significantly greater in PBBI animals compared to Sham animals. However, no differences in hNSC transplant survival were detected between groups. The transplant derived projections traversed corticofugal subcerebellar pathways akin to layer 5 cortical neurons.

These results suggest the PBBI milieu at one-week post injury supports hNSC engraftment independent of transplant location. Integration of transplanted hNSCs into the host may provide therapeutic benefit by salvaging endogenous tissue destined for secondary damage. The distribution of the transplant derived projections in the corticofugal subcerebellar motor pathway suggests a potential role for graft-derived cells in amelioration of motor deficits.

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Poster

337. Executive Function: Inhibitory Control

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 337.01/TT62

Topic: H.01. Animal Cognition and Behavior

Title: Primary motor cortex modulation during reactive and proactive response inhibition

Authors: *W. D. BYBLOW^{1,2}, M. J. COWIE¹, J. CIRILLO^{1,2}, H. J. MACDONALD³ ¹Dept. of Exercise Sci., ²Ctr. for Brain Res., Univ. of Auckland, Auckland, New Zealand; ³Sport, Exercise and Rehabil. Sci., Univ. of Birmingham, Birmingham, United Kingdom

Abstract: A central component of effective motor control is the ability to cancel a pre-planned movement, termed response inhibition. When stopping is unexpected, response inhibition is reactive and associated with a non-selectively reduction in motor excitability. If stopping is forewarned, response inhibition can be proactive and excitability may be selectively decreased. The modulation of GABA-ergic inhibitory networks within the primary motor cortex during

proactive versus reactive response inhibition is unclear. The present study investigated the modulation of long and short-interval intracortical inhibition using paired-pulse TMS in eighteen right-handed participants performing reactive and proactive response inhibition tasks. Measures of long and short latency intracortical inhibition (LICI and SICI respectively) were obtained from motor evoked potentials in task-relevant and task-irrelevant intrinsic hand muscles. When only a subcomponent of the response required stopping the remaining response was delayed, with the extent of delay greater in reactive versus proactively cued trials. LICI was reduced in both muscles during all types of response inhibition task compared with pre-task baseline. Additionally, on reactive trials task-relevant LICI positively correlated with behavioral response times of the right hand when responses were inhibited on the left side. Task-relevant SICI was reduced when proactive cues indicated responding was highly likely but was unchanged when response conditions were uncertain. These novel findings indicate that GABA_B receptor mediated pathways may be involved in setting inhibitory tone according to task expectations whereas GABA_A receptor mediated pathways may be recruited proactively with response certainty.

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Poster

337. Executive Function: Inhibitory Control

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 337.02/TT63

Topic: H.01. Animal Cognition and Behavior

Support: DA031695

Title: Modulation of putative dopamine neuron firing in rat ventral tegmental area during performance of a stop-change task

Authors: *S. TENNYSON¹, D. BRYDEN², N. WOYTOWITZ¹, N. HRICZ¹, M. R. ROESCH³ ¹Univ. of Maryland, College Park, MD; ²Univ. of Maryland Col. Park, College Park, MD; ³Univ. of Maryland at Col. Park, College Park, MD

Abstract: Inhibiting unwanted actions is essential for decision-making and executive control. Attention disorders such as addiction, attention deficit hyperactivity disorder (ADHD), schizophrenia, and obsessive-compulsive disorder (OCD), are marked by an inability to control inappropriate behaviors. The midbrain dopamine system plays a critical role in controlling behavioral impulses and encoding the value of reward. Numerous lines of evidence demonstrate that dopamine (DA) firing is modulated by reward, shown by an increase in firing to unexpected reward and a decrease in firing to reward omission. However, very little is known about how the

activity of single DA neurons are modulated by tasks that require response inhibition and induce response conflict when two opposing behaviors compete with one another. To answer this question, we recorded single neuron activity in the rat ventral tegmental area (VTA) while subjects performed a stop signal reward task. In this task, rats are trained to respond to a 'GO' cue on 80% of the trials. On 20% of the trials, rats are given an unexpected 'STOP' cue, where they must inhibit the ongoing GO response and respond in the opposite direction. Putative DA neurons were isolated based on waveform characteristics and analyzed using peri event time histograms. Data suggests that DA neurons exhibit similar firing on correct STOP and GO trials during cues and responses. However, DA firing was reduced after the STOP cue on error trials, prior to completion of the behavioral response. Further, firing to reward was slightly stronger during reward delivery on correct STOP trials compared to GO trials, possibly reflecting the uncertainty with obtaining reward on STOP trials. Together, these results suggest that DA neurons are not modulated during successful response inhibition when two responses are in competition with each other. In the context of this task, DA likely encodes the predicted value of cues that predict reward and missed reward during correct and incorrect trials, as well as the uncertainty of receiving reward on difficult trials.

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Poster

337. Executive Function: Inhibitory Control

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Program#/Poster#: 337.03/TT64

Topic: H.01. Animal Cognition and Behavior

Title: Clonidine mildly reduces impulsive behavior at sub-sedating doses that also impair sustained attention in male rats

Authors: *P. J. MCLAUGHLIN, M. C. NORMANN, J. E. JAGIELO-MILLER, T. M. PROPER, R. M. HARDY Dept. of Psychology, Edinboro Univ. of Pennsylvania, Edinboro, PA

Abstract: Clonidine is a nonselective noradrenergic $\alpha 2$ agonist that is approved as a nonstimulant therapy for Attention Deficit Hyperactivity Disorder (ADHD). Relative to psychostimulants, it is less efficacious in treating attention symptoms, and has even been found to reduce accuracy in preclinical models of attention. These counterintuitive findings may be better understood with a fuller investigation into the different behavioral processes that may be involved in clonidine's effects. It was therefore proposed that clonidine would ameliorate impulsive behavior, and impair sustained attention, in two models of different types of ADHD symptoms. Adult male Sprague Dawley rats were trained on a variable consecutive number task

with a discriminative stimulus (VCN-S_D), which requires a chain of paced responses on one lever, until a stimulus tone signals the availability of reinforcement following a response on a second lever. Premature responding on the second lever results in a timeout and reset of the count. A different group of animals performed a fixed-lever, variable ratio 15 (VR15) auditory signal detection task incorporating features of human sustained attention assessments, including minimal feedback, and infrequent, unpredictable signals, relative to frequent irrelevant stimuli. At up to 0.03 mg/kg, clonidine modestly improved percentage of completed chains in the VCN-S_D model of impulsivity. As a positive control, the 5HT_{1A} agonist 8-OH-DPAT produced stronger, biphasic effects: more impulsive behavior at a low dose, followed by less impulsivity at higher doses, relative to vehicle control. In the sustained attention task, clonidine dosedependently impaired sensitivity (d'), without altering bias (c), replicating previous findings. However, when comparing only the first lever press of each trial, clonidine reduced bias, and overall, reduced no-signal lever responding to a greater degree than signal lever responding. Doses of clonidine used in these tasks were just below those that significantly altered observational ratings of sedation. It is concluded that clonidine may have clinical efficacy in ADHD more via reductions in impulsivity than via direct enhancement of attention. Impairments on the attention task appear to have been mitigated somewhat by a slight reduction in bias; we speculate that this may have been due to motor slowing reducing behavioral momentum, an effect not seen in the impulsivity assay.

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Poster

337. Executive Function: Inhibitory Control

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Topic: H.01. Animal Cognition and Behavior

Support: Neurological Foundation NewZealand

Title: Involvement of hippocampal, orbitofrontal and subthalamic oscillations in behavioural inhibition in the stop signal task in rats

Authors: *A. BANSTOLA, C. K. YOUNG, N. MCNAUGHTON Psychology, Univ. of Otago, Dunedin, New Zealand

Abstract: Rhythmicity allows communication between distributed brain regions to occur in a specific frequency band, potentially binding structures into a functional circuit. Local field potential (LFP) oscillations represent synchronous activity of thousands of neurons and so can assess circuit involvement in behavioural and cognitive functions. Frontal-subthalamic and

frontal-hippocampal circuits have been separately implicated in behavioural inhibition; but their possible interactions have not been assessed. We implanted four adult Long-Evans rats to record LFPs simultaneously from the hippocampus (HPC), orbitofrontal cortex (OFC) and subthalamic nucleus (STN) during a Stop Signal Task (SST). We found increase of beta band coherence across all recording areas when rats inhibit their responses, with correct stopping associated with lower (~20 Hz) beta frequencies compared to failed stopping (~30 Hz). In addition, we observe a decrease in theta and alpha coherence between the HPC and OFC following failed stopping, which coincide with an increase of theta and alpha coherence between the OFC and STN. These observations support the notion that the HPC also receives pro-stopping signals through beta oscillations communicated between OFC and STN. We speculate changes in theta/alpha coherence across the structures may be involved in error-monitoring processes.

Disclosures: A. Banstola: None. C.K. Young: None. N. McNaughton: None.

Poster

337. Executive Function: Inhibitory Control

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 337.05/TT66

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH109334

Title: A cross-species neurophysiological assay of cognitive control: Development of a touchscreen-based rodent analog of the Flanker Task

Authors: *M. A. ROBBLE¹, S. NICKELS², L. WOOLDRIDGE², S. PERLO², E. CÁRDENAS², B. KANGAS², J. BERGMAN², W. A. CARLEZON, Jr², D. A. PIZZAGALLI² ¹Behavioral Genet. Lab., McLean Hospital, Harvard Univ. Med. Sch., Somerville, MA; ²McLean Hospital, Harvard Med. Sch., Belmont, MA

Abstract: Deficits in cognitive function, such as reward sensitivity and cognitive control, are a common feature of virtually all neuropsychiatric disorders. While perturbations in cognitive control have been studied extensively in humans, it has been more challenging to examine these complex processes in model organisms. Stagnation in the development of animal-based tasks to assess these processes has coincided with a decline in the development of effective therapeutics for neuropsychiatric disorders. As part of a larger effort to create reliable and valid cross-species assays of cognitive function, we have begun developing a rodent version of the Eriksen Flanker Task to assess cognitive control.

Using fading and correction procedures combined with touchscreen-based response technology, Sprague Dawley rats were trained to discriminate between several distinct pairs of visual stimuli. These stimuli included arrows (</>) and letters (H/S), as well as colored stripes and more detailed pictures (cherries/leaf). While rats did not meet the required criteria of 80% response accuracy for arrow or letter stimuli, successful discrimination was observed using the red/green stripes and cherry/leaf pictures.

Following training, rats were presented with flanker probe trials. Intriguingly, when increasing the number of flanked trials in a session from initially 20% to 100%, performance remained high and the Flanker interference effect of lower reduced accuracy for incongruent stimuli was confirmed. Notably, a response bias on incongruent trial types was observed in which performance on trials with a red target was reduced, regardless of stimulus type. Presently, efforts are underway to reduce this bias by altering the intensity of the green stimulus in each condition. In addition, other color schemes that may be more advantageous for visual discrimination in the rat are also being tested. Following these design modifications, EEG data will be collected during the rodent Flanker Task and the relevant ERPs will be compared to those observed in human studies.

In parallel, 45 EEG data sets were collected from human subjects using the cherry/leaf stimuli and the more traditional arrow stimuli. Relevant outcome measures such as the N200 component, theta power, and the error-related negativity (ERN) were comparable across stimuli, raising confidence that the stimuli ultimately found suitable for rodents will also be appropriate for studies in humans. Cross-species behavioral and neurophysiological correspondence will serve to validate the rodent task and provide the predictive power to screen potential therapeutics for numerous neuropsychiatric conditions.

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Poster

337. Executive Function: Inhibitory Control

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 337.06/UU1

Topic: H.01. Animal Cognition and Behavior

Title: Neuronal correlates of motivated inhibition in monkey motor cortices

Authors: *M. GIAMUNDO^{1,2}, F. GIARROCCO¹, E. BRUNAMONTI¹, M. MATTIA³, P. PANI¹, S. FERRAINA¹

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Abstract: The ability to rapidly inhibit actions is critical to the effective and flexible interactions with the environment, and it has been widely studied by using the countermanding (stop-signal) task. An open question is how this ability can be affected by other cognitive functions, such as

motivation, and relatedly, which are the neural correlates of this influence. To address this question we designed a combined countermanding/motivation task intended to modulate the motivation to stop and to move by using the reward prospect, and we recorded the Multi-Unit Activity (MUA) by means of Utah arrays (2x48 channels) from the dorsal premotor cortex (PMd) and the primary motor cortex (M1) of a macaque monkey while engaged in the task. Indeed previous data have shown that motor cortices neuronal activity signals both movement inhibition (Mattia et al., 2013) and reward (Roesch and Olson, 2004), but it is unclear whether and how these phenomena are combined.

In the countermanding/motivation task, the monkey was instructed to reach to a target after a go signal (no-stop trials) but to withhold his response when a stop-signal followed after a variable delay (stop trials). In every trial, one out of three possible reward cues (RC) was presented 1 sec before the go signal to inform about the amount of reward that would have been delivered if a correct response had been produced. RC conditions were: Go+Stop- with a higher reward for nostop trials compared to the stop trials; Go-Stop+, with reversed reward amounts; and Go Stop with equal amounts delivered. We found that the monkey strategically adapted his behavior to the different reward prospects: reaction times (RTs) diminished and the probability of errors in the stop trials increased from Go-Stop+, to Go Stop and then to Go+Stop- conditions.Recorded MUA in both PMd and M1 was modulated by task conditions. In some channels (22/96), MUA was significantly modulated before go signal by the RC value. Of these channels, 41% showed a progressive increase of MUA after the RC presentation until movement onset; this activity was progressively higher for Go-Stop+, Go Stop and Go+Stop-. Fifty-five percent showed a decrease during the RT epoch and a further increase of MUA just before or after movement onset. Others channels (48/96) showed RC related modulations around movement onset and/or after reaching the final target but not before go signal. Interestingly, in correct stop trials, some of the channels showed an early stop-signal response which magnitude was affected by the RC value. These results suggest that motor cortices can signal and integrate motivational aspects into movement control and inhibition.

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Poster

337. Executive Function: Inhibitory Control

Location: Halls A-C

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Program#/Poster#: 337.07/UU2

Topic: H.01. Animal Cognition and Behavior

Title: Study of global and selective inhibition of upper and lower limb by a stop signal task

Authors: *E. BRUNAMONTI, P. DE SIMONE, M. PAOLONI, P. PANI, S. FERRAINA Sapienza Univ., Rome, Italy

Abstract: The stop signal task and the related horse race model have been widely used to study the processes underlying motor inhibition of the eyes and of several segments of the upper limb (finger, wrist or arm), while it is poorly investigated whether and how comparable principals account for inhibition of the lower limb segments. Indeed, we count on the control of lower limbs to accomplish many of our daily actions. In addition, we usually engage a complex coordination between the upper and lower limbs that often requires the selective inhibition of only one of the effectors. For example, during driving, one can be required to suppress the command on the gas pedal but to keep acting on the wheel to avoid an obstacle that suddenly appears on the road. In the present work, we used different variants of the stop signal task, presented in experimental contexts of different complexity, to study the inhibition of lower limbs and its mutual interaction with the inhibition of the upper limbs, during actions in which their movement is associated. Specifically, we investigated: 1) whether the inhibition of the foot fitted the predictions of the race model, as described for other motor effectors; 2) whether these predictions were kept, when it was required either a selective or a combined inhibition of the two effectors; 3) if the complexity of the context influenced the selective inhibition of each of the two different effectors. Ten participants were tested in separate blocks in a conditional stop signal task requiring always to lift both their index finger and their foot in response to a go signal (70% of trials), but to suppress the movement of both (global inhibitory block), or only one of the two effectors (either foot or finger selective inhibitory block), when a conditional stop signal was unpredictably presented in 30% of the trials. In an additional block, all the inhibitory conditions were randomly presented (and the effector cue instructed) to test whether the occurrence of an unpredictable stopping condition affected inhibition. Our results revealed that the race model correctly accounted for the selective inhibitory performance of both effectors, when tested in the unmixed conditions, but that it failed its predictions, when the selective inhibition of the effectors was required in the more complex mixed block condition. On the contrary, inhibition in global condition accomplished the model predictions in the mixed block and was significantly more efficient than in selective conditions. The present results are in line with the hypothesis that when the selective inhibitory system is engaged in a complex context the go process interferes with the efficiency of the stop process.

Disclosures: E. Brunamonti: None. **P. De Simone:** None. **M. Paoloni:** None. **P. Pani:** None. **S. Ferraina:** None.

Poster

337. Executive Function: Inhibitory Control

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Program#/Poster#: 337.08/UU3

Topic: H.01. Animal Cognition and Behavior

Title: Premotor neuronal correlates of voluntary limb movement initiation

Authors: *P. PANI, F. GIARROCCO, M. GIAMUNDO, E. BRUNAMONTI, S. FERRAINA Sapienza Rome Univ., Roma, Italy

Abstract: In reaction time tasks, when a signal instructing to move (Go) is presented the movement is generated after a variable latency (reaction time; RT). Typically the RT varies across trials with identical instruction conditions, and this variability has been explained within the general framework of accumulator models. These models posit that the movement occurs after a signal, that represents the evidence for the type of movement to perform, has accumulated to a response threshold. These models can account for RT variability in terms of four basic parameters: starting point (initial state of the accumulating signal); encoding time (time necessary to perform sensory and perceptual processing before evidence starts to accumulate); drift rate (mean rate of evidence accumulation), and threshold (level of accumulation). The variability of each of these parameters can explain the RT variability. For example, in the variable threshold account, RT will be shorter when the threshold is lower and longer when the threshold is higher. Neurophysiological studies have linked the evidence accumulation to the dynamic of firing rates of single cortical neurons recorded while monkeys were performing RT tasks. The above parameters have been translated respectively into the mean level of activity before Go (starting point); into initial rising of neural activity after Go signal (the encoding time); into slope of neural activity growth (the drift rate); and, finally, into the level of activity just before movement generation (threshold). This approach has been useful in finding that saccades are initiated when in frontal eye fields (FEF) movement neurons reached a fixed threshold and that the saccadic RT variability is due to a variable drift rate across trials (Hanes and Schall 1996). By using a similar approach we studied single neurons recorded from the dorsal premotor cortex (PMd) while 3 monkeys performed a countermanding task, that required in most trials reaching movement towards peripheral targets, and in some of the trials in try to refrain from moving after a stop signal. This task is able to generate wide RT distributions. We found that in PMd different neural dynamics, and thus different accumulator accounts, are at play when analyzing single unit activity. After the Go signal, we found that different subsets of neurons showed different profiles, i.e. different accumulator instantiations, thus corroborating the idea that RT variability cannot be explained exclusively by a single neural dynamic. Indeed RT variability correlates with different features at the same time, thus showing that in PMd movement initiation is related to diverse dynamic contributions from single neurons

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Poster

337. Executive Function: Inhibitory Control

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Program#/Poster#: 337.09/UU4

Topic: H.01. Animal Cognition and Behavior

Support: KAKENHI-PROJECT-26710001

Title: The nigrostriatal dopamine pathway transmits a stop signal during the performance of a saccadic countermanding task in monkeys

Authors: *T. OGASAWARA^{1,3}, M. TAKADA³, M. MATSUMOTO^{2,1}

¹Grad. school of Comprehensive Human Sci., ²Fac. of Med., Univ. of Tsukuba, Tsukuba-Shi, Japan; ³Primate Res. Institute, Kyoto Univ., Inuyama, Aichi, Japan

Abstract: Response inhibition is the ability to withhold planned or ongoing motor actions that would lead to unwanted outcomes. Although previous studies have shown that the prefrontal cortex plays a crucial role in response inhibition, the basal ganglia might also contribute to this ability because they form loop circuits with the prefrontal cortex that regulate body movements not only in a facilitative but also in a suppressive manner. To investigate whether and how the nigrostriatal dopamine pathway, an important component of the basal ganglia circuitry, might contribute to response inhibition, we recorded single-unit activity from projection neurons in the caudate nucleus and dopamine neurons in the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) in monkeys performing a saccadic countermanding task. While the monkey was gazing a central fixation point, the point disappeared and a saccadic target was presented on the right or left side of the point. In 70% of the trials, the monkey was required to make a saccade to the target. In the remaining 30%, the fixation point reappeared as a "stop signal" with a random delay after the onset of the target. The monkey was then required to cancel a planned saccade to the target. We recorded the activity of 200 striatal projection neurons, and found that 59 of the neurons showed a significant excitation to the stop signal while 72 of them exhibited a significant inhibition. We further recorded the activity of 70 dopamine neurons, and found that 28 of the neurons showed a significant excitation to the stop signal whereas no neurons displayed a significant inhibition. Notably, the dopamine neurons excited by the stop signal were observed mainly in the SNc, but not in the VTA, that send massive projections to the caudate nucleus. These results suggest that dopamine neurons in the SNc transmit a signal that could inhibit saccadic eye movements to the caudate nucleus. To test the causal relationship between the capacity of response inhibition and nigrostriatal dopamine signaling, we injected SCH23390, a dopamine D1 receptor antagonist, or haloperidol, a dopamine D2 receptor antagonist, into the caudate nucleus. We found that the monkey often failed to cancel a saccade not only after the SCH23390 but also after the haloperidol injection. The overall data indicate

that the nigrostriatal dopamine pathway plays an important role in suppressing a planned saccade in the saccadic countermanding paradigm.

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Poster

337. Executive Function: Inhibitory Control

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Topic: H.01. Animal Cognition and Behavior

Support: NIMH grant R01 MH068073

Title: Insular cortex activity is implicated in appetitive inhibitory conditioning

Authors: *K. R. LIGHT¹, Z. TEK¹, R. BOWLER², V. WINIGER², A. WANAR¹, B. COTTEN¹, M. R. BAILEY², A. KALMBACH¹, E. H. SIMPSON², P. D. BALSAM¹ ¹Barnard Col., New York, NY; ²Columbia Univ., New York, NY

Abstract: While a great deal is understood about the mechanisms involved in excitatory conditioning both for aversive and appetitive stimuli, far less is known about the neural underpinnings of inhibitory conditioning. Inhibitory conditioning is the process of learning to inhibit a behavior when a signal is presented that indicates that behavior will not produce a previously expected outcome. Rodent research on inhibitory conditioning has suggested a possible involvement of the prelimbic (PL), and infralimbic (IL) subregions of the medial prefrontal cortex (mPFC) as well as the insular cortex. In two parallel experiments we investigated the neural activity in PL, IL, and insular cortex related to conditioned inhibition and tested the functional requirement of PL and IL in appetitive inhibitory learning. To examine the neural activity related to conditioned inhibition, we measured *c-fos* immunoreactivity during initial learning (after 3 days of training) and when learning was asymptotic (after 15 days of training). Behavior and neural activity specific to inhibitory learning about a tone that signaled the non-availability of reward was identified by comparing inhibitory learning groups to control groups in which tones had no consistent relationship to reward or non-reward. One control group received an equivalent number of tone and reward presentations and one received an equivalent rate of tone and reward presentations. Analysis of behavior showed clear inhibitory learning in only the inhibition group. Further, the inhibitory learning group demonstrated far more inhibition on Day 15 than Day 3. Neural activity of the insular cortex as measured by *c-fos* expression did not differ between groups on Day 3 but by day 15 it was significantly elevated in the inhibitory training group relative to controls. Further, *c-fos* expression was significantly higher on Day 15 than Day 3 in the conditioned inhibition group. No significant differences in neural activity were observed in PL or IL at either time point. The results of the neural activity experiment suggest

that the insular cortex is involved in the learning and/or performance of inhibitory learning. In a parallel experiment, we explored the functional role of the PL and IL in appetitive inhibitory conditioning. Specifically, we selectively inactivated these structures with the inhibitory DREADD hM4Di and injected CNO before each daily training session.

Neither inactivation of PL or IL affected the acquisition of inhibitory learning. Together, these data suggest that the circuit required for inhibitory learning in our appetitive conditioning task includes the insular but not medial prefrontal cortex.

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Poster

337. Executive Function: Inhibitory Control

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Topic: H.01. Animal Cognition and Behavior

Support: Toxicology Scholar- Interdisciplinary Environmental Toxicology Program

Campus Research Board, University of Illinois at Urbana Champaign

Title: Two forms of circadian disruption impact response inhibition and attention in adult longevans rats

Authors: *R. C. BALACHANDRAN¹, M. L. SIEG², K. M. HATCHER², M. M. MAHONEY¹, P. A. EUBIG¹

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Abstract: Circadian rhythms, which are endogenous rhythms that synchronize behavior and physiology, can be disrupted by external factors, such as alterations in the ambient light cycle. Common forms of circadian disruption in human populations include working outside of the regular hours of '9 to 5' (shift work) or untimely exposure to light at night (artificial light-at-night). We are studying the effects of two models of circadian disruption on attention and response inhibition using a 5-choice serial reaction time task (5- CSRTT). Adult Long-Evans rats of both sexes were maintained on a 12h:12h light:dark cycle and tested under three circadian conditions: 4h into the dark phase with no exposure to a pulse of light for 1h at the time of testing (a model of light-at-night), and 4h into the light phase (a model of shift work). We hypothesized that rats tested under the two models of circadian disruption would be less attentive

and have reduced response inhibition (i.e., be more impulsive) than controls. Our preliminary results reveal that rats tested under both circadian disruption conditions were equally less attentive (had a reduced percent accuracy in responding to visual cues) than control rats. Furthermore, there was a differential effect of circadian disruption on response inhibition. Darkphase tested rats that experienced a pulse of light had significantly greater premature responding on 5-CSRTT than light-phase tested rats, and both groups were significantly more impulsive compared to control rats. Sex-related differences in behavior were not detected. To summarize, our findings demonstrate that different forms of circadian disruption can affect attention and differentially affect response inhibition, the latter being an effect not previously reported. While acetylcholine (ACh) is an important modulator of circadian rhythms and attention, dopamine is a key modulator of response inhibition. Thus, as we begin to investigate underlying mechanisms responsible for our findings, we hypothesize that an interaction between ACh and dopamine systems is important in the relationship between circadian rhythmicity and cognitive functions such as attention and response inhibition, particularly in the prefrontal cortex, where nicotinic ACh receptors (nAChRs) modulate dopamine release. We will also present early results from pharmacologic challenges examining the response to combinations of ACh and dopamine agonists and antagonists on 5-CSRTT performance as we proceed to examine the hypothesized ACh-dopamine interaction.

Disclosures: R.C. Balachandran: None. M.L. Sieg: None. K.M. Hatcher: None. M.M. Mahoney: None. P.A. Eubig: None.

Poster

337. Executive Function: Inhibitory Control

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Robin and Richard Patton through the E. Bronson Ingram Chair in Neuroscience

Title: Microcircuitry of agranular frontal cortex: Laminar organization of saccade performance monitoring signals in supplementary eye field

Authors: *A. SAJAD, J. D. SCHALL Psychological Sci., Vanderbilt Univ., Nashville, TN

Abstract: To effectively interact with the environment, it is essential to monitor the consequences of our visually guided behavior and make appropriate adjustments. Despite much

investigation on this topic the mesoscopic circuit mechanisms accomplishing these processes remain poorly understood. To investigate this, we sampled neural activity from primate supplementary eye field (SEF), an agranular cortical area that contains neural signals related to error and reinforcement, using linear electrode arrays. During recordings monkeys performed a countermanding saccade paradigm where on~50% of trials they made saccadic eye movement towards a visual stimulus (correct; rewarded) but on a proportion of trials (~25%) made erroneous saccades despite an instruction to stop (error; unrewarded). After a short delay following the saccade an auditory tone was presented which indicated the absence or presence of an upcoming juice reward. Spiking activity (N = 293 units), field potentials, and current source density from 16 sessions with perpendicular electrode penetrations into the gray matter (three sites in two monkeys) were analyzed to determine the laminar structure of three signal types: visual, error, and reinforcement. Our analyses revealed that task-related visual signals were largely confined to L2/3 and L5. Error signals had the shortest latency in intermediate layers (lower L3 and upper L5) with a later appearance in upper (L2/3) and lower layers (L6). Reinforcement-related signals were observed throughout cortical depth but exhibited different spatial profile during different task epochs. Entirely predictive units (modulated following the tone but before juice) were mainly concentrated in depths spanning lower L3 to L6b while those that also modulated following juice delivery were mainly observed in upper layers L2/3 and L5. Furthermore, reinforcement units that were positively modulated for reward (L5 and upper L6) showed a complementary (and inverse) distribution to those that were negatively modulated (L2/3 and lower L6). Overall, these data are inconsistent with a general canonical cortical microcircuit derived from sensory areas, and they contribute to understanding the contributions of laminar-specific cortical and subcortical as well as feedforward and feedback processes, and to constraining circuit-level models of executive control.

Disclosures: A. Sajad: None. J.D. Schall: None.

Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant MH085828

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Title: Dynamic striatal and medial temporal lobe interactions are mediated by varying demands on pattern separation

Authors: *A. FRITHSEN¹, S. M. STARK³, S. NIKOLOVA⁴, C. E. STARK² ¹Neurobio. & Behavior, Univ. of California Irvine, Orange, CA; ²Univ. of California Irvine, Irvine, CA; ³Neurobio. & Behavior, ⁴Univ. of California, Irvine, Irvine, CA

Abstract: Our theoretical framework for understanding striatal responses and striatal-medial temporal lobe (MTL) interactions during conditional motor associative learning is that motor learning proceeds in a gradual transition between two parallel but cooperative phases within the striatum. In the early, more flexible phase, spatial sequences are coded in visuospatial coordinates that is subserved by a cortical-striatal loop involving the associative striatum. The MTL constitutes a parallel and cooperative learning pathway for conditional motor associative learning that is primarily involved in the "flexible" early phase of learning and specializes in learning new associations between arbitrary elements in memory. This framework predicts that early in new associative learning the striatum together with the MTL provide different kinds of parallel and cooperative learning signals: we predict the strongest interaction in the form of coordinated activity and strong functional correlations will be seen between the MTL and both the limbic striatum and the associative striatum. In contrast, we predict little or no interaction between the MTL and the sensorimotor divisions of the striatum. Young adults performed an associative learning task using kaleidoscopic images in two conditions: 1) images with high perceptual similarity and 2) images with low perceptual similarity. Participants learned to associate an arbitrary response pairing with each image, resulting in a continuous probability correct measure across learning: a learning slope that reflects memory strength. We found robust hippocampal and nucleus accumbens (a core part of the striatum) activity that varied across memory strength, regardless of high vs. low similarity. In order to evaluate the functional relationship between the hippocampus and MTL with the limbic, associative, and sensorimotor striatal systems, we performed a seed-based functional connectivity analysis in both the low and high similarity conditions. Interestingly, we found that functional connectivity between the hippocampus and MTL with the associative striatal system was modulated by high vs low similarity, bilaterally. These data suggest that the interactions between memory systems are very dynamic and may reflect competition between memory systems, which may be exacerbated when pattern separation demands are high.

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Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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Program#/Poster#: 338.02/UU9

Topic: H.02. Human Cognition and Behavior

Support: Alberta Innovates Health Solutions Polaris Award

Title: Dynamics of sharp wave/ripple-triggered cortical and subcortical high frequency local field potential in humans

Authors: *I. SKELIN^{1,2}, J. ZHENG³, B. L. MCNAUGHTON^{1,2}, J. J. LIN⁴ ¹Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada; ²Neurobio. and Behavior, ³Biomed. Engin., ⁴Neurol., Univ. of California Irvine, Irvine, CA

Abstract: Sharp wave/ripples (SWRs) are brief high frequency events (<100 msec; 80-150 Hz in humans), occurring in hippocampus during non-REM sleep and quiet wakefulness. SWRs are considered to represent the temporal windows for efficient hippocampal-cortical communication during memory reactivation, potentially orchestrating activity of anatomically disparate brain structures, which store different aspects of recent and consolidated memory. Therefore, information on the dynamics of SWR-triggered activity throughout the human brain would improve understanding of the role of SWRs in memory consolidation. Functional magnetic resonance imaging (fMRI) revealed SWR-triggered increases in hemodynamic signal throughout cortex (Logothetis et al., 2012). The patterns of SWR-triggered cortical hemodynamic responses also depend on the SWR waveform signature and phase coupling to hippocampal delta or sharp waves (Ramirez-Villegas et al., 2015). However, the more precise interpretation of these results was hampered by the relatively slow temporal resolution of fMRI. The high frequency (300-900 Hz) local field potential (HF-LFP) represents a reliable indirect measure of neuronal spiking activity (Wilber, Skelin et al., unpublished). Taking advantage of excellent spatiotemporal resolution of electrophysiological recordings, as well as the ability to simultaneously record from large number of subcortical and cortical structures in human epileptic patients undergoing presurgical evaluation, we recorded hippocampal SWRs and SWR-triggered HF-LFP in the amygdala, frontal, cingulate, temporal and parietal cortices from 4 patients, during 8 overnight sleep sessions. The HF-LFP power in amygdala, temporal and parietal cortices was increased around the time of SWRs, with the ipsilateral increases present both before and after SWRs and contralateral increases following SWRs. We also observe delta-phase dependent SWR-triggered effects on HF-LFP power in cingulate cortex. In addition, we observed that the relative proportions of SWRs locked to different delta or sharp wave phases are stable across multiple days, but differ between the hippocampal locations. The present data reveals hemispheric differences in SWR-triggered cortical activity, as well as hippocampal location-dependent patterns of SWR phase locking to delta or sharp waves.

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Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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Program#/Poster#: 338.03/UU10

Topic: H.02. Human Cognition and Behavior

Title: Interaction between the hippocampus and neocortical regions during associative memory formation: An fMRI study

Authors: *T. ISHII, T. ASO, K. NAKAMURA

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Abstract: The case report of patient H.M. and a series of subsequent works have revealed that the medial temporal robe (MTL) including hippocampus is critical for forming memory. Meanwhile, many animal studies have uncovered that memory trace is stored in cortical regions which are responsible for processing specific information. However, how the MTL and each cortex interact to form new memory remains to be elucidated especially in human. We aimed to investigate interaction between the MTL and cortical regions during associative memory formation in human using fMRI (functional magnetic resonance imaging). We scanned thirteen healthy participants with a 3 tesla MRI scanner while they performed a face-name association task. During encoding blocks, participants viewed serially presented unknown faces which were paired with names. They were instructed to commit pairs to memory. In recall blocks, participants viewed the faces only and attempted to answer the associated names. To prevent rote rehearsal between encoding and recall blocks, we used the Odd/Even Digit task in which participants were shown single digits and asked to indicate whether the digit was odd or even. We performed PPI (psycho-physiological interaction) analysis to see the effect of memory formation process on the functional connectivity between the MTL and related cortices. The result showed that functional connectivity between the hippocampus and fusiform gyrus, which is thought to be specialized for the perception of faces, was enhanced during both encoding and recall sessions although activation of the hippocampus decreased with repetition of the same pair. The present result lend support to the view that the hippocampus and neocortical regions which engage in processing specific information interact dynamically and wire together to form new associative memory.

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Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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Program#/Poster#: 338.04/UU11

Topic: H.02. Human Cognition and Behavior

Title: Resting-state medial temporal connectivity with reward centers predicts how motivation impacts learning

Authors: *L. FRANK¹, A. R. PRESTON³, D. ZEITHAMOVA² ¹Psychology Dept., ²Univ. of Oregon, Eugene, OR; ³The Univ. of Texas At Austin, Austin, TX

Abstract: Memory is influenced by motivation, such as a promise of future monetary reward for remembering an event. Reward-based memory modulation has been shown to rely on interactions between brain structures sensitive to reward, such as the midbrain, and those supporting episodic memory, such as the medial temporal lobe (MTL), during motivated encoding. Here, we tested whether intrinsic connectivity between the memory-related (MTL) and reward-related (midbrain, striatum, orbitofrontal cortex) regions during rest can predict reward modulation and if these connections increase as a function of motivated encoding. Subjects underwent resting-state functional MRI before and after a monetary incentive encoding task in which a potential monetary reward cue (penny, dime, or dollar) was followed by a pair of objects to be remembered. The associated monetary value was awarded to the subject for each correctly recalled object pair. Resting-state fMRI revealed that MTL connectivity with reward regions significantly increased from pre- to post-learning, specifically driven by MTL-midbrain and hippocampus-striatum connectivity. These findings suggest that interactions between reward and memory systems can be upregulated in response to a motivated encoding task. Intrinsic connectivity further tracked individual differences in reward modulation, such that subjects who demonstrated greater sensitivity to reward also showed significantly greater intrinsic MTLorbitofrontal connectivity when collapsed across the pre- and post-learning scans. These results suggest that intrinsic connectivity between reward- and memory-related regions underlies individual differences in reward modulation and highlight the dynamic interactions between these systems that extend beyond the period of learning.

Disclosures: L. Frank: None. A.R. Preston: None. D. Zeithamova: None.

Poster

338. Human Long-Term Memory: Medial Temporal Lobe

Location: Halls A-C

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Topic: H.02. Human Cognition and Behavior

Support: NSF GRFP

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Program Project Development Grant

Title: Changes in memory representations are related to features in prior night's sleep

Authors: *E. COWAN¹, A. A. LIU², S. KOTHARE², O. DEVINSKY², L. DAVACHI³ ¹Ctr. For Neural Science/ New York Univ., New York, NY; ²New York Univ. Sch. of Med., New York, NY; ³Dept Psychology, NYU, New York, NY

Abstract: Theories of systems memory consolidation posit that memories are stabilized as they become more distributed throughout the cortex. Sleep has been linked with successful memory consolidation, and evidence suggests particular features in the architecture of sleep may relate to sleep-dependent memory enhancements. Recent evidence suggests a 24-hour delay promotes more distributed memory traces, as measured by greater hippocampal-cortical functional connectivity, in a manner predictive of subsequent behavioral resistance to forgetting, indicating a specific role in memory stabilization. However, it remains unknown how aspects of sleep architecture might be related to the representation of memory traces the next day, and how this relates to behavioral measures of memory. To investigate this relationship, we designed a threeday experiment utilizing overnight polysomnography recordings, fMRI, and behavior. Subjects encoded two lists of word-image pairs twice, either with an intervening period of overnight sleep (Sleep List), or a brief wakeful period (New List), such that the lists differed in the opportunity for consolidation processes. During the re-study session, subjects were presented with the previously seen word-image pairs, and a new list of pairs (Single Study List) while in the scanner. Cued source recall was probed immediately following the scan and after a 24-hour delay, providing a measure of memory stability over time. Univariate analyses revealed that for the Sleep List in particular, time spent in NREM sleep is positively related to greater activation in the hippocampus. Using multivariate pattern similarity analysis, we examined the representations of trials from each encoding list. Within the anterior hippocampus, later remembered Sleep List pairs were represented more distinctly compared to those later forgotten, while in posterior hippocampus, remembered pairs were more similar compared to forgotten. This significant interaction was not observed in either of other encoding lists, indicating a potential dissociation along the long-axis of the hippocampus that develops with sleep.

Disclosures: E. Cowan: None. A.A. Liu: None. S. Kothare: None. O. Devinsky: None. L. Davachi: None.

Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant AG053040

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Title: Measuring neural activity in the MTL using simultaneous BOLD and functional MR spectroscopy

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Abstract: We have used BOLD fMRI extensively to characterize behavioral and neural (structural and functional) profiles of neurocognitive aging in the context of the medial temporal lobe (MTL) memory system. However, the spatial and temporal limitations of BOLD are well known and appreciated, as are the limitations imposed by the fact that it is fundamentally a contrastive measure (e.g., Condition X vs. Y vs Z). The effect of this contrastive nature of BOLD is magnified greatly by the fact that factors clearly outside of cognitive or neural changes can alter the BOLD signal. Here, we investigated a functional variant on magnetic resonance imaging spectroscopy (fMRS) that collects metabolite data while participants engage in various cognitive tasks, dynamically measuring excitatory transmission during a memory task by quantifying a composite measure of glutamate and glutamine (Glx) while simultaneously measuring BOLD fMRI at the same location. We scanned 20 young adults during 30 second blocks of 4 conditions: 1) viewing novel images of scenes, 2) viewing familiar images of scenes, and 3) a perceptual baseline task, and 4) unconstrained rest. We collected both BOLD and MRS data from two large voxels: one in the right parahippocampal cortex (PHC) (~12mL) and one in the right hippocampus (~3.75mL). Consistent with previous findings from standard BOLD fMRI, we observed greater BOLD activity for novel images than for familiar ones (i.e. the repetition suppression effect) in both the hippocampus and PHC. Likewise, we found decreased BOLD signal for the perceptual baseline task and a modest decline for rest. Thus, our proxy for BOLD is consistent with prior results using more typical BOLD fMRI scans. NAA and Cr concentrations served as negative controls and, as expected, showed no task modulation, but Glx concentrations showed clear task modulations, demonstrating a reliable functional MRS signal. In both regions, novel and familiar pictures elicited higher levels of Glx than rest. In the hippocampus, the perceptual baseline was associated with very low levels of Glx while in the parahippocampal cortex, Glx levels were elevated. Thus, in both BOLD and Glx, novel and familiar pictures elevated their respective measures of activity. Yet, clear differences emerged. In particular, our two control conditions (simple rest and perceptual baseline) differed dramatically in the parahippocampal cortex (highest levels of Glx vs. lowest levels of BOLD). Therefore, even in a healthy, young population, there are both clear points of contact between these two measures of activity and clear points of departure, providing us with an interesting new tool for evaluating neural activity.

Disclosures: C.E. Stark: None. S. Nikolova: None. S.M. Stark: None.

Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: H.02. Human Cognition and Behavior

Title: Functional connectivity segregation along the long-axis of the hippocampus

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Abstract: In primates and humans, the development of the hippocampus and surrounding cortex facilitated an axial change which resulted with an elongated, longitudinal shape whose posteriorto-anterior axis corresponds to the dorsal-to-ventral axis of the rodent hippocampus. Despite its distinct uniform structure, the hippocampus may not, in fact, function as such, but rather consists of distinct sub-structures along its long axis. Given the apparent diversity of sub-hippocampal functionality, the computational principles of the hippocampus as a whole remain to be elucidated. Here we suggest two anatomically-guided functional connectivity methods aimed at delineating cortical and sub-cortical networks that are specifically co-active with discrete subhippocampal areas. These methods were run on resting-state blood-oxygenated-level-dependent (BOLD) data of 31 healthy volunteers from the publicly available NKI Enhanced dataset. Following the parceling of the anatomical MRI datasets using FreeSurfer (http://surfer.nmr.mgh.harvard.edu), each participant's hippocampi were delineated for further analysis. Next, BOLD signal time-courses were extracted for each and every voxel of the entire brain, and Pearson correlations were computed between the time-courses of each voxel in the brain and the time-courses of each and every voxel in the hippocampi. Finally, each brain voxel was assigned the relative location along the anterior-to-posterior hippocampal axis with which it was maximally correlated. Results of this analysis demonstrate that distinct brain networks coactivate with distinct hippocampal regions, such that posterior hippocampus highly co-activates with primary sensory and motor regions (including primary visual, auditory, and taste cortices), the anterior hippocampus is preferably connected with amygdala, temporal poles, and ventromedial prefrontal cortex, whereas the middle hippocampus is highly correlated with core regions of the default-mode network (DMN). Corroborated by converging findings using an anatomically-guided seed-based general linear model approach, these results provide direct evidence as to the distinct cortical and sub-cortical networks that converge into subcompartments of the hippocampus, arguing in favor of a functional segregation along the long axis of the hippocampus.

Disclosures: A. Mendelsohn: None. S. Gabay: None.

Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

Support: SAF2015-65982-R

Title: Travelling waves along the long-axis of the hippocampus

Authors: *M. YEBRA¹, O. JENSEN², N. AXMACHER³, B. A. STRANGE^{1,4} ¹CTB, Madrid, Spain; ²Sch. of Psychology, Univ. of Birmingham, Birmingham, United Kingdom; ³Neuropsychology, Inst. of Cognitive Neurosci., Bochum, Germany; ⁴Dept. of Neuroimaging, Alzheimer's Dis. Res. Centre, Reina Sofia-CIEN Fndn., Madrid, Spain

Abstract: Travelling waves are present in different brain structures at different frequency ranges and in different species. The relationship of the phase of the traveling waves with neuronal firing or other oscillations is thought to represent a mechanism to code information by means of the expression of different phases along the same structure or between structures at the same time. Traveling waves have been shown in the theta range in the hippocampal formation of rodents traveling along the long-axis (Lubenov & Siapas, 2009; Patel et al., 2012). Similar patterns were found in humans . We report iEEG recordings along the long-axis of the hippocampus from epilepsy patients. We used a version of the Von Restorff paradigm. Participants studied pictures of faces and houses shown in grayscale against a red or green background. While the majority of items came from *expected* category, a small proportion belongs to *unexpected* category. We used a fine grained analysis, including the traditional convention in humans for different delta, theta, alpha and beta frequency ranges. We confirm the existence of theta traveling waves along the long-axis of the hippocampus and go beyond previous work by showing that this frequency range extends to the alpha band. We did not see a modulation of the travelling wave patterns by novelty processing suggesting that hippocampal activity shows similar anterior to posterior phase differences regardless of unexpectancy. If encoding is more likely to occur at the peak of the theta signal and retrieval at the trough and if the place field size increases from posterior to anterior hippocampus in humans - as has been shown in rodents -, the existence of a theta traveling wave suggests that while the tail is experiencing a peak (representing small place field size -details- and encoding state) the head is experiencing a trough (representing big place field size -context- and retrieval state) and vice versa. This suggests that the hippocampus puts details into context and contextualizes details every ~200ms.



Figure 1. Theta and alpha travelling wave pattern for one representative patient A) Theta and alpha traveling waves across neighbouring electrodes. Displayed filtered signals in theta (4-8Hz) alpha (8-13Hz) locked to the peaks of the most anterior hipocampal electrode and averaged across trials for expected and unexpected conditions. B) Polar histogram across trials for the theta (upper row) and alpha (lower row) phase difference between the most anterior electrode as a reference channel and each electrode along the longitudinal axis of the hippocampus for expected and unexpected conditions.

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Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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Title: Preferential viewing of old scenes reflects conscious memory for which scenes are old or new and is impaired after medial temporal lobe damage

Authors: *C. N. SMITH^{1,2}, Z. URGOLITES^{1,2}, L. R. SQUIRE^{1,2,3,4} ¹Veterans Affairs San Diego Healthcare Syst., San Diego, CA; ²Dept. of Psychiatry,

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Abstract: When individuals try to select the recently studied (and familiar) item during a multiple-choice memory test, they direct a greater proportion of viewing time towards the selected item when their choice is correct than when their choice is incorrect. Thus, for both correct and incorrect choices, individuals indicate that the chosen item is old, but viewing time differentiates between old and new items. What kind of memory supports this preferential viewing effect? One possibility is that automatic, hippocampus-independent memory supports this effect because viewing time differentiates old items from new items independently of overt behavioral choice. Alternatively, conscious, hippocampus-dependent memory might support this effect. If so, the preferential viewing effect should be related to established measures of declarative memory (i.e., accuracy scores, confidence ratings, and response times). In two experiments, we tested whether the preferential viewing effect reflects conscious memory or unconscious memory (Experiment 1, 30 young adults) and whether the effect is dependent on the medial temporal lobe (Experiment 2, 5 patients with damage limited to the hippocampus or limited to the medial temporal lobe and 6 age-matched, older controls). In Experiment 1, participants made three-alternative, forced-choice, recognition memory judgments for targets (200 photographs of scenes studied 30 min earlier) and foils (each target was presented together with two thematically-related novel scenes). We replicated the preferential viewing effect: individuals looked longer at the selected item when it was correct than when it was incorrect. Importantly, the effect was stronger when individuals were more confident in their decisions and when their response times were shorter. Moreover, the size of the preferential viewing effect was strongly correlated with overall accuracy scores. Thus, the preferential viewing effect reflects conscious memory for which items are old and which items are new. In Experiment 2, participants made recognition memory judgments for targets (100 photographs of scenes studied 1 min earlier) and foils. Patients exhibited impaired declarative memory. Specifically, they were less accurate and exhibited smaller differences in confidence ratings and response times for correct versus incorrect trials. The controls exhibited the preferential viewing effect, but the patients did not. Taken together, these findings suggest that the preferential viewing effect reflects conscious, medial temporal lobe-dependent memory.

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Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

Title: Mnemonic discrimination tuning: A potential mechanism underlying the other-race effect

Authors: *J. YAROS, ESQ¹, D. A. SALAMA¹, M. A. YASSA² ¹UC Irvine, Irvine, CA; ²Neurobio. and Behavior, Univ. of California Irvine Dept. of Neurobio. and Behavior, Irvine, CA

Abstract: The Other-Race Effect (ORE) is a social phenomenon in which people are better at recognizing individuals within their own, relative to other races. While behavioral research suggests the ORE is due to extensive experience with one's own race group, the neural mechanisms underlying the effect remain unclear. We believe that the enhancement in same-race (SR) recognition typical of the ORE, is due to enhanced mnemonic discrimination (MD), a process driven by pattern separation of similar inputs. In the present study, we sought to identify unique MD response profiles for subjects performing an encoding and memory task for faces of several different races. During encoding blocks, subjects viewed and were asked to remember a series of computer generated Caucasian, Asian, and Black faces. During the following test, subjects made 'Old'/'New' judgements, if they believed to have seen the faces before, or not, respectively. Some of the faces shown in test were parametrically altered so that the dissimilarity between encoded and test faces could be quantified. This allowed us to tax mnemonic discrimination selectively for numerous levels of interference between stimulus presentations. Given prior results in the lab, we anticipated performance for SR recognition accuracy to be sigmoidally tuned as a function of face similarity, and OR accuracy to increase linearly with face dissimilarity. This may reflect differences in mnemonic strategies employed for SR vs OR recognition. To further expound upon the role of experience in tuning these mnemonic strategies, subjects are being trained in OR faces, and will subsequently be re-evaluated for SR and OR recognition performance. We expect to see a tuning in the post-training MD function for OR faces, such that it approaches the sigmoidal function synonymous with SR recognition. This will serve as mechanistic evidence that experience alters the efficacy of discrimination in the face of overlapping face inputs.

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Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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Title: Acute mild exercise improves memory by enhancing hippocampal-neocortical connectivity

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Abstract: While a growing number of studies have demonstrated that physical exercise has beneficial effects on hippocampal functions especially on spatial memory, there are few studies on pattern separation associated with the dentate gyrus (DG). Additionally, the most effective exercise regimen (e.g., intensity) for improving hippocampal functions is undetermined. We recently showed that acute mild-intensity exercise without any stress responses has the potential to activate hippocampal neurons including those in the DG (Soya et al., BBRC, 2007) and that such mild training regimens cause neurogenesis (Okamoto et al., PNAS, 2012, Inoue et al., PLoS One, 2016). This led to the hypothesis that acute mild exercise may also enhance DG-mediated pattern separation, a hypothesis supported in part by our recent findings in humans that acute moderate-intensity exercise improves the performance of a mnemonic discrimination task designed to selectively tax pattern separation (Suwabe et al., Hippocampus, 2017). Using the same experimental protocol, here we aimed to investigate whether acute mild exercise improves hippocampal memory, and if so, to explore the exact neuronal substrate in terms of functional activation in the medial temporal lobe (MTL) using high-resolution functional MRI. Healthy young adults underwent a mnemonic discrimination task after 10 minutes of very light intensity exercise (30% VO_{2peak}; corresponds to "very light" in the ACSM's definition) on a recumbent cycle ergometer or rest (control) on separate days in randomized order. We compared neural activity between exercise and control conditions in the pattern separation contrast (lure correct rejections minus lure false alarms). Moreover, we explored cortical regions that were functionally correlated with the hippocampal subfield activity using psychophysiological interaction (PPI) analysis. Exercise improved mnemonic discrimination performance and enhanced fMRI activity in the DG/CA3 and surrounding neocortex. Exercise also enhanced the functional connectivity between the DG/CA3 and cortical regions including the parahippocampal, fusiform and angular gyri, regions that are involved in representing highprecision memories. Critically, the magnitude of the enhancement in functional connectivity predicted the extent of behavioral enhancement at the individual subject level. Thus, we

concluded that acute very light intensity exercise, which is comparable to traditional oriental bodywork such as Yoga and Tai Chi, is capable of enhancing hippocampal memory, and enhancement of the DG/CA3-neocortical functional connectivity is likely the neuronal substrate underlying the effects.

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Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

Support: NINDS R3721135

UCI School of Medicine Bridge Fund

Title: Amygdala-hippocampal-orbitofrontal network dynamics support contextual modulation of facial expression

Authors: J. ZHENG¹, S. VADERA², R. T. KNIGHT⁴, *J. LIN³

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Abstract: When interpreting an ambiguous facial expression, the context or "emotional" background provides situation specific cues to resolve ambiguity and guide interpersonal behavior. Thus contextual processing allows the meaning of a facial expression to be understood according to the emotional backdrop in which it is retrieved. We examined the spatiotemporal dynamics of contextual face processing by presenting a neutral face against a background scene from the International Affective Picture System (IAPS) that varied parametrically by emotional valence (positive, negative, neutral). During the task, the subject was asked to rate the emotional valence of the facial expression, which was displayed immediately after the IAPS background image. We recorded local field potentials (with high temporal and spatial resolution) from depth electrodes implanted in the orbitofrontal cortex (OFC), amygdala (AMYG) and hippocampus (HPC) of 5 patients undergoing evaluations for epilepsy surgery. We quantified how high gamma (HG, 70-250Hz) activity encoded different task-related factors at a single trial level (Fixation, Background IAPS Image, Face, Valence Rating). We found that: 1) the subjects' valence ratings of facial expression were positively correlated with the IAPS valence ratings; 2) electrodes from different brain regions were selective to distinct computational factor at different task stages and exhibited a systematic temporal propagation from AMYG to HPC to OFC; 3)

Single stacked trials (N=120) of high gamma activity in the OFC sorted by response time showed that the peak latency of the high gamma activity correlated with reaction time. These findings provide evidence of a circuit-level interaction within AMYG-HPC-OFC network that supports contextual processing of facial expressions.

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Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

Support: NIH NINDS NS 45540

NIMH R01MH102392

NIA R21 AG049220

Title: Gamma and theta activity in the human medial temporal and prefrontal cortices predicts performance on a spatial learning task

Authors: *R. F. STEVENSON¹, J. ZHENG¹, A. S. MOON¹, S. VADERA¹, R. T. KNIGHT², J. J. LIN¹, M. A. YASSA¹ ¹UC Irvine, Irvine, CA; ²UC Berkeley, Berkeley, CA

Abstract: The ability to learn novel associations is a critical feature of episodic memory. Accumulating evidence suggests that this ability involves local processing within the medial temporal lobe (MTL) and prefrontal cortex (PFC) as well as functional interactions between these two regions. However, the ways in which the MTL and PFC contribute to associative learning in humans as well as the dynamics of MTL-PFC interactions are still not well understood. To address this question, we tested pre-surgical epilepsy patients with depth electrodes implanted in both the MTL and PFC using a spatial learning task in which subjects attempted to learn object-location associations over the course of three training blocks. During encoding, thirty objects were presented at different locations on a computer screen and subjects were asked to try to remember the location of each object. For each training block, the same objects were presented at the top of the screen and subjects were asked to use the mouse wheel to move the object to where it appeared during encoding. After subjects finished placing each object, the object was shown in the correct location for one second as feedback. Following the three training blocks there was a final test during which no feedback was given. During retrieval, we found that there was a greater increase in gamma (40-100 Hz) power in the MTL and PFC for highly precise trials. In contrast, at feedback there was a greater increase in MTL gamma power for low precision trials. Together, these results suggest that while MTL activity during retrieval reflects the precision of an association, activity during feedback signals the amount of error. We also examined theta (3-8 Hz) activity in the MTL-PFC network during retrieval and feedback and found that both power and phase synchronization predicted spatial memory precision. Overall, these data suggest putative mechanisms for the learning of object-location associations.

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Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

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NIMH R01 MH102392

Title: Performance on object pattern separation task predicts cognitive status and is linked to anterolateral entorhinal cortical thinning in cognitively normal older adults

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Abstract: Developing cognitive tasks and biomarkers that are sensitive to the earliest stages of decline in Alzheimer's disease (AD) are one of the highest priorities for research. MRI studies in humans have shown that entorhinal cortical (EC) thinning is a highly sensitive measure of structural change in both mild cognitive impairment (MCI) and AD. Recent studies have suggested that human EC can be divided into functional subdivisions: the anterolateral EC (alEC) is thought to comprise an object/item information pathway, whereas posteromedial EC (pmMEC) is thought to comprise a spatial/contextual information pathway. In this study, we tested a sample of cognitively normal (MMSE 25 to 30) older adults using high resolution structural MRI and a novel cortical thickness segmentation pipeline as well as a suite of behavioral tasks that selectively tax mnemonic discrimination (pattern separation) of object identity or spatial location. The EC was subdivided into alEC and pmEC using expert anatomical

segmentations on a set of atlases that were propagated to the study cohort using a joint label fusion (JLF) and SyN diffeomorphic registration implemented within Advanced Normalization Tools. Behaviorally, we found poorer performance in object relative to spatial discrimination, replicating our prior work. We next calculated the magnitude of difference between performance on each task to establish a measure of asymmetry in response (i.e. an object vs. spatial performance bias), and found that this bias metric was a reliable predictor of scores on the Mini Mental State Exam (MMSE), a measure of global cognitive function. The higher the asymmetry, the more intact the participants' MMSE, suggesting that early changes in impairment are indexed by decreasing asymmetry. We also observed a relationship between performance on the object task and thickness of the alEC, which was not the case in the spatial task or the pmEC, linking alEC decline specifically to memory deficits for object/item information. These findings support the idea that deficits in object memory manifest prior to spatial memory, and that asymmetry in performance may be linked to earlier stages of cognitive decline. Thus, the contrast of object and spatial memory performance on a task designed to test these specific computations may serve as a useful behavioral assay in identifying subtle neurocognitive changes related to neurodegeneration. The results additionally suggest that alEC thickness may be an early biomarker that is associated with these cognitive changes. The combination of sensitive cognitive assay and biological marker offer promise for optimizing diagnosis and/or outcomes for clinical trials.

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Poster

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Title: Hippocampal integration and separation processes are driven by the strength of memory reactivation during learning
Authors: *R. J. MOLITOR, K. R. SHERRILL, N. W. MORTON, A. R. PRESTON Univ. of Texas At Austin, Austin, TX

Abstract: Efficient learning requires encoding of both detailed information from individual events and generalities across related experiences. In service of these goals, it is hypothesized that the hippocampus can create distinct, orthogonal memory representations through pattern separation or form integrated representations that code the common features among memories. To optimize learning, it is critical to identify how these seemingly opposing representational strategies coexist within the hippocampal network, as well as to determine the conditions that promote each type of representational strategy. One factor that may influence whether the hippocampus integrates or separates related memories is the strength of memory reactivation during new learning. Recent evidence indicates that when individuals learn about new events that overlap with past experience, they reactivate memories about related events. Such reactivation has been shown to promote integration of the new information into existing memory representations. Other data suggest, however, that memory reactivation during learning may lead to competition between co-active memories, thus requiring pattern separation to mitigate interference. Here, we test the hypothesis that the strength of memory reactivation may be an important determining factor in whether new events are integrated with or separated from reactivated memories within hippocampus. Participants initially studied a set of associations (AB pairs comprised of face-object or scene-object pairs), and then during fMRI scanning, they encoded overlapping associations (BC object-object pairs). Both prior to and immediately after learning, participants also viewed images (A and C) in isolation during scanning. We used representational similarity analysis to assess whether activation patterns for indirectly related images (A and C) become less similar (i.e., pattern separated) or more similar (i.e., integrated) in hippocampal subregions after learning. Additionally, we used a pattern classifier to measure the reactivation of related memories (A items) in ventral temporal cortex during overlapping event (BC) learning. Consistent with our hypothesis, we found that the degree of memory reactivation in neocortex predicted whether indirectly related memories were pattern separated or integrated in hippocampus after learning. Furthermore, this relationship between reactivation and representational change differed across hippocampal subregions. Together, these findings provide insight into the basis of the divergent yet complementary neural codes formed by the hippocampal memory system.

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Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

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NIH Grant R01-MH48832

Title: The role of sleep in generalizing across disjointly presented information

Authors: *A. C. SCHAPIRO, M. BAYDA, E. CHO, R. COX, R. STICKGOLD Psychiatry, Beth Israel Deaconess Med. Ctr. / Harvard Med., Boston, MA

Abstract: Interleaving the presentation order of complex information is important for learning useful representations of its structure, but waking experience does not normally offer such an optimized exposure order. The Complementary Learning Systems theory (McClelland, McNaughton, O'Reilly, 1995, Psychol Rev) argues that sleep provides this function, helping the cortex uncover structure through interleaved replay of hippocampal traces. We tested this idea by exposing participants to information in a suboptimal order and assessing whether subsequent periods of sleep and wake result in differential understanding of the underlying structure. Participants learned visual and verbal features of three categories (A, B, and C) of "satellite" objects, where satellites in the same category shared most of their features. At the very end of this training, we exposed them to two additional satellites, each composed of a combination of features from categories A and B. These satellites provided a bridge between the two categories, supporting the potential generalization that other combinations of features from A and B could also produce plausible satellite objects. Because the satellites were presented after the categories were already learned, it should be difficult to update category representations to accommodate this new information without going back and revisiting already-learned objects. We reasoned that if sleep helps interleave information offline, participants who sleep should improve in their generalization to novel objects combining features from A and B, relative to participants who do not sleep, and relative to objects that combine features from categories B and C or categories A and C. Our tests of generalization involved asking if a novel satellite 1) had been previously viewed and 2) seemed like it would be "functional." Testing occurred immediately after training and again 12 hr later. Our predictions were supported for both test questions: Participants in the sleep, but not wake, condition showed increased generalization to novel combinations from the bridged categories in the second test session, falsely believing they had seen these satellites before, and judging them to be more functional. However, we additionally found differences in first session performance between the sleep and wake conditions, suggesting a potential influence of circadian factors. Contributions of sleep features and stages to learning based on high density EEG and PSG sleep recordings will also be discussed. The results provide evidence in support of the theory that sleep is important for interleaving information in order to better understand its structure.

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338. Human Long-Term Memory: Medial Temporal Lobe

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Title: The medial temporal lobe and topographical memory

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Abstract: The medial temporal lobe (MTL) is essential for the formation of long-term (declarative) memory. There has been interest in the idea that the MTL might be especially important for spatial processing and spatial memory. We tested the proposal that the MTL has a specific role in topographical memory as assessed in tasks of scene memory where the viewpoint is shifted from study to test. Building on materials used previously for such studies and generously provided by Neil Burgess, we administered three different tasks in a total of nine conditions. Participants studied a scene depicting four hills of different shapes and sizes and made a choice among four test images. In the Rotation task, the correct choice depicted the study scene from a shifted perspective. MTL patients succeeded when the study and test images were presented together but failed the moment the study scene was removed (even at a 0-sec delay). In the No-Rotation task, the correct choice was a duplicate of the study scene. Patients were impaired to the same extent in the No-Rotation and Rotation tasks after matching for difficulty. Thus, an inability to accommodate changes in viewpoint does not account for patient impairment. In the Nonspatial-Perceptual task, the correct choice depicted the same overall coloring as the study scene. Patients were intact when test images were presented together with the study scene and also when the test image was presented 2 sec after the study scene was removed, but failed at longer, distraction-filled delays. We suggest that the impairment in these tasks reflects a broad deficit in the ability to remember both spatial and nonspatial material. We further suggest that the different results for the spatial and nonspatial tasks are related to differences in demand on working memory.

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Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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Support: James S. McDonnell Foundation

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UC Irvine Bridge Fund

Title: Human hippocampal pre-activation predicts behavior

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Abstract: The response to an upcoming salient event speeds up when the event is expected given the preceding events – i.e. a temporal context effect. For example, naming a picture (e.g. "boat") following a strongly constraining temporal context (e.g. "We sailed in a wooden") is faster than naming the picture after a weakly constraining context (e.g. "He looked at a wooden"). The human hippocampus represents mnemonically expected stimuli in their absence. Using intracranial electroencephalogram (iEEG) from the human hippocampus (n = 5), we tested if the hippocampal representation of expected stimuli emerges prior to the stimuli onset and if this pre-activation predicts behavior. Participants named pictures that were preceded by incomplete sentences presented auditorily. In each trial, naming the picture completed the sentence. The sentence manipulated the expectation on the upcoming picture. We observed more power in the hippocampal high-frequency band activity (50-250 Hz) prior to the picture onset (pre-picture interval) of strongly expected pictures than of weakly expected pictures (p = 0.014). We then applied pattern similarity analysis on the temporal pattern of hippocampal high frequency band activity in single contacts. We observed that the similarity between the prepicture interval and the expected picture interval predicted the picture-naming latencies (p = 0.008). Additional pattern similarity analyses indicated that the hippocampal representations reflect a semantic cognitive map. We conclude that hippocampal pre-activation of expected stimuli is a facilitating mechanism underlying the powerful contextual behavioral effect.

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Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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Title: Acute physical exercise improves memory consolidation in humans via BDNF and endocannabinoid signaling

Authors: *K. IGLOI, B. MARIN BOSCH, A. BRINGARD, G. FERRETTI, S. SCHWARTZ Neurosciences, Univ. of Geneva, Geneve, Switzerland

Abstract: Regular physical exercise enhances memory functions and neurogenesis in the hippocampus. In animals, a single session of physical exercise boosts BDNF (Brain Derived Neurotrophic Factor) levels, with parallel increases in neurogenesis and plasticity in the hippocampus. Furthermore, acute exercise promotes the release of endocannabinoids (especially anandamide, AEA), which are small lipophilic molecules that affect dopaminergic transmission and also enhance BDNF release. Whether these effects are also observed in humans and how they influence memory remain unknown. Here we combined blood biomarkers, behavioral measures and fMRI to assess the impact of medium and high intensity acute physical exercise on memory and underlying biomechanisms in humans. We hypothesized that medium intensity exercise improves memory consolidation and enhances hippocampal activity via BDNF and/or endocannabinoid signaling, while high exercise intensity causes physical stress detrimental to cognitive functioning. We tested associative memory performance in twenty healthy participants across three visits: each visit consisted of a learning and a test part performed in fMRI separated by a period of exercise (moderate or high intensity) or rest. All participants also came back for a single retest session three month after the last visit. Two blood samples were taken at each visit before and after the exercise or rest periods. Our results show a general increase in memory performance (measured by accuracy, reaction times, and confidence) after exercise at moderate intensity but not high intensity, compared to after rest. Levels of BDNF and AEA were higher after exercise compared to rest. Crucially, AEA increase after medium and after high exercise correlated with hippocampal activity during retrieval suggesting that AEA may have an acute effect on synaptic plasticity in human. Finally, long-term memory at retest was significantly better for elements learnt during the medium exercise visit than during rest or high exercise visits. Overall, these findings demonstrate that a single session of medium intensity exercise enhances long-term associative memory and shed light on the biomechanisms underlying the beneficial influence of acute physical exercise on human cognitive functions.

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Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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NIH Grant AG034613

Title: Neural substrates of mnemonic discrimination: A whole-brain fMRI investigation

Authors: J. KLIPPENSTEIN¹, S. M. STARK², C. E. STARK², *I. J. BENNETT¹ ¹Dept. of Psychology, Univ. of California, Riverside, Riverside, CA; ²Univ. of California Irvine, Irvine, CA

Abstract: A fundamental component of episodic memory is the generation of discrete memory representations for unique events. Because we frequently encounter highly similar events (e.g., eating breakfast today versus yesterday), accurate memory depends on our ability to dissociate these memory representations, termed mnemonic discrimination. Numerous functional magnetic resonance imaging (fMRI) studies have revealed that the hippocampus, and specifically the dentate gyrus subfield, is involved in this type of mnemonic discrimination. However, few studies have assessed neural activity associated with mnemonic discrimination in regions beyond the medial temporal lobe. Therefore, the current study examined brain-wide fMRI activity in 11 younger adults (19-23 years, 3 female) during performance of a mnemonic discrimination task. In the incidental encoding phase, participants made "indoor"/"outdoor" judgments to a series of to-be-remembered objects. In the test phase, they made "old"/"new" judgments to a series of probe objects that were either repetitions from the memory set, similar to objects in the memory set, or novel. Behavioral results revealed that participants correctly endorsed repeated objects as "old" and novel objects as "new". Mnemonic discrimination was observed as a decrease in "old" responses as objects became less similar to those in the memory set. Mnemonic discrimination was also seen as a significant difference in sensitivity (d' > 0) when comparing distributions of "old" responses to repeated versus similar objects. Imaging data during the test phase were first examined during three task conditions ("old" repeated, "old" or "new" similar, "new" novel) relative to a baseline condition. Results revealed that both repeated and similar objects elicited significant activity in bilateral hippocampus, occipital cortex, and medial frontal cortex. Mnemonic discrimination-related activity was then assessed by comparing these task conditions, which revealed a trend for greater activity to similar versus repeated objects in these regions.

These preliminary findings add to a growing body of work suggesting that mnemonic discrimination is mediated by a broader memory network that extends beyond the hippocampus.

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Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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UL TR00157

Title: Increased aerobic fitness is related to increased anterior dentate gyrus/CA3 volume in healthy young adults following exercise training

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Abstract: Converging evidence suggests a relationship between aerobic exercise and hippocampal neuroplasticity that is centered on adult neurogenesis in the dentate gyrus (DG) subregion of the hippocampus. In humans, this relationship has not been elucidated at the level of the hippocampal subregions, nor has it been examined functionally. Using structural MRI and a hippocampal-dependent behavioral task requiring disambiguation of similar stimuli, we tested the hypotheses that 1) aerobic fitness levels are positively associated with volume and function of DG/CA3 in humans, and 2) a 12-week exercise intervention will increase the volumes of the entorhinal cortex and DG/CA3 and increase disambiguation task accuracy. Healthy young adults (ages 18-35) participated in either an aerobic or resistance training intervention three times per week for twelve weeks. Changes in aerobic fitness were determined via a submaximal, incremental treadmill test using a modified Balke protocol prior to and following the intervention. We conducted volumetric analysis following manual segmentation of T₂-weighted MR images with high in-plane resolution (0.4 mm²; Philips 3 T Achieva). At baseline, greater aerobic fitness was associated with greater volume in the entorhinal cortex and anterior subregions of the hippocampus (DG/CA3 and subiculum). Aerobic fitness at baseline did not correlate with disambiguation task accuracy. Following the exercise intervention, results show

that an increase in fitness percentile selectively predicted greater volume change in the left anterior DG/CA3. Behaviorally, individuals who were lower fit at baseline showed a positive correlation between increased changes in fitness percentile and improved corrected accuracy for trials requiring the highest level of disambiguation. Extending our previous work (Whiteman et al. 2016), this suggests that improving fitness may lead to increased volume within the hippocampus in young adults, and lower-fit individuals may receive the most cognitive benefit.

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Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

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Title: Simulated memory impairment: Neural correlates of memory decisions made in the face of conflict

Authors: *E. J. MAHONEY¹, D. E. HANNULA²

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Abstract: Questions are addressed in the current investigation about the neural correlates of memory decisions that are made in the face of conflict, which requires increased cognitive control. Participants in this experiment studied several scene-face pairs and were tested with three-face displays preceded by studied scene cues. The associate of the scene cue was either present in the three-face display (target-present trials) or not (target-absent trials). On every trial, participants were to indicate whether the trial was target-present or -absent by making a button press. Half of the participants (controls; n=20) were instructed to perform optimally and the remainders were told to simulate memory impairment (simulators; n=20). fMRI and eye tracking data were acquired during encoding and test, and a final post-test, on which all of the participants were instructed to perform their best, was completed outside of the scanner. Consistent with instructions, simulators performed at chance levels when they were asked to make present/absent decisions during the test phase; controls outperformed simulators, as expected. In contrast to these between-groups differences in explicit recognition decisions, early eye movement behavior was comparable - participants from both groups spent a disproportionate amount of time looking at associates (target-present trials) within 500-750ms of 3-face display onset. Results from preliminary fMRI analyses show that activity differences in the hippocampus during presentation of the scene cue predicted early high viewing of the associate, even when explicit recognition decisions were incorrect, among simulators. Activity differences associated with explicit recognition performance during presentation of the 3-face display distinguished the two groups. While hippocampal activity was greater for correct than incorrect present/absent decisions among controls, the opposite pattern was evident in simulator data. This pattern - activity differences greater for incorrect than for correct responses - was also evident in anterior cingulate cortex for simulators, an outcome that likely reflects conflict between memory retrieval and decision making. Together, these results indicate that participants can successfully conceal memory in explicit recognition responses, but that eye movements and hippocampal recruitment are sensitive to retrieval of learned associations none-the-less. Furthermore, during the decision making process, the act of simulating memory impairment results in recruitment of brain regions implicated in cognitive control.

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Poster

338. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

Support: ERC-2015-CoG 682591

Title: The role of spatial context representations in medial temporal lobe regions in fear generalization: A virtual reality approach

Authors: *L. D. DE VOOGD¹, *L. D. DE VOOGD¹, Y. P. J. MURRAY¹, R. M. BARTE¹, A. VAN DER HEIDE¹, G. FERNÁNDEZ¹, C. F. DOELLER^{1,2}, E. J. HERMANS¹ ¹Donders Inst. For Brain, Cognition and Behaviour, Nijmegen, Netherlands; ²Kavli Inst. for Systems Neuroscience, NTNU, Trondheim, Norway

Abstract: Correctly using contextual information to predict aversive events from environmental cues is a critical ability that protects from overgeneralization of fear responses. Animal models demonstrated the importance of spatial context representations within the medial temporal lobe (MTL) in contextualization of fear learning. In humans, this relationship has not been investigated. We hypothesized fear generalization is a result of a weak context representation. The present study was designed to test this hypothesis in humans using functional MRI and a virtual environment. Twenty-four healthy participants underwent a context training paradigm within the virtual environment. This environment involved a rich landscape including two buildings the participants were able to enter. The buildings had distinct features from the outside, but were identical on the inside. Within this environment, participants underwent a differential

delay contextual fear conditioning paradigm with spatial context as occasion setter. Two different cues (CS+ and CS-) were presented in both buildings in the same way, but the CS+ was consistently (reinforcement rate of 100%) paired with a mild electric shock in the threat context, but never in the safe context. The CS- was never paired with a shock. To confirm the effectiveness of our fear conditioning paradigm, we analyzed skin conductance responses (SCR) in response to the CS+ and CS- in both contexts. We found stronger SCR amplitudes for the CS+ compared to CS-. As expected this differential response was stronger within the threat compared to the safe context, but also present within the safe context. Similarly, we observed robust differential BOLD responses in regions commonly (de)activated in fear conditioning paradigms. These were stronger in the threat compared to the safe context, but also still present in the safe context. These results indicate that differential fear generalized across contexts. Further analyses should reveal whether interindividual differences in fear generalization can be explained from poor spatial context representations. These representations will be estimated using a linear SVM classifier that we will train on MTL patterns obtained during context training and test during fear conditioning. Higher classification accuracies can be interpreted as a stronger context representation. In conclusion, we successfully developed a paradigm that allows us to investigate spatial context-dependency of fear learning by combining neuroimaging with virtual reality. This is relevant because deficits in suppressing fear responses in safe contexts is a hallmark of fearrelated disorders.

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Poster

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Topic: H.02. Human Cognition and Behavior

Title: Reward modulates memory encoding for objects and scenes in the medial temporal lobe

Authors: *H. SCHULTZ^{1,2}, J. YOO², D. MESHI^{2,3}, H. R. HEEKEREN² ¹Sch. of Psychology, Univ. of Birmingham, Birmingham, United Kingdom; ²Biol. Psychology and Cognitive Neurosci. Unit, Freie Univ. Berlin, Berlin, Germany; ³Dept. of Advertising and Publ. Relations, Michigan State Univ., East Lansing, MI

Abstract: The Medial Temporal Lobe (MTL), which is comprised of the hippocampus (HC) and MTL cortex, is critical for the processing of episodic memory. Human fMRI studies support a framework in which anterior and posterior MTL cortex act as parallel pathways processing object-related and spatial memory components, respectively, which are then integrated by the HC. A complementary line of research has established a functional loop between the HC and the

dopaminergic midbrain as crucial for the formation of new memories, and human fMRI studies show that these regions interact during reward-related memory encoding. However, it is unknown whether reward differentially modulates the encoding of distinct memory components (e.g., object-related vs. spatial) in anterior vs. posterior MTL cortex.

To address this, healthy adult participants (n=22, 8 male, mean age 25, range 18-32) solved a novel encoding task crossing category and reward while undergoing fMRI (Siemens 3T, 2x2x2 mm EPI sequence, TR 1.8s, multiband factor 2). In each trial, they deduced the value (high vs. low) of a stimulus from a combination of stimulus category (object vs. scene) and frame color (blue vs. yellow) (counterbalanced over blocks). Correct identification of the value resulted in immediate high vs. low reward feedback for that stimulus. The next day, participants returned for a surprise recognition test. FMRI encoding data were analyzed using a 2x2x2 model with the factors category (object vs. scene), reward (high vs. low), and subsequent memory (remembered vs. forgotten). We expected differential effects of high reward on encoding activity in anterior vs. posterior MTL cortex for objects vs. scenes, respectively.

Behaviorally, reward enhanced memory (F=17.4, p<.001), which was more pronounced for objects than scenes (F=13.6, p=.001). On the neural level, reward modulated memory encoding in a category-independent manner in the anterior MTL cortex (-26 -5 -33, t=3.86), posterior MTL cortex (29 -26 -26, t=3.27) and HC (-25 -30 -9, t=3.44). Importantly, category-specific subsequent memory effects complemented the behavioral asymmetry: successful scene encoding in the posterior MTL cortex (-31 -38 -11, t=5.00) and HC (-34 -25 -11, t=4.55) was not modulated by reward, while successful object encoding was enhanced by reward in the anterior MTL cortex (15 0 -42, t=3.15) (all p<.001 unc.).

To our knowledge, this is the first study investigating the differential effects of reward on category-specific MTL memory encoding. Our data suggest that, on both the behavioral and neural level, reward enhancement of memory is not independent of the stimulus category.

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Poster

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Canada Research Chair (MDB)

Title: Anterolateral entorhinal cortex volume affects both intra-item and inter-item configural processing

Authors: *L.-K. YEUNG¹, R. K. OLSEN², H. E. P. BILD-ENKIN¹, B. HONG¹, V. MIHAJLOVIC¹, M. C. D'ANGELO², A. KACOLLJA², D. A. MCQUIGGAN², A. KESHABYAN¹, J. D. RYAN², M. D. BARENSE¹ ¹Psychology, Univ. of Toronto, Toronto, ON, Canada; ²Rotman Res. Inst., North York, ON, Canada

Abstract: In humans, the anterolateral entorhinal cortex (alERC) is of particular importance, as it overlaps with regions where Alzheimer's disease pathology first appears (Braak & Braak, 1991; Khan et al, 2014). Further, its volume is reduced in participants who perform poorly on neuropsychological tests selective for AD (Olsen, Yeung, et al, in press). However, few human studies to date have investigated its cognitive role. Based on experimental findings in rodents and its anatomical connections, we hypothesized that it may play a role in configural processing for items. To test this hypothesis, we took high resolution (0.43x0.43mm in-plane) structural scans of the medial temporal lobes of a group of 40 older adults. We performed manual segmentation following the Olsen-Amaral-Palombo protocol (Olsen et al, 2013), supplemented by the protocol of Maass et al (2015) - which subdivides the entorhinal cortex based on functional connectivity with other MTL regions - to obtain structural volumes for the alERC and neighboring MTL regions. We then compared these structural measures to behavioral performance on two eyetracking-based incidental viewing paradigms designed to test intra-item, and inter-item configural processing. In the first study, participants viewed conjunctive objects with distinct top and bottom halves. We showed that among all the MTL regions we assessed, only alERC volume was associated with the proportion of fixations directed to the configurallyimportant central region, which served as a measure of intra-item configural processing. In the second study, participants studied scenes associated with one particular critical object, and were subsequently shown either repeated scenes, manipulated scenes (where the critical object has been moved), and novel scenes. We showed that alERC and parahippocampal cortex volumes were selectively related to the proportion of fixations directed to the critical object previously associated with a particular scene in the manipulated condition only, a behavioral measure of inter-item configural processing. Together, these two studies suggest that the alERC may play a cognitive role that includes configural processing for the spatial relations between the parts of a single object, as well as its spatial relations relative to other objects.

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338. Human Long-Term Memory: Medial Temporal Lobe

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Topic: H.02. Human Cognition and Behavior

Title: Changes in item representations following category learning

Authors: *S. ASHBY, C. R. BOWMAN, D. ZEITHAMOVA Univ. of Oregon, Eugene, OR

Abstract: Learning about category membership can alter representations of individual items resulting in increased perceived similarity of items within a category and decreased perceived similarity of items from different categories. The current study aimed to investigate changes in subjective similarity ratings and neural pattern representations before and after category learning. Stimuli were faces constructed as 50/50 blends of never-seen "parent" faces. Across two experiments, two or three parent faces were selected to determine category membership, presented as a family surname. These family surname-relevant faces were blended with other parent faces, to generate face stimuli for presentation. Pairs of faces could share a parent face relevant for family membership, a parent face irrelevant for family membership, or not share a parent face. Participants first passively viewed the stimuli during functional MRI and then rated the similarity of pairs of faces prior to category learning. The same passive viewing and subjective similarity ratings were repeated after participants were trained to categorize the face stimuli into families (categories). Prior to category learning, subjective similarity ratings were the same for pairs of faces from the same category as for pairs of faces from different categories that had a common parent. After category learning, similarity ratings for items within a category increased, while rated similarity of faces that shared a common parent but belonged to different categories decreased. Similarity ratings for faces that had no shared features also decreased. Similar changes in perceived similarity within vs. across categories was observed even for a separate group of participants that were trained to remember item-specific information (faceunique full name) where category information (a common surname) was present but not emphasized. Preliminary fMRI data suggest the behavioral effects may be mediated by the formation of category representations in the ventromedial prefrontal cortex, where distributed neural patterns differentiated faces belonging to different families after learning but not before. These findings elucidate the neural mechanisms underlying the changes in item representations resulting from category learning.

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Title: Disinhibiting inhibitory engrams elicits memory interference

Authors: *H. BARRON¹, R. KOOLSCHIJN¹, A. PANTELIDES², U. EMIR¹, T. BEHRENS¹ ¹Univ. of Oxford, Oxford, United Kingdom; ²Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Individual memories are thought to be stored by ensembles of neurons that are reactivated during recall. However, it remains unclear how a given ensemble of neurons can contribute to more than one memory without causing confusion at the time of recall. Here we show evidence to suggest that inhibition plays a critical role in protecting overlapping memories from interference. Using non-invasive methods in humans, we first train subjects to acquire two memories that are context specific but otherwise overlapping. We then assess interference between these two memories at both a behavioural and neural level. By combining ultra-high field MR spectroscopy, fMRI and transcranial direct current stimulation (tDCS), we then show that confusion between overlapping memories can be induced when neocortical GABA is reduced using brain stimulation. These results are consistent with evidence to suggest that neocortical excitation and inhibition are tightly calibrated except during sensory exposure or memory recall. Furthermore, the data suggest that deficits in inhibitory gating may underlie hallucinatory experiences observed in clinical conditions such as schizophrenia where the precise balance between excitation and inhibition is poorly maintained.

Disclosures: H. Barron: None. **R. Koolschijn:** None. **A. Pantelides:** None. **U. Emir:** None. **T. Behrens:** None.

Poster

339. Computational Approaches to Understanding Interactions Between Short- and Long-Term Memory

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NSF CAREER Award 1056019

Title: Opportunity to link related memories during encoding reveals adolescent-specific neural strategy

Authors: *M. L. SCHLICHTING¹, K. F. GUARINO², H. E. ROOME³, A. R. PRESTON³ ¹Psychology, Univ. of Toronto, Toronto, ON, Canada; ²Loyola Univ. Chicago, Chicago, IL; ³The Univ. of Texas at Austin, Austin, TX

Abstract: Previous work shows that hippocampus (HPC) and prefrontal cortex (PFC) support the formation of relational memory networks. Such networks can be created during encoding itself, when related memories are linked to, or integrated with, current experience. It is known that adults bring related memories to mind during encoding to form integrated memories, and that doing so results in increased behavioral flexibility. Yet, how existing knowledge impacts encoding over development remains virtually unstudied. Both HPC and PFC undergo continued structural change well beyond childhood, with PFC maturation in particular continuing into adulthood. Children also show less memory flexibility than adults, suggesting that they may not retrieve and link related knowledge during learning. Whether adolescents show similarly rigid memory signatures remains to be seen. Here, human participants aged 7-30 years encoded AB and XY object-object associations across three study repetitions during high-resolution functional MRI scanning. Participants also studied related BC pairs, which included an object (B) that was part of an AB pair, as well as a new C item from one of two visual categories (face or scene). We used a pattern classifier to measure reactivation of related (C) memories across the three AB study repetitions. Adults showed sustained reactivation of related content through the third repetition, while children show no reactivation at all. Interestingly, both younger and older adolescents showed an intermediate pattern, with initial reactivation followed by decreases as learning continued. The relationship between reactivation and behavior—specifically, the ability to link indirectly related A and C items-also changed with age, with sustained reactivation slowing performance early and speeding performance later in development. These results suggest important developmental differences in the tendency to bring to mind related knowledge during new events. In particular, the intermediate signature observed in adolescents might suggest that when HPC-driven reactivation is mature but PFC control is not, suppressing an initially reactivated memory is beneficial. This adaptive suppression may protect adolescents from interference among related memories. In a broader sense, our results highlight the importance of considering that differences in neural mechanism may still be present during adolescence, even when adult-like memory behavior has been reached. These data also open the door to future investigations that use memory models to quantify developmental shifts in the neural strategy deployed during specific encoding events.

Disclosures: M.L. Schlichting: None. **K.F. Guarino:** None. **H.E. Roome:** None. **A.R. Preston:** None.

Poster

339. Computational Approaches to Understanding Interactions Between Short- and Long-Term Memory

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Topic: H.02. Human Cognition and Behavior

Support: DFG Research Fellowship

Title: Reinforcement learning over time: Spaced versus massed training leads to longer-lasting value associations

Authors: *G. WIMMER¹, R. A. POLDRACK²

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Abstract: Over the past few decades, neuroscience research has illuminated the neural mechanisms supporting learning from reward feedback, demonstrating a critical role for the striatum and midbrain dopamine system. Simple feedback learning paradigms are increasingly being extended to understand learning dysfunctions in mood and psychiatric disorders as well as addiction ("computational psychiatry"). However, one potentially critical characteristic that this research ignores is the effect of time on learning: human feedback learning paradigms are conducted in a single rapidly paced session, while learning experiences in the everyday environment and in animal research are almost always separated by longer periods of time. Recent work has shown that rapidly paced learning sessions rely predominantly on short-term working memory. Importantly, spacing is also known to have strong positive effects on item memory across species and in reward learning in animals, suggesting a quantitative or qualitative shift in the underlying learning mechanisms. Remarkably, the effect of spaced training on human reinforcement learning has not been investigated. In our experiments, we examined reward learning distributed across weeks vs. learning completed in a traditionally-paced single session. Participants learned to make the best response for landscape stimuli that were either associated with a positive or negative expected value. In our first study, as expected, we found that after equal amounts of extensive training, performance was near ceiling and equivalent between the spaced and massed conditions. Critically, in a final online test 3 weeks later, we found greater value association discrimination for spaced-trained stimuli. Our fMRI results suggest that in contrast to spaced value associations, massed associations elicited greater PFC engagement and differential putamen activity. In our second study, we found a significant positive relationship between early learning performance and working memory, supporting a role for short-term

memory in single-session tasks. Further, spaced training again showed a benefit: the rating difference for reward and non-reward stimuli in spaced-trained stimuli was maintained at a level of over 89%, versus only 31% for massed-trained stimuli. Our results indicate that single-session tasks may not lead to the kind of robust value associations that are characteristic of "habitual" value associations. Overall, these studies begin to address a large gap in our knowledge of fundamental processes of reinforcement learning, with potentially broad implications for our understanding of learning in mood disorders and addiction.

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Poster

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Title: Memory load modulates the dynamics of visual working memory

Authors: *M. F. PANICHELLO¹, B. D. DEPASQUALE¹, J. W. PILLOW^{1,2}, T. J. BUSCHMAN^{1,2} ¹Princeton Neurosci. Inst., Princeton, NJ; ²Dept. of Psychology, Princeton Univ., Princeton, NJ

Abstract: Working memory is a dynamic process - it unfolds over periods of seconds to minutes and memories become less accurate with time. The nature of these dynamics places significant constraints on the nature of working memory representations and the neural mechanisms supporting working memory. Here, we combine psychophysical methods and computational modeling to precisely describe the dynamic forces governing working memory. We show that the accumulation of error in working memory over time is not random but reflects underlying

attractor dynamics. Furthermore, these dynamics are modulated by memory load. To identify these attractor dynamics, we trained both monkeys and humans to perform a continuous-report working memory task. In brief, subjects were asked to remember 1 to 3 colored squares. After a short memory delay, subjects had to report the color of a cued stimulus on a continuous color wheel. Dynamic models fit to the behavioral responses of both monkeys and humans showed random diffusion dominated the dynamics at low memory load but strong attractors dominated dynamics at high memory load. In other words, memory dynamics are load-dependent: attractor depth increased with the number of items in working memory. These results provide a mechanistic explanation of the severely limited capacity of working memory - the observation that as the number of items to be remembered (memory load) is increased, the accuracy of memory reports is reduced (Bays, Catalao, & Husain, 2009; Luck & Vogel, 1997; van den Berg et al., 2012; Zhang & Luck, 2008). Furthermore, the finding of increasing attractor depth with memory load may provide a normative way of reducing interference among memory items when memory load is high.

Disclosures: M.F. Panichello: None. **B.D. DePasquale:** None. **J.W. Pillow:** None. **T.J. Buschman:** None.

Poster

339. Computational Approaches to Understanding Interactions Between Short- and Long-Term Memory

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Title: Medial prefrontal cortex compresses concept representations through learning

Authors: *M. L. MACK¹, A. R. PRESTON², B. C. LOVE³ ¹Univ. of Toronto, Toronto, ON, Canada; ²The Univ. of Texas At Austin, Austin, TX; ³Univ. Col. London, London, United Kingdom

Abstract: Prefrontal cortex (PFC) is sensitive to the complexity of incoming information and theoretical perspectives suggest that a core function of PFC is to build efficient codes that highlight goal-relevant features by filtering out irrelevant content. At the heart of these accounts is the hypothesis that during learning, medial PFC (mPFC) performs data reduction on incoming information, compressing less critical features to emphasize encoding of goal-relevant information structures. Although emerging evidence suggests that goal-specific representations occur in the rodent PFC, such coding in human PFC remains poorly understood. Here, we directly assess the data reduction hypothesis by leveraging an information-theoretic approach to measure how learning is supported by neural compression processes. Participants learned to classify the same insect images composed of three features across three different problems. These problems were defined by rules for which 1, 2, or 3 stimulus dimensions had to be considered to successfully classify. By varying the number of relevant dimensions, we manipulated the complexity of the problems' conceptual spaces. For example, whereas a 1dimensional rule that depends on the insects' leg width means the dimensions of antennae length and pincer shape are irrelevant and should be compressed during learning, a 3-dimensional rule requires that all stimulus dimensions are represented with no compression. Critically, this design allowed us to test the prediction that learning shapes the dimensionality of neural concept representations: Brain regions that learn to compress will represent simpler problems with fewer dimensions. We recorded fMRI data while participants learned the three problems and measured the degree that multivoxel activation patterns were compressed through learning using principal component analysis. Throughout the entire brain, only mPFC showed the predicted relationship between compression and conceptual complexity: Greater neural compression was found for problems with lower dimensional concepts, and importantly, this effect emerged over learning. Furthermore, by leveraging a computational learning model, we found that individual differences in neural compression predicted attentional strategies to the stimulus dimensions. Specifically, learners with greater problem-specific neural compression showed more optimal attention such that relevant dimensions were attended to and irrelevant dimensions ignored. These findings support the view that mPFC is critical for transforming information into low-dimensional representations useful for the task at hand.

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Poster

339. Computational Approaches to Understanding Interactions Between Short- and Long-Term Memory

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Topic: H.02. Human Cognition and Behavior

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R01-EY019882 R01-MH110378 P30-EY08126 T32-EY007135

Title: Neural bases of automaticity

Authors: M. SERVANT, G. D. LOGAN, *G. F. WOODMAN Dept Psychol, Vanderbilt Univ., Nashville, TN

Abstract: Automaticity allows us to perform tasks in a fast, efficient, and effortless manner after sufficient practice. Theories of automaticity propose that across practice processing transitions from being controlled by working memory to being controlled by long-term memory retrieval. Recent event-related potential (ERP) studies have sought to test this prediction, however, these experiments did not use the canonical paradigms used to study automaticity. Specifically, automaticity is typically studied using practice regimes with consistent mapping between targets and distractors and spaced practice with individual targets, features that these previous studies lacked. The aim of the present work was to examine whether the practice-induced shift from working memory to long-term memory inferred from subjects' ERPs is observed under the conditions in which automaticity is traditionally studied. We found that to be the case in 3 experiments, firmly supporting the predictions of theories. In addition, we found that the temporal distribution of practice (massed versus spaced) modulates the shape of learning curves. The ERP data revealed that the switch to long-term memory is slower for spaced than massed practice, suggesting that memory systems are used in a strategic manner. This finding provides new constraints for theories of learning and automaticity.

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Poster

339. Computational Approaches to Understanding Interactions Between Short- and Long-Term Memory

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Title: Dependence of facial attractiveness perception on categorization induced informational constraints: A Bayesian Statistical account

Authors: *C. K. RYALI¹, A. J. YU²

¹Computer Sci. and Engin., UC San Diego, LA Jolla, CA; ²Cognitive Sci., UC San Diego, La Jolla, CA

Abstract: Though interactions between high-level perception and memory are commonplace in real life, they are typically studied separately in neuroscience. However, recent experimental findings have indicated that processes dependent on both long-term memory and short-term memory significantly alter high-level perception in complex manners. Specifically, the classical perceptual phenomenon of Beauty in Averageness (BiA), in which a face morphed from two original faces is typically judged by humans to be more attractive than the originals, significantly diminishes when the original faces are recognizable (based on long-term memory), or when the attractiveness judgment is preceded by a judgment of the similarity of the morph to the two original faces (based on working memory). A previous qualitative account of these results hypothesized that a negative affect associated with the difficulty of "disfluency" of categorizing morphed faces (either explicitly by being asked to make similarity judgments, or implicitly by automatically matching morphed faces to well-known faces) is misattributed by subjects to be associated with the attractiveness perception, thus resulting in a diminution of the BiA. However, this disfluency account cannot explain why performing the similarity judgment leads to a subsequent increase in the perceived attractiveness of the original faces, nor the original BiA effect. Here, we propose a statistical theory of how humans use a latent low-dimensional representation of intrinsically high-dimensional data (e.g. faces) to efficiently perform high-level perceptual judgments (attractiveness rating), utilize working memory to compare such items (similarity judgment) and encode and retrieve such items in long-term memory (celebrity recognition). Essentially, the model assumes that the extent to which a face typifies or represents the faces stored in memory contributes positively to the perceived attractiveness of the face and that a concurrent perceptual categorization task selectively enhances the importance of certain featural dimensions in the typicality judgment, thus differentially modulating the perceived attractiveness of different faces. This model explains both the original BiA effect, as well as its modulation by short-term and long-term memory-dependent categorization processes. It also gives hints as to the nature of the neural representation of complex sensory data, as well as its transformation under top-down modulation of concurrent cognitive processes.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: John Templeton Foundation

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Title: Drift diffusion modeling of interactions between episodic and working memory

Authors: *A. NOVICK, A. M. BORNSTEIN, K. A. NORMAN, J. D. COHEN Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: A primary question in memory research is how different forms of memory interact. Previous work has shown episodic memory (EM) supports short-term memory tasks when working memory (WM) is overloaded or maintenance is interrupted (e.g., Brown, 1958; Peterson & Peterson, 1959). However, EM reactivation can occur automatically, regardless of its relationship to immediate behavioral goals (Gupta et al., 2010). We hypothesized that these ongoing EM reinstatements could affect WM maintenance, even in the absence of interference. We used a distraction-free delayed non-match to sample (DNMS) task. While accuracy in such tasks is typically at ceiling, we reasoned that response times might reveal the influence of EM. Four target words were followed by an 18 second delay, after which participants were asked whether a probe word matched the targets. Word stimuli used in the task were pre-trained in sets, each associated with a different context (face or scene picture). Temporal context models of memory predict that triggering one memory from a given context, or the context itself, will lead to other recalls from the same context (Howard & Kahana, 2002). We predicted that reinstating the context associated with the probe word during the delay period would introduce the probe word into WM, even if it was not one of the target words. We predicted that this errant presence of the probe in WM would slow response times when participants were asked whether the probe word was one of the targets (i.e., whether it matched the contents of WM) when it was not. To measure the influence of context reinstatement on performance in a specific trial, we used multivariate pattern analysis of fMRI BOLD signal in ventral temporal cortex to generate a trialby-trial measure of which specific contexts were reactivated during the delay period. We modeled WM search at response as a thresholded evidence accumulation process using drift diffusion models (Ratcliff, 1978). Using this approach, we fit a model in which drift rate (reflecting the consistency of information in WM) varied with neural evidence for context reinstatement, compared it to several alternative, behavior-only models, and found that the neural model provided a superior fit (p=0.01).

These results reveal a previously hidden effect of EM on WM, opening new questions about both WM representations and the adaptive benefits of EM replay.

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339. Computational Approaches to Understanding Interactions Between Short- and Long-Term Memory

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Topic: H.02. Human Cognition and Behavior

Support: NSF EPSCoR Award Number 1632738

Title: Efficient learning: Manipulating context to enhance (or diminish) memory

Authors: *J. R. MANNING¹, K. ZIMAN², A. C. HEUSSER³

¹Dept. of Brain and Psychological Sci., ²Psychological and Brain Sci., ³Psychology, Dartmouth Col., Hanover, NH

Abstract: Our memory systems leverage the statistical structure of our experience (context) to organize and store incoming information and retrieve previously stored information. Here we explore the extent to which (and the circumstances under which) these processes may be manipulated, and how they are similar and different across individuals. We developed a series of word list learning paradigms that enabled us to systematically manipulate the context in which each word is learned. Each word was imbued with a set of features (word category, size of the object the word referred to, location on the screen, word length, starting letter, font color, etc.). In Experiment 1, we had participants study a series of 16 lists. The words on the first 8 lists were presented in an order sorted along one feature dimension (e.g. words presented alphabetically), and the words on the second 8 lists were always studied in a random order. We could then ask: how does imposing this structure on early lists affect memory performance for those early lists, and to what extent do those effects linger on later lists where that structure is no longer maintained? In Experiment 2, we had our participants study a series of randomly ordered lists to estimate each person's tendency to organize their recalls along each feature dimension. For example, even though all of the words on those lists were presented in a randomized order, some participants were more likely to group their recalls by the word categories, others were more likely to recall the words in alphabetical order, etc. Given the participants' tendencies, we adapted the presentation orders of subsequent lists. We found that when participants studied words in an order that matched their recall tendencies on previous lists, they remembered more words overall. We also present preliminary data showing that people's memory tendencies may be decoded from electrophysiological data recorded as they study each word, suggesting that neuroimaging data might be incorporated into adaptive learning experiments to further enhance memory.

Disclosures: J.R. Manning: None. K. Ziman: None. A.C. Heusser: None.

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant F32AG054204

Title: Prototype model correlates in the VMPFC during concept generalization

Authors: *C. R. BOWMAN, D. ZEITHAMOVA

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Abstract: Healthy memory function involves both the ability to remember specific details of prior events and the ability to integrate common event features to form generalized concepts. The ventromedial prefrontal cortex (VMPFC) has been shown to support the latter integrative memory function in episodic memory tasks. However, formation of integrated representations in these tasks is dependent on specific memory for individual components to be integrated. Whether this dependence is always the case has been of considerable debate. Some computational models of concept learning have proposed that abstract information is extracted from individual instances and need not be maintained as a distinct representation (exemplar models). Other models propose that representations of central tendency form separately or in the absence of exemplar representations (prototype models). We used a category-learning task to simultaneously measure abstract and event-specific representations that may dissociate. Participants were trained to sort exemplars into two categories, then underwent fMRI during a generalization test that probed for category knowledge applied to novel exemplars. Exemplar and prototype models were fit to behavioral data in individual subjects and then used as regressors in neuroimaging data to identify the neural correlates of each. Results showed predominant prototype use in behavior accompanied by robust prototype correlates in the VMPFC. Further, the strength of prototype representations in behavior correlated with the strength of category representations detected in VMPFC. Despite weaker exemplar model fits to behavior, exemplar correlates were found in visual and lateral prefrontal regions. Thus, results suggest that neural mechanisms supporting memory integration do so across memory domains and that multiple memory representations may be detected even when one dominates behavior.

Disclosures: C.R. Bowman: None. D. Zeithamova: None.

339. Computational Approaches to Understanding Interactions Between Short- and Long-Term Memory

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Support: UT Systems Neuroscience UT BRAIN seed grant

Title: Adaptive cognitive flexibility improves both prospective and long-term remembering

Authors: *S. KOSLOV¹, J. A. LEWIS-PEACOCK^{1,2} ¹Psychology, Univ. of Texas, Austin, TX; ²Inst. for Neurosci., Austin, TX

Abstract: Prospective memory describes the ability to remember to perform goal-relevant actions at an appropriate time in the future. The multiprocess view of prospective memory posits that two separable control mechanisms underlie this ability: proactive control, which involves maintenance of goal information in working memory and monitoring of the environment for relevant cues; and reactive control, involving the formation of cue-response associations which are later automatically retrieved from episodic memory. Individuals will often engage proactive and reactive control strategies appropriately when the demands of the task environment are stable. For instance, individuals tend to rely on reactive control in situations with a high cognitive load, whereas proactive control is favored in situations with fewer cognitive demands (Lewis-Peacock, Cohen, & Norman, 2016). When task demands vary, sub-optimal strategy use can lead to performance costs and memory failures. Being able to adaptively implement control strategies in response to changing task demands is important, but little is known about if and how people accomplish strategy flexibility. Here, we sought to characterize the flexibility of control strategy use for prospective remembering in situations with rapidly varying task demands. Participants were asked to identify the reappearance of picture targets (a small set of faces and scenes, one per trial) while at the same time performing an orthogonal visual search task that fluctuated in difficulty. After the experiment, participants were given a surprise memory test for the target items. Behavioral results indicate that the degree to which subjects adapted their control strategy on a moment to moment basis (e.g., switching from a proactive strategy to a reactive strategy for the target when the search task became more difficult) predicted better delayed execution of goals and better long-term retention of those goals. Conversely, sticking with a non-adaptive strategy hurt both performance metrics. Preliminary neural results, using multivariate pattern analyses of fMRI data to track working memory use during each trial, corroborated the behavioral findings; subjects allocated cognitive resources in response to changing task demands in a fluid and flexible manner. Moreover, neural decoding of working memory content also demonstrated that the degree to which individuals adaptively adjusted

strategies was linked to improvements in memory performance. These results highlight an important link between cognitive control and memory, and motivate the use of adaptive cognitive flexibility as a target for cognitive training paradigms.

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Poster

339. Computational Approaches to Understanding Interactions Between Short- and Long-Term Memory

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Topic: H.02. Human Cognition and Behavior

Support: The Templeton Foundation

Title: Rational use of long-term and working memory in goal-directed behavior: A normative account of prospective memory

Authors: *I. MOMENNEJAD¹, K. NORMAN², J. D. COHEN², S. SINGH³, R. L. LEWIS⁴ ¹Princeton Neurosci. Inst., ²Princeton Univ., Princeton, NJ; ³Univ. of Michigan, Michigan, MI; ⁴Psychology and linguistics, Univ. of Michigan, An Arbor, MI

Abstract: The execution of planned action demands adaptive interaction between noisy streams of memory and perception. We propose a normative approach to modelling and understanding the strategic use of long-term memory (LTM) and working memory (WM) in planned dual tasks. We formulate such tasks as problems of computational rationality, asking how a rational agent would integrate information across noisy perceptual and memory streams to maximize goaldirected task performance. For instance, successful realization of non-immediate plans typically requires the organism to hold on to a future plan while accomplishing other intermediate activities, a capacity known as prospective memory. Thus we have selected a number of classic human experimental findings on prospective memory (PM), where the agent performs an ongoing task while monitoring the environment for rare goal states (target stimuli) upon which the agent performs a planned prospective task. We present a model of a dual-task setting involving a prospective memory task and an ongoing working memory task and assess model predictions against human performance reported in the prospective memory studies. To solve the WM-EM strategic allocation problem in these tasks, we adopt a Bayesian optimal control formulation in which both probabilistic task structure and bounds on the agent's memory and perception determine optimal behavior. We address the computational challenges of finding optimal behavioral policies using reinforcement learning algorithms, and explore the implications of varying the constraints on memory, perception, and policy space. The normative model offers a principled framework for understanding how LTM and WM should be used to

realize planned action. However, the nature of optimal behavior may vary across populations with different WM capacities, neural impairment, or memory disorders. Our approach can be extended to study model parameters that govern the bounded rationality of memory use in planned action in neuroimaging data, and across different populations.

Disclosures: I. Momennejad: None. **K. Norman:** None. **J.D. Cohen:** None. **S. Singh:** None. **R.L. Lewis:** None.

Poster

339. Computational Approaches to Understanding Interactions Between Short- and Long-Term Memory

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Topic: H.02. Human Cognition and Behavior

Title: The precision of context-based prediction biases memory pruning

Authors: ***H. KIM**¹, M. L. SCHLICHTING², A. R. PRESTON^{3,4}, J. A. LEWIS-PEACOCK^{3,4} ¹Dept. of Psychology, Univ. of Texas At Austin, Austin, TX; ²Dept. of Psychology, Univ. of Toronto, Toronto, ON, Canada; ³Dept. of Psychology, ⁴Ctr. for Learning and Memory, Univ. of Texas at Austin, Austin, TX

Abstract: When the statistics of our experiences change, our memory is updated by weakening ("pruning") unreliable memories that generate automatic, but incorrect, predictions (Kim et al., 2014). Here, our goal was to determine how the precision of context-based memory predictions (i.e., item vs. category) impacts memory pruning. We hypothesized that contexts that tend to generate specific predictions (e.g., "that striped tiger") would produce prediction errors that lead to pruning of those memories when an unexpected item appeared instead (e.g., a breakfast taco). However, contexts that tend to generate broader predictions (e.g., "some kind of animal") would not lead to pruning of the previous memory if the unexpected item satisfied the broader prediction. We tested this hypothesis by asking observers to make subcategory judgments (e.g., female vs. male) on a sequence of items for which we manipulated the transition probabilities. During this incidental learning task, certain items ("cues") appeared four times followed each time by a new item. Cues were either followed always by an item from a different category (e.g., Beyonce-tiger, Beyonce-taco, etc.), or followed by items from a single category (e.g., the cue is followed by "animals" such as Adele-deer, Adele-owl, etc.). We reasoned that when the reappearance of a cue reliably predicted the category of the following item, the precision of context-based predictions generated from this cue would broaden from item to category, and thus item-specific prediction errors would decrease. Consistent with our hypothesis, we found that cues which generated item-specific predictions led to pruning of the predicted items more often than when a cue reliably predicted the category of the upcoming item. fMRI results indicated that only for cues in the different-category condition that were followed by new items from new categories did stronger predictions of previous items lead to more forgetting of those items. This suggests that pruning occurs based on the prediction errors between specific items. These results also suggest that when memory predictions are broadened from item to category, as in the same-category condition, prediction error decreases and memory of the previous items are more likely to be preserved. However, once the predictions were broadened, this also reduced the encoding specificity and later recognition of the subsequent items, highlighting a tradeoff between preserving old memories and building new ones.

Disclosures: H. Kim: None. M.L. Schlichting: None. A.R. Preston: None. J.A. Lewis-Peacock: None.

Poster

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Title: Contributions of working memory and across-trial probability learning to context-based decisions

Authors: *O. LOSITSKY¹, M. SHVARTSMAN¹, J. D. COHEN¹, R. C. WILSON² ¹Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; ²Univ. of Arizona, Tucson, AZ

Abstract: Context (such as our location or current goal) can inform our decisions by predicting upcoming stimuli and relevant responses. However, we often face a trade-off between developing predictions that maximize reward for specific contexts vs. priors that generalize across contexts. We investigated this trade-off in a simple decision-making task (AX-CPT) in which a contextual cue (A or B) signals the correct response (left or right) to a probe (X or Y) that appears seconds later (AX or BY => right, BX or AY => left). To test when people form specific priors for each cue, subjects were given different probabilities of seeing X and Y depending on the cue. For A cues, all subjects saw X more frequently than Y (pressed the left button more). For B cues, some subjects saw more X's (pressed the right button more) while others saw more Y's (pressed the left button more). We hypothesized that subjects who pressed the left button more often for B would form a general expectation to press left, while subjects who pressed the right button more often for B would develop separate priors adapted to each cue.

Our hypothesis was partially correct. Using a drift diffusion model we had built, we measured people's priors (tendency to expect the left response) as a function of the cue. We found that response priors were averaged across A and B cues when they made sufficiently similar predictions, sacrificing reward rate in the service of simplicity. This averaging of response probabilities across cues was consistent with their explicit reports of the trial frequencies during debriefing. Explicit probability reports also revealed that, when A and B predicted the same probe but different responses, subjects did not form distinct priors for each cue as we had predicted: instead, they now averaged the probability of seeing X, instead of the probability of pressing left, across cues. In other words, subjects appeared to organize observations differently depending on which dimension was most predictive. To verify that this averaging reflected their internal representation of the task, we ran a follow-up study in which the probabilities of X / Y for B cues changed in the middle of the experiment. This study showed that subjects continued to group their observations according to the same dimension they learned earlier in the experiment, even when the environment no longer favored such groupings. Our work helps dissociate the contributions of across-trial learning from within-trial context memory to behavior on this task, which has been used to diagnose working memory deficits while leaving contributions from long-term memory unexplored.

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Poster

339. Computational Approaches to Understanding Interactions Between Short- and Long-Term Memory

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Title: A Bayesian approach to inferring latent connectivity patterns from spike trains reveals that working memory maintenance induces rapid synaptic plasticity

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Abstract: Recent theoretical models suggest that rapid, temporary changes in effective synaptic connectivity (i.e., synaptic facilitation) might form a powerful potential substrate for working memory (WM) maintenance. According to these models, incoming information to be maintained in WM results in a rapid reorganization of the connectivity pattern of the neural network. The resulting WM-specific connectivity pattern will cause any future input to be processed according to the previous stimulation history, thus enabling present behaviour to be flexibly guided based on past input. Such a WM substrate is energy-efficient, robust to interference, and elegantly bridges short- and long-term memory systems.

Despite theoretical appeal, however, directly testing these models remains difficult, as it requires the simultaneous recording of large numbers of neurons and their connectivity strengths. A robust estimate of synaptic connectivity is particularly hard to obtain in populations of sparsely firing PFC neurons. We now overcome these challenges by (1) pooling data from a large archive of experiments involving simultaneous spiking recordings from multiple neurons of non-human primate PFC; and (2) developing a novel method for the detection of latent synaptic connectivity states in such recordings.

Our method to extract connectivity patterns from spike train recordings is based on Bayesian inference for generalized linear modelling (GLM) of stochastic point processes. We demonstrate that our method is highly sensitive in recovering latent connectivity states, even in the presence of considerable noise and potentially confounding fluctuations in firing rate. Furthermore, the GLM aspect of our approach enables the straightforward characterization of dynamics in the latent states, over and above 'static' connectivity.

Applying this novel method to our database of neural recordings, we discovered a significant subset of neurons that modulate their synaptic connectivity pattern as a function of WM contents. We thus identify, for the first time, an important role for short-term synaptic facilitation in the maintenance of WM. Our findings thereby extend classical models of WM maintenance based solely on persistent changes in firing rate.

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Poster

339. Computational Approaches to Understanding Interactions Between Short- and Long-Term Memory

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Support: NIH Grant MH100121

Title: Memory reactivation modulates encoding and retrieval of relational memories

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Abstract: Memories are not formed in isolation, but rather are learned in the context of a vast store of existing knowledge. In order to use this store of information flexibly, the memory system is thought to code information about relationships between different events. Formation of relational memories may be facilitated by complementary processes of memory integration, where existing memories are updated with information about related events, and active separation, where competing memories are stored with distinct codes to limit interference during retrieval. Recent evidence suggests that a network of regions within medial prefrontal cortex (MPFC), ventrolateral PFC (VLPFC), and hippocampus supports these complementary processes. To examine the role of memory integration and active separation in encoding of related events, we first had participants learn initial face-object and scene-object (denoted AB) pairs. Then fMRI data were collected while participants learned overlapping object-object (BC) associations, where one object (B) overlapped with the initial set of pairs. Finally, participants performed an associative inference task where they were tested on associations between A and C items, which were related indirectly through their association with the same B item. Prior evidence suggests that retrieval of indirect associations is faster when memories have been integrated into a relational network. Using multivariate pattern analysis, we found evidence of reactivation of initial pair memories during learning of overlapping object-object associations; the degree of reactivation predicted individual differences in reaction time on the inference test, consistent with our prediction that memory reactivation would support successful memory integration, facilitating flexible use of memory. We compared models of potential links between reactivation and accuracy on the inference test, and found that intermediate levels of reactivation were associated with decreased performance. Taken together, our findings suggest that intermediate levels of memory reactivation may lead to active separation of related memories, while higher levels of reactivation support memory integration. We also found that MPFC, VLPFC, and hippocampus were more active during indirect test trials where there was greater memory reactivation during encoding of the corresponding object-object (BC) association. This network of regions may serve an important role in organizing memories of related events.

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Poster

340. The Human Language Singularity

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Title: Sensitivity to written word structure is associated with diffusivity in ventral white matter pathways

Authors: M. YABLONSKI¹, K. RASTLE², J. S. H. TAYLOR³, *M. BEN-SHACHAR¹ ¹Bar Ilan Univ., Ramat Gan, Israel; ²Royal Holloway, Univ. of London, London, United Kingdom; ³Aston Univ., Birmingham, United Kingdom

Abstract: Morphological processing, the ability to extract information about the internal structure of words, is an essential component of skilled reading. For example, recognizing the morpheme govern inside the word government facilitates access to its meaning. Functional MRI studies have identified several cortical regions involved in morphological processing, yet the white matter underpinnings of this skill remain largely unknown. We analyzed the relationship between microstructural properties of white matter pathways, identified using diffusion MRI (dMRI), and behavioral sensitivity to written word structure, in adult English readers (N=49). To this end, we used the morpheme interference paradigm (Crepaldi et al., 2010) that quantifies sensitivity to real morphemes within invented stimuli (e.g., *addment = add+ment; compare with *addmant = add + *mant). We hypothesized that morphological processing relies primarily on ventral reading pathways, as morphemes provide cues for mapping between print and meaning. Accordingly, we targeted the inferior fronto-occipital fasciculus (IFOF), inferior longitudinal fasciculus (ILF) and uncinate fasciculus (UF). We further analyzed two dorsal pathways: the long and anterior segments of the arcuate fasciculus. Participants completed dMRI scans at Royal Holloway (3T Siemens scanner, b=1000 s/mm², 64 diffusion directions, voxel size: 2x2x2mm³) and a behavioral battery that included the morpheme interference task and measures of phonological and orthographic processing. Tracts of interest were identified bilaterally, using deterministic tractography and automatic tract segmentation (Yeatman et al., 2012). Fractional anisotropy (FA) and mean diffusivity (MD) profiles were calculated along each pathway, and Spearman's correlations were calculated between these profiles and morphemic sensitivity. Significant negative correlations were found between morphemic sensitivity and FA in the IFOF, bilaterally. Additionally, significant positive correlations were found between morphemic sensitivity and MD in the left UF and left ILF. Post-hoc analyses revealed that these effects all stemmed from positive associations with radial diffusivity. The correlations remained significant after partialling out nonword repetition scores, suggesting some level of cognitive specificity. Morphological processing thus appears to rely on ventral pathways, primarily in the left hemisphere. The results support the contribution of morphological processing to recognition and comprehension of complex words.

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340. The Human Language Singularity

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grants NS064033

Title: Neural dynamics of verbal working memory in sentence comprehension

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Abstract: Sentence comprehension processes would be expected to require humans to exert verbal working memory operations in addition to semantic and syntactic processing. We attempted to dissect cortical activities attributable to verbal working memory from those involved in answering simple auditory questions. We studied 19 patients who underwent electrocorticography recording, and measured cortical high-gamma activity during both sentence comprehension and working memory tasks. The sentence comprehension task consisted of overt naming of an object (e.g.: 'Bird') relevant to a spoken question (e.g.: 'What flies in the sky?'). The working memory task, consisting of brief maintenance of a verbally provided set of two or four letters followed by deciding whether a subsequent target letter had been included, effectively allowed us to identify regions involved in verbal working memory maintenance and scanning processes. Superior-temporal high-gamma activity was augmented bilaterally during stimulus presentation commonly in both tasks. Left inferior precentral high-gamma activity began to be augmented in the middle of question stimuli and gradually built up afterwards in the sentence comprehension task, whereas activity was augmented throughout the maintenance period in the working memory task. Around the stimulus offset of the sentence comprehension task and during the scanning period of the working memory task, high-gamma activity was augmented commonly in widespread areas of the left temporal-parietal-frontal association neocortex. High-gamma augmentation specific to the sentence comprehension task was noted in the inferior-temporal gyrus, fusiform gyrus, occipital lobe as well as pars orbitalis of the inferiorfrontal gyrus of the left hemisphere. In summary, early left inferior-precentral high-gamma augmentation during the sentence comprehension task may be, in part, attributed to working memory maintenance function. Substantial proportions of subsequent high-gamma augmentation in the left temporal-parietal-frontal association neocortex may be attributed to working memory scanning function. High-gamma augmentation in the left ventral visual pathway following

question offset cannot be attributed to verbal working memory function and may reflect processing of mental images semantically relevant to the task. High-gamma augmentation in the pars orbitalis of the left inferior-frontal gyrus may reflect syntactic or semantic processing of sentence questions.

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Poster

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Title: Searching for the categorical structure of abstract concepts

Authors: *C. WALSH, S. J. GOTTS, A. MARTIN Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Object concepts fall naturally into easily identifiable and distinct categories such animals, tools, furniture, flowers and the like. In contrast, our large corpus of abstract concepts do not easily fall into distinct categories. In order to make progress on understanding the neural underpinnings of abstract concepts it seems that the first questions that must be addressed concern distinctions among abstract concepts. Do abstract concepts show a categorical structure? Do clear boundaries exist between these different categories? In this study we sought to address these questions using a novel behavioral sorting paradigm and a measure of automatic semantic priming.

We completed two experiments. In the first experiment, subjects (n=20) performed a computerized clustering task using 35 words that referred to abstract concepts. All words were rated very low on concreteness and imageability based on standard published norms (Coltheart, 1981; Brysbaert et al., 2014). Participants were presented with the words randomly distributed around a circular array on a computer screen and asked to group them in any way they saw fit. A k-means clustering analysis identified four consistent and robustly separated abstract word clusters, which we called: 1) cognition and thoughts, 2) negative qualities, 3) morality and personality, and 4) aesthetics and morals. When compared to chance (determined through random shuffling of rows/columns for each individual subject in permutation testing, 5000 iterations), within-cluster distances were all significantly smaller than chance (P<.0002, for all) and between-cluster distances were larger than chance (P<.002, for all but cluster 2 with cluster 3, P<.13). These results indicated that abstract concepts can be reliably categorized. We then used these clusters in an automatic semantic priming paradigm with 15 new subjects in

order to determine whether they were behaviorally relevant and automatically processed. Prime words were briefly presented (100 msec), pattern masked (50 msec followed by 100 msec blank screen), with the probe word presented for 250 msec and participants judging whether the probe was a concrete or abstract word. The prime-probe relationship did indeed influence response time, but not in the expected direction: within-cluster pairs yielded significantly slower, rather than faster responses relative to the across-cluster pairs (paired t-test: t=3.259 (df=14), P<.01). We are currently evaluating this inhibitory effect using different priming paradigms (e.g., lexical decision) and different subsets of abstract words.

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Poster

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Title: Decoding how the human brain parses continuous speech into linguistic representations

Authors: *L. GWILLIAMS, J.-R. KING, D. POEPPEL Psychology, New York Univ., New York, NY

Abstract: Language is generally described with multiple levels of description, building from speech sounds (e.g. /d/, /k/) to lexical items (e.g. dog, cat) to syntactic structures (e.g. dogs [SUBJECT] chase [VERB] cats [OBJECT]). How does the human brain encode and combine these hierarchical representations to ultimately derive meaning from continuous speech? Twenty-one native English participants listened to four fictional narratives that were fully annotated - from speech sounds to syntactic structures - such that each of these levels could be correlated with brain activity. This naturalistic but controlled setup allowed us to decode, localise and track phonemes, part of speech (POS) and syntactic operations from magnetoencephalography recordings (MEG) using machine learning algorithms. First, low-level phonetic features (e.g. voicing, manner, place of articulation) can be successfully discriminated from a sequence of

neural responses unfolding between ~100 ms to ~400 ms after phoneme onset. Second, POS (e.g. verb, noun, adjective), indicative of lexical processing, can be decoded between ~150 ms and ~800 ms after word onset. Third, we could decode and track both syntactic operations (e.g. number of closing nodes) and syntactic states (e.g. depth of tree) as predicted by theoretical linguistics. Interestingly, some of these syntactic representations are clearly present several hundreds of ms before word onset, whereas others maximally peak ~300 ms later. Overall, these sustained and evoked MEG responses show that the human brain explicitly represents each level of description, as proposed by linguistic theories. The corresponding neural assemblies overlap in space and time, which suggests that speech comprehension is implemented with a cascade architecture. Additionally, our study illustrates how the combination of machine learning and traditional statistics can bridge the gap between spatiotemporally-resolved neuroimaging data and rich but tractable naturalistic stimuli.

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Poster

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Topic: H.02. Human Cognition and Behavior

Title: Processing syntactic and prosodic information during sentence comprehension

Authors: *C. L. VAN DER BURGHT¹, T. GOUCHA¹, A. D. FRIEDERICI¹, J.

KREITEWOLF^{1,2}, G. HARTWIGSEN¹

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Abstract: In everyday conversation, various types of information need to be brought together. When processing a sentence, we understand 'who is doing what to whom' not only by the words used, but also by how the utterance is spoken. Both word forms and speech melody (prosody) establish which words belong together, creating syntactic boundaries between different parts of a sentence. For example, words can be grouped by syntactic cues (a particular word form) or by prosodic boundaries (a sentence pause). The neural correlate of how these different types of information contribute to sentence comprehension are yet to be specified. We used fMRI to address this issue, hypothesizing that if a prosodic boundary is processed as a syntactic boundary rather than only as a prosodic event this would lead to left hemispheric activation, possibly in the inferior frontal gyrus (IFG). In our experiment, we measured the processing of different types of utterances in which the message was established by either the word form, a sentence pause, or a combination of these. Subsequently, participants made a decision about which role a particular agent fulfilled in the action in the sentence, basing their response either on the syntax or the
prosody. Our results show that the contribution of the left IFG to speech processing depends on the information that is available and required for sentence comprehension. We found that taskrelated activity in the left IFG was increased when the syntactic boundary was marked by only one element (either syntactic or prosodic), as contrasted to sentences in which both syntactic and prosodic elements were marking the boundary. In accordance to the literature, a strong contribution of the left IFG was revealed when the syntactic cue was decisive for sentence meaning. As hypothesized, when a sentence boundary was indicated by a prosodic boundary only, the main activation was also found in the left IFG, as opposed to the right hemispheric activity commonly found for purely prosodic events. Our results suggest an involvement of the left IFG in function of the processing demands during sentence comprehension, regardless of whether a listener relies on what is being said, or how it is said.

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Poster

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Title: The neural bases of syntactic processing in American Sign Language: Evidence from fMRI and MEG

Authors: *A. K. VILLWOCK, W. MATCHIN, A. ROTH, D. ILKBASARAN, M. HATRAK, E. HALGREN, R. MAYBERRY UCSD, La Jolla, CA

Abstract: Language does not consist merely of sequences of words, but rather hierarchical linguistic structure. Previous research has illustrated that despite the fact that signed languages use a different modality than spoken languages, they involve the same syntactic properties. Neuroimaging studies on spoken languages using magnetoencephalography (MEG) and functional magnetic resonance imaging (fMRI) have routinely identified a core combinatorial language network consisting of left hemisphere brain regions, including the posterior and anterior temporal lobe and the inferior frontal gyrus by contrasting linguistically structured with unstructured stimuli. However, to date, the neural network for syntactic processing in signed languages is unknown. The present study investigated the neural bases of combinatorial linguistic processing in American Sign Language (ASL) in a group of congenitally deaf native

signers (N = 13). We conducted a parametric study of the processing of hierarchical linguistic structure using fMRI and MEG. The stimuli were sequences of six signs presented at three levels of syntactic complexity: unstructured word lists, simple two-word sentences, and six-word complex sentences. Stimuli were presented in blocks of three, six-word sequences. During both the fMRI and MEG experiments, participants determined whether a probe picture presented following a stimulus displayed the meaning of a sign in the sequence. We predicted increased activity for higher linguistic complexity in the same left hemisphere regions that have been identified in previous studies on spoken languages. The fMRI results showed that activity in both anterior and posterior portions of the left superior temporal sulcus increased parametrically with degree of structure. The MEG results showed the contrast of six-word sentences minus unstructured word lists to display increased activity for sentences in the temporal pole within the N400 time window (300–500 ms). These results are the first to demonstrate that the underlying neural basis of abstract syntactic processing is not altered by the sensory-motor channel of language reception and expression.

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Poster

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Intramural Research Program of the National Institute of Mental Health

Title: Cerebellar Crus I and caudate nucleus volumes are associated with deficits in verbal working memory in FOXP2 point mutation

Authors: *G. P. ARGYROPOULOS¹, K. SCHULZE², M. MISHKIN³, F. VARGHA-KHADEM^{1,4}

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Abstract: Introduction: We previously reported¹ an impairment in the phonological loop² component of working memory (WM) in affected members of the KE family (aKE), who have a verbal and orofacial dyspraxia resulting from a *FOXP2* point mutation³. We now report the

relationship between this deficit and volumes of the caudate nucleus and cerebellar Crus I. regions with pronounced structural/functional abnormalities in aKE members^{4,5}.

Methods: We assessed the WM of 5 aKE members with a test⁶ based on the Baddeley and Hitch WM model². We calculated their Crus I and caudate nucleus volumes (T₁-weighted MRIs), and correlated these volumes with their verbal (backward digit recall; counting recall; listening recall; word list recall; word list matching; digit recall) and non-verbal WM measures (block recall; mazes memory).

Results: Verbal WM scores were selectively lower for aKE members, and negatively correlated with their caudate nucleus and Crus I volumes. Non-verbal WM scores showed no decrement or correlation with those volumes (fig. 1).



Fig. 1: Crus I and caudate nucleus volumes correlate negatively with verbal (a-d) but not nonverbal WM (e-g); *: p < .05.

Discussion: Our results suggest an association between impaired articulatory rehearsal in WM¹ and the cerebellar/striatal abnormalities in aKE members^{4,5}, consistent with early FOXP2 expression in these structures³ and their reciprocal connectivity⁷. Our findings are in line with previously reported negative correlations of both volumes with measures of complex non-word repetition^{4,5}, and may reflect ontogenetic compensatory mechanisms resulting in abnormally increased GM density in basal ganglia structures of aKE members (e.g. putamen⁴).

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Poster

340. The Human Language Singularity

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Title: The effect of overt and covert speech on visual word recognition

Authors: *C. KOHEN, K. WINSLER, K. J. MIDGLEY, P. J. HOLCOMB Psychology, San Diego State Univ., San Diego, CA

Abstract: Efference copies are the internal representations of the perceptual consequences of a motor action. This copy is thought to be a crucial link between sensory and motor systems, however its top-down role in higher level perceptual processing remains overlooked. Recently Tian and Poeppel (2013) demonstrated an early role of efference copies in speech processing using overt and covert (imagined) production of single spoken syllables. Similarly, the current study investigated the role of efference copies in visual word recognition using event-related potentials (ERPs). Thirty participants viewed 40 trials in which they were cued to do nothing or produce speech (either overtly) of a repeated or unrelated monosyllabic three-letter word. This was split into two blocks, an overt block (say) or a covert (imagine saying) block. Results showed reduced negativities to repeated words as early as 150ms only if participants produced speech (either overtly or covertly; p = .02). Additionally, repeated words produced less negativity throughout the N400 (p < .01) and this effect was larger if either overt or covert speech was produced (p < .01). No statistical differences were found between overt and covert production. Overall, the results suggest an early, perhaps amodal, top-down influence of spoken word efference copies, comparably elicited via speech or imagined speech, on the perception of written words.

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Poster

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Title: Dynamics of start and stop signals during language production

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Abstract: The starting and stopping of movement plays a central role in many human behaviors. Previous research has revealed that starting and stopping motor behavior relies on a complex cortical-subcortical circuit involving, among others, the Sub-Thalamic Nucleus (STN), the Right Inferior Frontal Gyrus (RIF), and the pre-Supplementary Motor Area (pre-SMA). However, these conclusions are based almost exclusively on tasks that require manual responses (e.g., Aron et al., 2014). Here we examined the involvement of these areas in the starting and stopping of human speech using a verbal response task. We examined the temporal dynamics and functional connectivity between areas using a novel fMRI technique that permits the extraction of a more accurate and high temporal resolution BOLD signal (Slice-Based fMRI; Janssen et al., submitted).

We examined starting and stopping behavior in a blocked naming task in which 30 participants produced the names of objects presented at a high frequency (1.25 Hz). The high demands of this task required quick starting and abrupt stopping behavior. We first verified the involvement of various cortical and sub-cortical areas among which the STN, RIF, and pre-SMA in the task by extracting standard whole-brain activation maps during the start and stop periods. We then examined the temporal dynamics of processing in each area by obtaining time-relevant measures from Gaussian curve-fitting to the BOLD signals in start and stop periods. Finally, we used an AAL parcellation of the brain to examine the task-based functional connectivity and hierarchical clustering between the three areas and the rest of the brain at high temporal resolution (0.954 ms).

Our results revealed that whereas subcortical areas such as Caudate Nucleus, Putamen, and STN and cortical areas such as Primary Motor Cortex, RIF, and pre-SMA were active during both starting and stopping, only STN, RIF, and pre-SMA were more active during stopping than during starting. In addition, Gaussian curve fits revealed that the STN had an early time-to-peak that was similar to the RIF, while the pre-SMA had a slower time-course. Functional connectivity analyses revealed clusters in which STN and RIF were interconnected with areas

involved in motor control (putamen, thalamus, cerebellum, operculum), whereas pre-SMA was connected to areas involved in response evaluation (caudate, mid-cingulum, amygdala). Together these results provide evidence for a system of starting and stopping that generalizes across response modalities, and speak to on-going debates on the neural implementation of response inhibition (e.g., Duann et al., 2009; Forstmann et al., 2008; Wiecki & Frank, 2013).

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Poster

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Support: Wellcome Trust PhD scholarship

Title: Neural entrainment to acoustic edges in native and foreign speech

Authors: *M. CUCU, N. KAZANINA, C. HOUGHTON Sch. of Exptl. Psychology, Univ. of Bristol, Bristol, United Kingdom

Abstract: Cortical neural oscillations in response to speech were found to show theta activity which is synchronous to the syllabic rhythm (Luo and Poeppel, 2007), yet the exact mechanism which triggers this synchronisation is yet to be discovered. One hypothesis is that the onset of the syllable provides an 'acoustic edge' to which neural populations may preferentially respond. These edges would act as landmarks in the speech stream, triggering phase locking between the sound and neural oscillations. In the current experiment, we manipulated the strength of the syllable edge by varying the nature of the consonant in the syllable onset: 'strong' stop consonants: 'b', 'd', 'g', 'k', 'p', 't' vs. 'weak' fricatives or approximant consonants: 'f', 'l', 'r', 's', 'v', 'z'. This manipulation yielded Strong-edge and Weak-edge conditions respectively, that contained length- and stress-matched sentences consisting of words in which every syllable started strong vs weak consonant.

Participants listened to four repetitions of 40 Strong-edge and 40 Weak-edge sentences while their EEG activity was recorded. Half of the sentences were in English, half in Russian, a language not understood by the participants. The sentences were recorded in the lab by a bilingual English-Russian speaker. In line with previous findings (Doelling et al, 2014), we predicted more entrainment in the Strong-edge then Weak-edge condition (see Figure 1), as well as more entrainment between the speech stream and the EEG signal when the language is understood by the participant (Peele and Davis, 2012). Entrainment will be assessed in terms of the phase coherence between the EEG signal and the envelope of the acoustic signal. The results will outline the importance of the acoustic quality of the syllable edge in resetting the theta rhythm, and potentially shed light onto the mechanisms of theta-gamma coupling during speech perception.

Figure 1.

Disclosures: M. Cucu: None. N. Kazanina: None. C. Houghton: None.

Poster

340. The Human Language Singularity

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Topic: H.02. Human Cognition and Behavior

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Title: Effects of language proficiency on cognitive control: Evidence from Resting-State functional connectivity

Authors: *X. SUN¹, L. LI², G. DING², P. LI³, R. WANG¹

¹Sch. of Psychology, South China Normal Univ., Guangdong, China; ²State Key Lab. of Cognitive Neurosci. and Learning, Beijing Normal Univ., Beijing, China; ³Pennsylvania State Univ., University Park, PA

Abstract: Cognitive studies have suggested that bilingualism plays an important role in the development of cognitive control, specifically in that bilingualism has been found to promote cognitive domains in switching and inhibition. In recent years functional neuroimaging studies suggest that bilingual experience changes neural activity in the cognitive control network. The present study used resting-state functional connectivity (RSFC) to explore the impacts that bilingualism, especially language proficiency, had on the networks of executive function. Seed regions centering on different components were selected for the RSFC analysis. Our RSFC analyses of high versus low proficiency bilinguals show that bilingualism has effects on distinct components of executive control. Specifically, significant differences were observed in the left anterior cingulated cortex (left ACC) for cognitive switch, but in the right middle frontal gyrus (rMFG) for inhibition. No difference was found for components of working memory. Thus, language proficiency could be interpreted to cause changes on some but not others components of cognitive control in the neural network. Finally, our findings indicate decrease in the strength of functional connectivity in the cognitive control network in high proficiency bilinguals, suggesting that higher proficiency in the second language results in less effortful processing and lower cognitive effort.

Disclosures: X. Sun: None. L. Li: None. G. Ding: None. P. Li: None. R. Wang: None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: BMBF Grant 13GW0053D (MOTOR-BIC)

DFG Grant EXC1086 (BrainLinks-BrainTools)

Title: Spatial multiscale fMRI analysis of the human cortical language system

Authors: *P. KELLMEYER, R. BERKEMEIER, T. BALL

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Abstract: In the last two decades, neuroimaging research has provided important insights into structure-function relationships in the human brain. Recently, however, the methods and software used for analyzing fMRI data have come under increased scrutiny with studies questioning the cross-software comparability¹⁻³, the validity of statistical inference⁴⁻⁶ and interpretation⁷, as well as the influence of the spatial filter size on fMRI (and PET) analysis⁸⁻¹⁰. Here, we use a spatial multiscale analysis with a range of Gaussian filters from 1-20 mm at fullwidth half maximum (FWHM) in analyzing fMRI data from a speech repetition paradigm in 25 subjects with the widely used software "Statistical Parametric Mapping" (SPM). We show that analyzing the fMRI data over a range of Gaussian filter kernels ranging from 1 to 20 mm FWHM reveals substantial variability in neuroanatomical localization as well as the average signal strength (Fig. 1A) and size (Fig. 1B) of suprathreshold clusters depending on the filter size. We also demonstrate how small spatial filter kernels bias the results towards subcortical and cerebellar activation clusters and that the atlas-based anatomical assignment of suprathreshold clusters shifts in relation to the filter size. Furthermore, we found substantially different scale-dependent cluster size dynamics between cortical and cerebellar clusters (Fig. 1C and D).

Each of the different ensembles of regions at any given filter size could be discussed along the existing literature on speech processing. The vast majority of fMRI studies, however, only use a single filter for analysis within narrow range of filter sizes. Our findings suggest that this single-filter approach seems to severely constrain the interpretation of individual fMRI studies and the validity of meta-analyses based on this body of studies. The limited multiscale analysis used here may inform the interpretation of structure-function relationships in analyzing fMRI data. We propose to develop true spatial multiscale analyses to fully explore the deep structure of the BOLD signal in Gaussian scale space.

Disclosures: P. Kellmeyer: None. R. Berkemeier: None. T. Ball: None.

Poster

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Chinese Fundamental Research Funding for Central Universities ZYGX2015002

Interdisciplinary Development Project of University of Electronic Science and Technology of China Y03111023901014007 Title: Modular reconfiguration of brain networks underlying word masking

Authors: *S. GAO, Y. YIN, D. YAO UESTC, Chengdu, China

Abstract: While dynamic brain modularity has recently been proposed to explain the neural basis of consciousness it still remains unclear how modular networks specifically reconfigure to optimize their functional operation for conscious and non-conscious processing. Here we measure brain network flexibility by the allegiance of nodes to modules during a word masking procedure and show that the visibility of target words is characterized by shifts of certain nodes between three task-related modules recruited respectively for perception, action, and coordination. In our paradigm, participants were randomly presented with masked words, visible words, or corresponding control trials where word stimuli were replaced by blank screens, and asked to make a forced choice to categorize each stimulus as affectively positive or negative. Applying graph theory analysis to functional magnetic resonance imaging data, we defined individual voxels of activated clusters as nodes, functional connections between nodes as edges, and interconnected nodes as modules, and employed the modular allegiance of nodes as the index of dynamic reorganization of modular networks. Three modules were identified under each task condition and more importantly certain nodes updated their allegiance to modules as a function of task states. With words masked these flexible nodes, located mainly in the dorsal anterior cingulate cortex, were organized in the coordination module whereas they were reassigned to either the perception or action module when words were visible. These results suggest that the dorsal anterior cingulate cortex may act as a key region implicated in switching between conscious and non-conscious contexts. The masking-dependent reconfiguration of functional modules revealed in this study provides a novel insight into brain mechanisms supporting conscious access.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: CONACYT Frontiers Grant 225

Title: The role of social cognition on pragmatic language comprehension: A meta-analysis of fMRI studies

Authors: *M. GIORDANO, *M. GIORDANO, E. VALLES-CAPETILLO, A. REYES-AGUILAR

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Abstract: The use of language as a means of social communication in humans requires more than the comprehension of the meaning of the individual words and their syntax, it involves understanding the intentions of the speaker, and the context in which the words are uttered. In addition, the listener may need to decode additional meaning present in the indirect or pragmatic language used by the speaker. Thus, we proposed the hypothesis that comprehension of diverse forms of pragmatic language would implicate activation of social cognition and theory of mind brain areas. To test this hypothesis, we did a meta-analysis of 39 studies published between 2006 and 2017 available through databases that reported the results of contrasts between the pragmatic forms (PF) of interest versus literal language, in healthy adults, and that used fMRI scanning throughout the whole brain. The PF included speech acts, metaphor, idiom, irony, and sarcasm. We used the software Signed Differential Mapping (SDM version 5.12; Radua & Mataix-Cols, 2012) for creating a SDM map for each study within a mask of gray matter. With the aim of finding the mean of the voxel values of the areas involved in the processes of interest, a "mean analysis" and "subgroup analysis" were performed; the comparisons between PF, and between natural languages were performed with the general linear model analysis; and the consistency of the results was tested with the jacknife analysis. We present the results according to the cluster center. For the contrast pragmatic>literal language, our results showed significant activation of the superior and inferior frontal gyrus (left; BA 32, 45); middle temporal gyrus (bilateral; BA21, 22), and inferior frontal gyrus (right; BA 45). The contrast speech-acts>all PF showed significant activation of the superior and inferior frontal gyrus (right; pre somatomotor area BA8, and orbital, BA47), and inferior frontal gyrus (left, triangular BA45); for idioms>all PF, significant activation of the inferior frontal gyrus triangular (BA46), and for the contrast irony + sarcasm>all PF, significant activation of the superior frontal gyrus, rostro medial prefrontal cortex (right; BA11). Metaphors did not show significant activation in comparison with all other PF. We also observed differences between the areas recruited by different natural languages, for instance the Japanese language only recruited paralimbic areas (left paracingulate gyrus, BA 24). These results suggest that pragmatic language comprehension recruits the extended language network more than literal language, as well as cortical areas involved in social cognition, emotion processing, and theory of mind.

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Poster

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Natural Science Foundation of Guangdong Province China 2016A030313327

Title: Phonological but not orthographic awareness is related to oral performance in post-stroke aphasia

Authors: *Z. CHEN¹, J. CAO², Y. CAI³, W. QIU¹

¹Rehabil. Med., ²Ultrasound Med., The Third Affiliated Hosp. of Sun Yat-Sen Unive, Guangdong, China; ³Otorhinolaryngology, Sun Yat-Sen Mem. Hosp., Guangdong, China

Abstract: The activation of right hemisphere was proposed to represent a compensatory visual/orthographic strategy when language networks were damaged in patients with post-stroke aphasia. Given that Chinese is a logographic language which emphasize orthography deeply, it is reasonable to hypothesize that aphasic patients speaking Chinese prefer to retrieve their ability of oral communication by switching toward visual/orthographic strategy following stroke. However, this hypothesis has not been confirmed and it remains unclear whether visual/orthographic strategy allows fully recovery of oral performance as what the original audio/phonological pathway dose. Here we addressed these questions by exploring a negativegoing event-related potential at ~400 ms (N400) indicating orthographic and phonological awareness in 18 post-stroke aphasic patients and 15 controls. Participants viewed line-drawing pictures of objects followed by a word in Chinese or a sound of speech for two respective session, being required to decide whether the word or the sound matched the picture. Results showed that the magnitude of N400 elicited by picture-word matching task (N400w) was larger than that of N400 acquired in picture-sound matching task (N400s), suggesting that both aphasic patients and control subjects speaking Chinese rely more on visual/orthographic pathway when they process semantic information. Although both tasks were finished with high accuracy, only N400s magnitude was positively correlated with patients' verbal production and speech fluency. Besides matching tasks, patients were required to name the same objects that were used in eventrelated-potential experiments. We found that N400s existed for the objects that could be named successfully and was absent for those could not be named, whereas N400w failed to demonstrate this pattern. The association between oral performance and phonological but not orthographic awareness even in a logographic language suggests that visual/orthographic strategy could be an inefficient alternation of the neural mechanisms following stroke. The preservation and reactivation of the audio/phonological pathway should be emphasized in order to recover a higher oral performance of aphasic patients.

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Poster

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Support: 973 Program (2014CB846100)

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Title: Structural connectivity subserving verbal fluency revealed by lesion-behavior mapping in stroke patients

Authors: *M. LI, J. DING, Y. FANG, Y. XU, Z. HAN State Key Lab. of Cognitive Neurosci. and Learning, Beijing Normal Univ., Beijing, China

Abstract: Abstract Tests of verbal fluency have been widely used to assess the cognitive functioning of persons, and are typically classified into two categories (semantic and phonological fluency). While widely-distributed divergent and convergent brain regions have been found to be involved in semantic and phonological fluency, the anatomical connectivity underlying the fluency is not well understood. The present study aims to construct a comprehensive white-matter network associated with semantic and phonological fluency by investigating the relationship between the integrity of 22 major tracts in the whole brain and semantic fluency (measured by 3 cues) and phonological fluency (measured by 2 cues) in a group of 51 stroke patients. We found five left-lateralized tracts including the anterior thalamic radiation (ATR), inferior fronto-occipital fasciculus (IFOF), uncinate fasciculus (UF), superior longitudinal fasciculus (SLF) and frontal aslant tract (FAT) were significantly correlated with the scores of both semantic and phonological fluencies. These effects persisted even when we ruled out the influence of potential confounding factors (e.g., total lesion volume). Moreover, the damage to the first three tracts caused additional impairments in the semantic compared to the phonological fluency. These findings reveal the white-matter neuroanatomical connectivity underlying semantic and phonological fluency, and deepen the understanding of the neural network of verbal fluency.

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Poster

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Topic: H.02. Human Cognition and Behavior

Title: A quantitative analysis of speech kinematics during word learning in children with autism

Authors: *J. V. JOSE^{1,2,3}, D. WU⁴, L. GOFFMAN⁵, L. BROWN⁶, A. GLADFELTER⁷ ¹Indiana Univ., Bloomington, IN; ²Stark Neurosci. Inst., Indiana Univ., Indianapolis, IN; ³Cell. and Integrative Physiol., Indiana Univ. Sch. of Med., Indianapolis, IN; ⁴Physics, Indiana Univ. Bloomington, Bloomington, IN; ⁵Univ. of Texas at Dallas, Dallas, TX; ⁶Purdue Univ., West Lafayette, IN; ⁷Northern Illinois Univ., DeKalb, IL

Abstract: Autism Spectrum Disorder (ASD) is characterized by communication deficits and repetitive motor behaviors and routines. One core component of ASD relates to learning-with impaired word learning a central feature. Often, the delayed onset of first words is a parent's earliest concern and is a key factor in securing a diagnosis. Words are a crucial building block for language because they connect concepts, word forms, and articulatory movement. While behavioral measures of concepts and word forms are well established, there is no current quantification of the distinction in the learning associated changes in the structure of underlying articulatory movement in children with ASD compared to their neurotypical peers. In our study, we apply kinematic analyses to continuously collected articulatory movements to quantify changes in underlying features of movement as children with ASD and their typically developing peers acquire new words. To keep information in the recording, we did our analyses without manipulating or averaging the articulatory kinematic data. Three phases were included in the paradigm: a pretest, a learning phase, and a posttest. Kinematics of lip/jaw movements were continuously recorded using a Northern Digital camera system. We extracted lower lip and jaw movement while children produced six new words multiple times each. We evaluated learning phase effects by calculating the correlations between pretest and posttest and further quantified pattern changes across the two phases. Findings were that children with ASD, although they produced the words accurately, did not acquire the differentiated movement patterns demonstrated by their neurotypical peers. This lack of motor change during tasks of word learning in children with ASD may be contributing to the delayed language development in these children; it is well-established in the motor learning literature that responsivity to change (i.e., variability) during initial learning leads to greater gains in later learning and retention. If children with ASD lack speech motor pattern change during their earliest experiences with new words, gains in language learning, retention, and generalization will be more difficult later. In summary, our findings reveal that application of complex signal processing approaches provides critical insights into the process of word learning at the sensorimotor level, as well as

the underlying link between the core diagnostic features of ASD, namely communication delays and repetitive motor behaviors. Additionally, our study establishes an approach to measurement of articulatory change that is applicable generally to future speech learning studies.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: Autism Speaks High Risk/Hig Impact Grant #5664

Elizabeth H. Solomon Center for Neurodevelopmental Research

Title: Amplitude, latency and theta phase coherence of event-related source generators differ during visual processing for nonverbal/minimally-verbal children with autism and their controls

Authors: *S. ORTIZ-MANTILLA, T. REALPE-BONILLA, A. A. BENASICH Rutgers The State Univ. of New Jersey, Newark, NJ

Abstract: When children with Autism Spectrum Disorder (ASD) are nonverbal (NV) or minimally-verbal (MV), it is a challenge to assess how much language they perceive and comprehend. It is known that to acquire language, children must build phonemic maps of their native language, assemble a lexicon, and learn word-object associations by mapping auditory words to visual objects. This latter process consists of relating each object's features to its corresponding semantic information, including the object's name. Nine-month old infants understand word meanings and are also able to use visual cues of objects to facilitate phonetic and phonological learning. In a recent event-related (ERP) study using a picture-word matching paradigm, 4- to 7-year-old NV/MV children with ASD showed relatively intact early sensory processing but abnormal lexical-semantic processing compared to age/gender-matched controls. However, it is not clear whether the limited ability to express and perhaps comprehend language that is observed in NV/MV children with ASD is related to absent or deficient cortical representations of objects' features and/or deficits in linking an object with its semantic information. To investigate the oscillatory underpinnings of ERP responses to visual information source generators of the sensory/perceptual response were identified and peak amplitude and latency of the sources examined. In addition, inter-trial phase locking analyses were conducted within source space. During early (0-200 ms) and late (250- 500 ms) visual processing, 3 sources, located in left and right occipital cortices and frontally at the level of anterior cingulate

cortex (ACC), explained ~93% of variance. We found that as compared to typically developing controls, the ASD group exhibited smaller amplitudes and longer latencies particularly during late visual processing in the left occipital source, and smaller theta phase coherence during both early and late visual processing for all three sources. Reduced phase coherence in the ACC indicates the ASD group may allocate less attentional resources to process visual stimuli. These results also suggest that children with ASD may have difficulties encoding stimulus features and then mapping objects with their related information. We propose that poor memory representations of common objects accompanied by diminish attentional allocation could be a factor that might explain linguistic differences observed in ASD groups. Furthermore, these results may well advance our basic understanding of the neural mechanisms that support both typical and atypical visual processing in children.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: Vanderbilt Institute for Clinical and Translational Research VR23988

Title: Investigating neural bias to speech using auditory event-related potentials in children

Authors: *A. WHITTEN, A. P. KEY, A. S. MEFFERD, J. W. BODFISH Hearing & Speech Sci., Vanderbilt Univ., Nashville, TN

Abstract: Several previous studies have found that infants and children exhibit a preference for speech over other types of sounds using behavioral paradigms. The present study sought to determine whether neural evidence could be found for this bias using EEG/ERP in a sample of 23 eight – twelve-year-old children. In the first experiment, we measured the N1 and P2 sensory auditory ERPs and the P3a orienting ERP response to a rarely presented synthetic speech vowel and complex tone in a passive oddball task. We hypothesized a neural bias to speech would be evidenced by faster latency of the N1, P2, and P3a ERPs to the speech sound as well as larger P3a mean amplitude to the speech sound. In contrast to our hypotheses, we found a significantly longer latency of both N1 and P2 to the synthetic speech compared to the non-speech tone (N1: t(22) = 5.23, p < .001; P2: t(22) = 3.3, p < .01), and no significant differences in the P3a latency or mean amplitude. We concluded that our results may not have replicated the behavioral findings because our study (like many EEG studies) used synthetic speech rather than natural speech, and thus the behavioral findings may reflect a bias to the human voice in general, rather than the linguistic content of the speech signal. In the second experiment, we tested this theory

by measuring N1, P2, and P3a auditory ERPs to a naturally spoken vowel /a/ compared to an acoustically and complexity-matched cow utterance. Here, we found support for a neural bias to the natural speech over the non-speech with a significantly shorter latency of the N1, P2, and P3a ERPs to the natural speech (p < .05 for all contrasts). Our results provide neural evidence for a bias to natural speech, but not synthetic speech, that occurs within the first ~300 ms of cortical auditory processing. These findings have implications for theories of language acquisition in which an early bias for listening to speech is driven by a preference for the human voice. In addition, our results suggest that future studies should compare neural differences in the cortical processing of synthetic vs. natural speech in order to determine whether synthetic speech is a valid proxy for natural speech in EEG/MEG studies.

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Poster

340. The Human Language Singularity

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Topic: H.02. Human Cognition and Behavior

Support: Natural Sciences and Engineering Research Council of Canada

Title: Resting state alpha oscillations correlate with language ability in young children

Authors: *E. KWOK, J. ORAM CARDY, B. ALLMAN, P. ALLEN, B. HERRMANN Univ. of Western Ontario, London, ON, Canada

Abstract: Resting state α -oscillations as recorded using electroencephalography (EEG) have been proposed to be related to the excitability/inhibition status of cognitive processes. However the role of α -oscillations in the development of language abilities is less well understood. The objective of this study was to explore the relation between resting state α -oscillations in typical language development in order to establish a standard for the understanding of language disorders.

Resting-state EEG was recorded in forty-one children with typical development (4 years, n = 12; 5 years, n = 14; 6 years, n = 15). Data were recorded while children had their eyes either open or closed (three minutes each). All participants scored within normal limits on standardized measures of language (*Clinical Evaluation of Language Fundamentals*-4) and Performance IQ (*Wechsler Abbreviated Intelligence Scale*). We explored three measures of posterior α -oscillations (7-10 Hz): α -spectral power, α -band long-range temporal correlations (using detrended fluctuation analysis; DFA), and fluctuation/flexibility of α -frequency over time. The difference between eyes-closed and eyes-open conditions (α -spectral, α -band long-range correlations and α -frequency) was entered into regression analyses using age, language ability,

and Performance IQ as predictors. Regression analyses showed that all three α -measures were predicted by participants' language ability. Higher language scores correlated with: lower α -power, longer α -band long-range correlations, and greater fluctuations in α -frequency. α -measures were not predicted by age or by IQ (with the exception of a significant IQ effect for the temporal correlation measure).

To our knowledge, this is the first study exploring the relation between α -oscillations and language ability in 4–6 year old children with typical language development. Our findings revealed a unique relationship between language ability and resting state α -oscillations that is independent of chronological age and global cognitive development. Our current findings suggest that α -oscillations, which have received limited attention thus far in language development studies, may be a useful biomarker for developmental language disorders.

Disclosures: E. Kwok: None. **J. Oram Cardy:** None. **B. Allman:** None. **P. Allen:** None. **B. Herrmann:** None.

Poster

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NICHD 1F31HD087085

NIDCD 1F31DC014230

Title: Evidence of maturational processes in linguistic brain (fNIRS) and physiological emotional (Thermal IR) responses in hearing infants to signing virtual humans

Authors: *B. MANINI^{1,2}, G. KARTHEISER^{1,2}, A. STONE^{2,1}, A. MERLA^{3,4}, L.-A. PETITTO^{2,1} ¹Brain and Language Lab. For Neuroimaging, Washington, DC; ²Gallaudet Univ., Washington, DC; ³Dept. of Neuroscience, Imaging and Clin. Sci., Universita' Gabriele D'Annunzio, Chieti, Italy; ⁴Next2U s.r.l., Pescara, Italy

Abstract: Sensitivity to linguistic patterns depends on brain development and undergoes rapid change between ages 6 to 12 months (Petitto et al., 2012). Younger infants engage linguistic processes when exposed to any linguistic input (e.g. phonetic discrimination, activation of classic

language systems) regardless of native language (e.g. Kuhl, 2010), and signed vs spoken modalities (Baker, Golinkoff, Petitto, 2006, Petitto et al., 2011). By ~12 months, universal processes attenuate to sensitivity to infants' native language(s). Infants with reduced linguistic exposure within this developmental period are at risk for phonological, linguistic, and reading deficits (Petitto et al., 2016). (1) Can a Virtual Human (VH) using American Sign Language (ASL) be effective as an augmentative linguistic partner for *all* infants, even those without signed language experience (Petitto et al., 2017)? (2) Can ASL input from VHs (vs other types of human and non-human biological movements) engage the same neural sites associated with linguistic processing in infants and emotional arousal responses, and developmental change? Methods. 9 hearing babies (5 boys); 2 age groups: 6-7 mo. (5) and 10-11 mo. Three conditions: 1) Language: VH signing an ASL nursery rhyme (+human form, +language), 2) Dancer: Producing gentle arm movements (+human form, -language) 3) Cat: A cat playing (-human, language). Novel integration of time-locked neural (fNIRS, block design) and psychophysiological (Thermal IR) responses were measured. Results. Only the Language condition showed age-related changes and yoked neural activity and physiological responses. Younger infants showed significantly left-lateralized IFC activity, indicative of linguistic processing that co-occurred with a peaked psychophysiological response (parasympathetic) typical of social engagement. Older babies showed bilateral IFC activity and no peaked emotional responses to the *Language* condition. Hearing babies' sensitivity to language patterns even in a silent sign language to which they were never exposed, and even with a VH, supports the hypothesis that babies are predisposed to core patterns in language (Petitto et al., 2016). VH learning tools may be effective as argumentative language partners for young infants at risk of minimal language exposure, particularly deaf infants. However, our first-time discovery of *linked* higher cognitive (language) neural activity and physiological (emotional) responses typical of linguistic processing in younger vs older infants suggests that the human language learning system involves a more powerful neural circuitry than was previously understood in child language history.

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Poster

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Topic: H.02. Human Cognition and Behavior

Title: Using inter-subject correlation to probe the neural response to article readability during natural reading

Authors: *Y.-F. LIU¹, W.-J. KUO³, F.-H. LIN²

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Abstract: The neural basis of high-level language function has been studied using natural story listening and telling, but reading comprehension has seldom been studied using natural stimuli. Particularly, besides our prior study, functional areas associated with subtle article properties such as genre and level of readability still remain unexplored. In this study, we presented articles of fiction/non-fiction contents translated from English to Chinese by human and machine to subjects and BOLD-contrast fMRI was measured. We identified spatial distributions of intersubject correlation (ISC) modulated by texts of fictional/non-fictional contents and machine/human translations. In the inter-genre contrast, reading news articles resulted in significant decrease in ISC at ventral PCC (BA 23), right somatosensory cortex (BA 1-3), bilateral secondary visual cortex (BA 18), bilateral fusiform gyrus (BA 37), bilateral precuneus (BA 7), bilateral anterior insulas, and right dorsal aTL (BA 38). No cortical area was found with significantly increased ISC value. In the inter-translation contrast, reading machine translation resulted in significant decrease in ISC at right precuneus only. Significant increase in ISC was observed at bilateral primary motor cortex (BA4), ventral anterior cingulate cortex (BA32), right inferior frontal gyrus (BA45). The result of the inter-translation contrast is in accordance with the coarse coding hypothesis and the gradient salient hypothesis. The right IFG may be related to the comprehension of less readable articles. Moreover, the result of the inter-genre contrast implies that the left aTL processes a natural article by directly associating language inputs to their semantic meaning; on the contrary, the right aTL associates language inputs to elements beyond their literal meanings. Distributed brain activation clusters in our data suggest that reading comprehension is a multi-modal task. Complex brain processes related to natural reading is hopefully to be further elucidated by our experimental design and analysis.



This study was approved by the IRB. 19 subjects were asked to read 8 Chinese translated by human or Google translate (semantic errors corrected manually) with fictional (Reader's Digest) or non-fictional (NY Times) contents. Articles were presented using vertical scrolling (3 lines per screen with fade-in and -out at top and bottom margins). Each subject read 2 articles of each translation-genre combination. Each article contained around 1,300 Chinese characters and took about 3.5 minutes to read. EPI data were collected on 3T scanner using a 32-channel head coil array.

The ISC of the first EPI 94 scans was calculated. A general linear model (GLM) was then applied to the z-transformed correlation coefficients to estimate the article translation/ content effects. We specifically examined two contrasts: "non-fiction vs. fiction" contents and "human vs. machine" translation. FDR correction was performed.

Disclosures: Y. Liu: A. Employment/Salary (full or part-time):; National Taiwan University. W. Kuo: None. F. Lin: None.

Poster

340. The Human Language Singularity

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 340.23/UU72

Topic: H.02. Human Cognition and Behavior

Support: Startup

Title: A reexamination of click detection during statistical learning

Authors: *T. OVERATH¹, D. L. MURPHY²

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Abstract: Click-detection during segmentation of continuously presented nonwords has been proposed as an online measure of statistical learning during language acquisition. In such a task, subjects are instructed to respond to clicks superimposed over a stream of tri-syllabic nonwords. In a previous study, reaction times (RT) to clicks paired with syllables within words (syllable 2, S2) slowed more rapidly than clicks paired with syllables between words (syllable 1, S1; Gomez et al., 2011), over a 4 min exposure period. Later work (Franco et al., 2015) demonstrated that it is unclear if this effect is a reflection of statistical learning of word boundaries or of competing attentional demands during active or passive word segmentation. It is also unclear if postexposure measurements of statistical learning directly represent the efficiency of the segmentation process, subjects' short-term auditory memory, or a combination of both. Participants in this study listened to a stream of concatenated tri-syllabic non-words and were asked to detect clicks that were either placed between words (S1) or within words before the third syllable (S3), for which the composite within-word predictability is highest (previous studies placed within-word clicks before S2). The exposure time was also increased to 6 minutes to test the stability of statistical learning. The shift in click position to S3 was motivated by additional exploration of target detection during word segmentation: under explicit and implicit learning conditions, during which subjects were instructed to actively segment word boundaries, RTs were fastest during detection of target syllables occurring as the last in a trisyllabic word (Batterink et al., 2015). Here, we show that detection of S3 clicks is faster than S1 clicks during explicit learning, replicating the behavior observed in syllable detection tasks (Batterink, 2015), but contradicting previous studies comparing clicks at positions S1 and S2 (Gomez et al., 2011; Franco et al., 2015). This suggests that one syllable is insufficient to trigger learning-guided shifts in attentional demand, but two syllables, when part of a statistically generated model, have an immediate salience effect. We also find that in the 2AFC word recognition stage, accuracy is correlated with the relative changes in S1 and S3 RTs, and that differences in S1 vs. S3 RTs diminished during minutes 5-6. References: Batterink LJ, Reber PJ, Neville HJ, Paller KA. (2015). J Mem Lang 83: 62-78. Gomez DM, Bion RAH, and Mehler J (2011). Language and

Cognitive Processes 26: 212-223. Franco A, Gaillard V, Cleeremans A (2015). Behav Res 47: 1393-1403.

Disclosures: T. Overath: None. D.L. Murphy: None.

Poster

340. The Human Language Singularity

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Topic: H.02. Human Cognition and Behavior

Support: National Natural Science Foundation of China (31622030, 31270023, and 30900393)

Title: Neural synchronization of syntactic priming during face-to-face communications

Authors: *W. LIU, X. BAI, 100875, H. ZHAO, 100875, Y. LONG, L. ZHENG, C. LU BEIJING NORMAL UNIVERSITY, Beijing, China

Abstract: Syntactic priming is a unique manifestation in psychological linguistics and has been proven in many experimental paradigms and in or across several languages, but the neural mechanism behind this phenomenon remains unclear. In this study, functional near infrared spectroscopy (fNIRS)-based hyperscanning was employed to measure brain activities of two communicators simultaneously during face-to-face communication with eye contact, face-to-face communication without eye contact, and back-to-back communication. The two communicators either produced the same syntactic structure [Double-Object(DO) or Prepositional-Object(PO) separately]] or different syntactic structures (DO and PO alternately, as a control task). Results showed that, in both face-to-face with eye contact condition and without eye contact condition, there was a significant increase of interpersonal neural synchronization (INS) in the DODO task relative to the control task in the left pSTC. The face-to-face with eye-contact condition additionally recruited the left SMC, whereas the task without eye contact additionally recruited the right TPJ. There was no significant increase of INS in the back-to-back condition. Also, no significant results were found for the POPO task in any conditions. In addition, the increase of INS correlated significantly with the accuracy of task performance in the DODO task, but not in the POPO task. These findings suggest that neural synchronization may underlies the alignment of syntax representation during communication, and different types of syntax representation corresponds to different level of neural synchronization. Thus, the neural synchronization can be considered as a neural marker of syntax representation in communications.

Disclosures: W. Liu: None. X. Bai: None. H. Zhao: None. Y. Long: None. L. Zheng: None. C. Lu: None.

Poster

340. The Human Language Singularity

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Program#/Poster#: 340.25/UU74

Topic: H.02. Human Cognition and Behavior

Support: JSPS KAKENHI

Title: Neural networks for understanding the intention of a speaker in discourse with explicit and implicit contexts

Authors: *S. TOKIMOTO¹, N. TOKIMOTO², Y. MIYAOKA³

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Abstract: This study examines the neural basis for understanding the intention of a speaker in verbal communication. In a daily conversation, a speaker often expresses his/her intention indirectly, and the comprehender understands the implicit intention by pragmatic inference. For example, we often use an interrogative for asking something to others ('Will you open the window?'). To examine the neural activity elicited by pragmatic inference, we visually presented three types of Japanese discourse to participants to record the electroencephalogram. The discourses were manipulated in three ways: 1) control discourses in which the intentions of speakers were directly expressed, 2) experimental context-explicit discourses in which the contexts to derive the intentions from were given explicitly, and 3) experimental context-implicit discourses in which the relevant contexts were implicit. The recorded EEG was analyzed timelocked to the onset of the presentation of the utterance that required the participants to understand the intention of a speaker. As a result, negative event-related potentials were broadly observed around the latency of 500 ms for the two types of experimental discourse against the control discourses, but their amplitudes were greater and their topographies were broader in the context-implicit than the context-explicit discourses. We localized the generators of the negativity by dipole fitting to examine the relationship between the pragmatic inference and the ability for mind-reading (Theory of Mind (ToM)). The sources of the negativity of the two experimental discourses against the control discourses were localized in the medial frontal region, which is often pointed out to be a region of ToM. These localizations of the dipoles for the experimental discourses suggest a close relationship between the mechanisms of pragmatic inference and ToM. However, the sources corresponding to the difference in the negativity between the context-explicit and the context-implicit discourses were localized in the left frontal region. Moreover, the dipoles corresponding to the contrast between the context-explicit and the control discourses were localized in the right temporal region. Our results thus suggest that the

neural networks for pragmatic inference can vary according to the characteristics of the context, and that some part of pragmatic inference can be independent of ToM.

Disclosures: S. Tokimoto: None. N. Tokimoto: None. Y. Miyaoka: None.

Poster

341. Pharmacology in Schizophrenia Models

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Topic: H.03. Schizophrenia

Support: KAKENHI (18023009, 20023006, 20390098)

Lilly Grant Office

William Paterson University

Title: Environmental stresses and psychiatric disorders based on genetic dysfunction of cannabinoid CB2 receptor

Authors: *H. ISHIGURO¹, K. TABATA¹, C. MOCHIZUKI¹, E. S. ONAIVI² ¹Univ. of Yamanashi, Chuo, Yamanashi, Japan; ²William Paterson Univ., Wayne, NJ

Abstract: Major depression and schizophrenia are mental health problems associated with stressful events in life based on certain genetic background. Cannabinoid system seems to have an important role in stress induced psychiatric disorders, and also each kind of stressor develops those disorders differently. In addition, there must be certain periods in life when brain neural network show fragility and dysfunction to the stressors. High incidence of polymorphisms in the Cannabinoid CB2 Receptor gene (CNR2) which related to low function of the receptors were found in both of schizophrenia, depression and alcoholism in Japanese population. We further examined a functional relationship between stressors and those molecules using those knockout mice as schizophrenia and depression models, as we had confirmed that neither Cnr2 knockout mice did not show any difference in PPI and in Zero maze test. Thus, the CB2 receptor has behavioral roles in response to pharmacological, immunological and psychological stresses possibly via HPA axis and neuroimmune system. We tested several stressors in mice models. Methamphetamine was injected to those mice to examine their locomotion as acute response and as acquired reverse tolerance. The Cnr2 knockout mice showed more locomotive activity after the acute treatment and also dramatic enhancement in locomotion after development of reverse tolerance. Poly-IC was injected i.p. to Cnr2 knockout mice, and their locomotor activity was measured 72 hours after the injection and compared with those of the wild type controls. The heterozygote mice relatively reduce their locomotion in the test cage. Also heterozygote Cnr2 knockout mice show more anxiety in Zero maze than wild type after Poly-IC injection. In those

model mice for psychiatric disorders, HPA or neuroimmune system related molecules could show similar changes in psychiatric patients. As results, *Fkbp5* and some cytokines' in *Cnr2* heterozygote knockout mice brain were expressed differently in comparison to wild type mice brain. It was interpreted that mice with those genetic dysfunctions develop psychiatric behaviors when they experienced certain stress and correspondent responses in HPA and immune system. Further studies are required to determine specific fragile age to the stressors based on each genetic background in the etiology of those psychiatric diseases.

Disclosures: H. Ishiguro: None. K. Tabata: None. C. Mochizuki: None. E.S. Onaivi: None.

Poster

341. Pharmacology in Schizophrenia Models

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Topic: H.03. Schizophrenia

Support: IBRO-ISN Fellowship 2015

NIH Grant R03 MH 104851

Title: Neural CaMKIIa is lost in NMDAR hypofunction schizophrenia; putative opposing roles of IGF-1R and TLR4

Authors: *O. M. OGUNDELE¹, C. C. LEE²

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Abstract: Schizophrenia (SCZ) is a neuropsychiatric disorder associated with a deficiency in social behavior, among other deficits. Loss of Calcium-Calmodulin Dependent Kinase II alpha (CaMKII α) impairs hippocampal synaptic development, leading to behavioral symptoms of SCZ. However, since CaMKII α is involved in NMDAR-mediated synaptic plasticity, and inflammation, it is important to ascertain whether upstream receptors involved in specific aspects of synaptic function, and inflammation might be involved in altering CaMKII α during NMDAR hypofunction SCZ. This study was aimed at evaluating the expression of hippocampal CaMKII α in NMDAR hypofunction SCZ. Furthermore, we highlighted the role of neural IGF-1R and TLR4 in determining the fate of CaMKII α in this model. We found that WT-NMDAR hypofunction SCZ mice were deficient in sociability and social novelty tests. In addition to a loss of hippocampal CaMKII α (p<0.001), there was a significant decrease in IGF-1R versus the control (p<0.01). Subsequent analysis showed that the frequency of action potentials was reduced in SCZ neurons compared with controls (p<0.01). SCZ neurons showed a gap firing pattern, and a prolonged hyperpolarization leak current when compared with WT-control

(p<0.001). Since this current involves the movement of K^+ ions, we also found an increase in KCa2.2 expression in SCZ neurons versus the control (p<0.001). Owing to the role of TLR4 in synaptogenesis, we ask whether TLR4 contribute to the dysregulation of IGF-1R/CaMKIIα during NMDAR hypofunction. To elucidate this mechanism, NMDAR hypofunction was induced in TLR4^{loxp/loxp} and TLR4^{Cre/-} mice as described for WT. We found that IGF-1R/CaMKIIα loss was prevented in the hippocampus of TLR4^{Cre/-}, but not TLR4^{loxp/loxp} mice, after NMDAR hypofunction. Additionally, NMDAR hypofunction TLR4^{Cre/-} neurons showed no prominent change in the frequency of action potentials when compared with the control (WT). Likewise, KCa2.2-mediated AHP was attenuated. Ultimately, the TLR4^{Cre/-} mice recorded no substantive change in social interaction behavior when compared with a WT control. Taken together, the outcomes of this study indicate that loss of hippocampal CaMKIIα, and an increase in *KCa2.2*-mediated currents are characteristic of NMDAR hypofunction SCZ. Furthermore, the fate of hippocampal CaMKIIα- and TLR4 - which facilitates its depletion in NMDAR hypofunction.

Disclosures: O.M. Ogundele: None. C.C. Lee: None.

Poster

341. Pharmacology in Schizophrenia Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: H.03. Schizophrenia

Title: Clozapine activates autophagy through AMPK-ULK1-beclin1 signal pathway in the rat frontal cortex

Authors: *S. KIM, Y. KIM, S. PARK

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Abstract: Clozapine, one of the representative atypical antipsychotics, has superior efficacy and is used for the treatment of severe psychotic disorders. Therefore, studies on the action mechanisms of clozapine are important to unravel the therapeutic mechanisms of psychosis. Adenosine monophosphate-activated protein kinase (AMPK) is a serine-threonine kinase that plays a major role in maintaining metabolic homeostasis. Unc-51-like kinase (ULK1) is phsphorylated by AMPK and activates beclin1 which results in the activation of autophagy process. In this study, we have examined the effects of clozapine on AMPK-ULK1 and autophagy signaling in the rat frontal cortex. Clozapine (10 mg/kg) administration increased AMPK phosphorylation at Thr172 in rat frontal cortex at 1, 2, and 4 hr after injection, as we have previously reported. Phosphorylation of ULK1 at Ser317 and that of beclin1 at Ser93 was also increased at 2 and 4 hr after clozapine administration. At the same time, protein level of

LC3-II and ATG5-ATG12 conjugation were increased, which represent the activation of autophagy process. To investigate the involvement of AMPK in the clozapine-induced autophagy, the effect of intracerebroventriular injection of compound C, an AMPK inhibitor, was examined. Intrabrain administration of coumpond C efficiently inhibited the phoshporylation of AMPK substrates indicating the effective inhibition of AMPK pathway in the brain. In this condition, compound C attenuated the clozapine-induced increase in the phosphorylation of ULK1 and beclin1, LC3II, and ATG5-ATG12 in the rat frontal cortex. Taken together, clozapine induces autophagy signaling through AMPK-ULK1 signal pathway in the rat frontal cortex.

Disclosures: S. Kim: None. Y. Kim: None. S. Park: None.

Poster

341. Pharmacology in Schizophrenia Models

Location: Halls A-C

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Topic: H.03. Schizophrenia

Title: Identification of long-term gene expression changes in adult rat frontal cortex after neonatal treatment of NMDA receptor antagonist

Authors: *Y. KIM¹, H. PARK², S. KIM¹

¹Dongguk Univ. Med. Sch., Goyang-Si, Gyeonggi-Do, Korea, Republic of; ²Burke Med. Res. Inst., White Plains, NY

Abstract: Early postnatal administration of NMDA receptor antagonist induces long-term psychotomimetic behavioral changes in adult period, which have been used as one of the neurodevelopmental animal model of psychotic disorders. The model could provide the tool to understand the time-dependent alterations in the brain through the evolving process of psychosis development. In this study, we have examined the effects of MK-801 treatments on postnatal 7 day (PN7) of rats on the long-term changes in the gene expression and in the rat frontal cortex at PN60, and, subsequently, the effects of repeated treatments of electroconvulsive seizure (ECS) on these changes were investigated. MK-801 1.0 mg/kg was injected twice 8 hr apart at PN7. The rats showed reductions in the weight gain through developmental process, and showed dysfunctions in sensorimotor gating at PN60 as evidenced by prepulse inhibition (PPI). DNA microarray analysis using frontal cortical tissues at PN60 revealed that transcript of transthyretin gene was most highly up-regulated in MK-801 group compared to control group. Quantitative realtime PCR confirmed the increased expression of mRNA level of transthyretin in the rat frontal cortex of early-MK-801-treated group. Repeated ECS treatments for 10 days (E10X) were applied during PN50-PN59 period after MK-801 treatments at PN7, and the effects of ECS on MK-801-induced changes at PN60 were investigated. Reduced PPI in MK-801 group was

ameliorated by E10X and PPI in MK-801/E10X showed no significant difference compared to control group. In this condition, increased expression of transthyretin by MK-801 was reduced by E10X that mRNA level of transthyretin reached to the level in control group. AP-1 protein is involved in the transcriptional regulation of transthyretin. mRNA level of Egr1 and c-Fos, but not c-Jun, showed similar pattern of changes. In addition, expression of neprilysin gene which expression is regulated by histone acetylation concurrently with transthyretin is increased in MK-801 group and reduced to control level when E10X was applied. The findings suggest that transthyretin might play the important role in psychotomimetic behavioral changes induced by early postnatal treatment of NMDA receptor antagonist, and the investigations on underlying regulatory mechanisms could enhance the evolving process of psychotic disorders.

Disclosures: Y. Kim: None. H. Park: None. S. Kim: None.

Poster

341. Pharmacology in Schizophrenia Models

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Program#/Poster#: 341.05/UU79

Topic: H.03. Schizophrenia

Title: Mismatch negativity (MMN) in rats - A translational model in schizophrenia research

Authors: *N. UPTON¹, G. WADSWORTH¹, D. R. ANDERSON², T. PISER², S. C. LESIER² ¹TRANSPHARMATION LTD, LONDON, United Kingdom; ²CADENT THERAPEUTICS, CAMBRIGE, MA

Abstract: The mismatch negativity (MMN) component of the auditory event-related potential (AEP) is a key biomarker for the study of auditory sensory dysfunction in schizophrenia and other neuropsychiatric disorders. MMN reflects pre-attentive processing and is elicited clinically by an auditory oddball paradigm in which a physically different, deviant ('oddball'; DEV) stimulus occurs infrequently and unexpectedly within a sequence of repetitive identical stimuli ('standards', STD). Here we developed a system whereby we utilized the Data Sciences International telemetry system to provide the flexibility of wireless recording up to 8 male SD rats simultaneously in sound-attenuated chambers with the robust data handling and timestamping capabilities of the Cambridge Electronic Design micro1401 processor. A custom sequencer file enabled Spike2 software to generate tonal stimuli delivered to all rats simultaneously and timestamp the EEG data with digital precision. Rats were subjected to a standard oddball paradigm using 6kHz and 8kHz tones. This protocol elicited significantly larger components of the AEP during DEV than STD, specifically in the regions defined as equivalent to the clinical N1 and P2. Component values were calculated on the Difference Waves (DIFF) which were generated by subtracting STD from DEV. After validating rats elicited a robust MMN, we also set forth to demonstrate that the MMN could be pharmacologically impaired

using the NMDA antagonist MK-801. Given that NMDA receptors have a central role in the generation of the MMN, we hypothesized that MK-801 would impair MMN. Here we present this data. Under pre-drug conditions rats exhibited similar peak amplitudes for N1 (30-60ms after stimulus) and P2 (50-150ms) components of the difference wave. Between 40-60 minutes after administration vehicle-treated animals retained robust MMN, with DIFF wave component values for the N1 and P2 of $-10.6\pm1\mu$ V and $10.0\pm1\mu$ V, respectively. The MK-801 0.1mg/kg, s.c. dose elicited a slight, non-significant decrease in the DIFF wave (N1: $-8.1\pm1\mu$ V; P2: $7.5\pm1\mu$ V). Most importantly, however, MK-801 at 0.3mg/kg, s.c. significantly reduced the DIFF wave in comparison to vehicle, thereby demonstrating impaired MMN. Following 0.3mg/kg the DIFF N1 was $-4.6\pm1\mu$ V and P2 was $5.1\pm1\mu$ V. The 0.5mg/kg, s.c. dose elicited similar results as the 0.3mg/kg dose. In conclusion, we have demonstrated that rats have a clear electrophysiological correlate to clinical MMN and further that this potential can be impaired with NMDA antagonism. This establishes a key translational tool in the effort to detect novel therapeutics targeting the NMDA receptor to treat neuropsychiatric disorders.

Disclosures: N. Upton: A. Employment/Salary (full or part-time):; TRANSPHARMATION LTD. G. Wadsworth: A. Employment/Salary (full or part-time):; TRANSPHARMATION LTD. D.R. Anderson: A. Employment/Salary (full or part-time):; CADENT THERAPEUTICS. T. Piser: A. Employment/Salary (full or part-time):; CADENT THERAPEUTICS. S.C. Lesier: A. Employment/Salary (full or part-time):; CADENT THERAPEUTICS.

Poster

341. Pharmacology in Schizophrenia Models

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Topic: H.03. Schizophrenia

Support: NIMH Grant F31MH109238

Title: Simultaneous array recording in monkey MD thalamus, DLPFC, and the ACC related to cognitive control in a task measuring deficits in schizophrenia

Authors: *A. L. DENICOLA¹, M.-Y. PARK², J. L. ZICK¹, K. SCHULTZ³, D. A. CROWE⁴, M. V. CHAFEE⁵

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Abstract: Patients with schizophrenia experience a range of cognitive deficits that are strongly correlated with functional outcome but are not well treated by available therapeutics. The Dot-Pattern Expectancy Task (DPX) isolates a specific cognitive deficit in patients. First, a cue

stimulus is presented, followed by a delay, and then a probe stimulus that triggers a response. The cue provides contextual information that is required to properly respond to the probe. The four categories of 'Cue-Probe' pairs are 'AX', 'AY', 'BX', and 'BY'. 'AX' trials, 67% of the total trials, require a target response. The three other trials require a non-target response. Patients with schizophrenia perform selectively worse on 'BX' trials. Target brain areas associated with cognitive control and hypoactive in schizophrenia include the dorsolateral prefrontal cortex (dlPFC), the mediodorsal nucleus of the thalamus (MD), and the anterior cingulate cortex (ACC). These three brain regions are also intricately connected, creating a cognitive control network that may be directly affected in patients. Previous work in our lab has demonstrated that monkeys exhibit the 'BX' impairment when given an injection of Ketamine or Phencyclidine (PCP), N-methyl-D-aspartate receptor antagonists during DPX performance. The current study attempts to define the neural correlates of cognitive control in relation to DPX at baseline in the MD, dlPFC, and ACC, to further delineate the specific roles each of these brain structures play in cognition. Using 32 channel multi-electrode arrays, we recorded in two brain regions simultaneously, either MD-PFC or ACC-PFC, during DPX performance. Generally, task-related modulations in neural firing rate were found in parallel between the MD, dlPFC, and ACC, with some subtle differences. MD thalamus was strongly activated during the task, and MD neurons exhibit a 'switch' pattern of activity preferring B-cues during the cue period and A-cues during the probe period that we previously found was a hallmark of PFC activation in this task. MD neurons showed a prominent tendency to ramp to the response, and more neurons preferred the target (habitual) response in MD relative to PFC. This was found in ACC as well, where neural responses preferred target response over nontarget. Cue-selective and delay responses were notably weaker in ACC relative to the other two areas. Our results indicate an overarching distribution of DPX task-defined signals across MD, PFC and ACC with differences evident at the population level indicative of differential contributions to cognitive control, which may be affected in schizophrenia.

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Poster

341. Pharmacology in Schizophrenia Models

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JSPS JP14J01466 JSPS JP15K14964 JSPS JP17H03989 AMED the SRPBS

Title: Whole-brain mapping analysis of the NMDAR antagonist-induced neuronal activation

Authors: *K. SEIRIKI^{1,2}, A. KASAI², T. KUWAKI², M. NIU², Y. NAKA², H. IGARASHI², T. NAKAZAWA³, S. YAMAGUCHI⁴, Y. AGO², H. HASHIMOTO² ¹Osaka Univ., Suita, Japan; ²Grad. Sch. of Pharmaceut. Sciences, Osaka Univ., Suita, Japan; ³Grad. Sch. of Dentistry, Osaka Univ., Suita, Japan; ⁴Grad. Sch. of Medicine, Gifu Univ., Gifu, Japan

Abstract: Non-competitive N-methyl-D-aspartate receptor (NMDAR) antagonists impair neurobiological functions and induce psychotomimetic effects. Although pharmacological models of schizophrenia using NMDAR antagonists (e.g. MK-801) have been widely studied, brain regions responsible for the behavioral alterations are not well understood due to the ubiquitous expression of NMDARs. To explore the candidate brain regions responsive to NMDAR antagonists, we performed mapping of neuronal activation induced by MK-801 using Arc-dVenus reporter mice, in which a fluorescent protein, dVenus is driven by an Arc promoter. MK-801 increased dVenus expression in various cortical regions, and the most activated regions included the orbitofrontal cortex. Chemical lesions of this region attenuated the MK-801-induced hyperlocomotion without affecting the basal locomotor activity in the novel environment. We also performed whole-brain imaging of Arc-dVenus mice treated with MK-801 or methamphetamine, and compared the brain-wide activation patterns between these mice. The activation pattern induced by MK-801 was significantly different from that by methamphetamine, especially in the orbitofrontal cortex. We then examined the effects of antipsychotics on the MK-801-induced neuronal activation and found that the dVenus expression in the orbitofrontal cortex was effectively suppressed by an atypical antipsychotic, clozapine, but not by a typical antipsychotic, haloperidol. These results indicate that the orbitofrontal cortex is involved in the NMDAR-induced hyperlocomotion, and that the suppression of this region could be related to the therapeutic effects of clozapine. These findings will contribute to a better understanding of neural mechanisms for psychotic symptoms associated with NMDAR hypofunction.

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Poster

341. Pharmacology in Schizophrenia Models

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Title: COMT inhibitors improve cognitive flexibility in a modified version of the rat attentional set-shifting task

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Abstract: Impaired executive function is a core feature of many neurological and psychiatric disorders, including schizophrenia. Cognitive flexibility is a component of executive function that is highly predictive of outcome for patients with these disorders, making it an important target for drug development. The rat digging version of the attentional set-shifting task (ASST) is a validated measure of cognitive flexibility, but it is labor and time-intensive. Here we present a modified ASST protocol for testing cognitive flexibility. We validated our new protocol as a reliable assay for drug development using multiple catechol-O-methyltransferase (COMT) inhibitors. Our version of the ASST is a three-day protocol using adult, male Sprague Dawley rats. The first day consists of habituating rats to the testing room, task apparatus, and ensuring reliable digging for rewards. During day two, rats are trained to perform simple discriminations based on two perceptual dimensions (odor/digging medium). ASST testing occurred on day three, in which rats perform all seven stages of the task. Previously published habituation and training schedules were adapted in our task. These changes included a more structured habituation period and less restrictive time limits during training trials. Using neurochemical correlates of dopamine metabolism, we selected a couple of compounds to assess the utility of a COMT inhibition strategy as a means of increasing cognitive flexibility. Tolcapone, a potent COMT inhibitor, shows efficacy in a different version of the ASST and we used it as a positive control. Rats consistently dug for food rewards and learned positive environmental cues, allowing for a pre-testing dropout rate of <10%. The decrease in dropout rate did not affect group performance in the ASST as scoring in test stages was similar to those from previously published versions of the odor/medium ASST. Importantly, COMT inhibition increased cognitive flexibility measured as improved performance in the extradimensional shift stage, but did not affect performance in other stages, as had been previously reported. These results

confirm that COMT inhibition is a viable strategy for increasing cognitive flexibility in rats and the ASST is a useful screen for the procognitive effects of therapeutic candidates.

Disclosures: S.T. Byers: None. I. Buchler: None. M. DePasquale: None. J.C. Barrow: None. G.V. Carr: None.

Poster

341. Pharmacology in Schizophrenia Models

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Topic: H.03. Schizophrenia

Support: NIH MH107126

Title: Novel brain-penetrant COMT inhibitors alter peripheral and central dopamine metabolism

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Abstract: Catechol-O-methyltransferase (COMT) is a critical enzyme that metabolizes dopamine and other catecholamines in the periphery and central nervous system (CNS). COMT inhibitors have served as an adjunct treatment in Parkinson's disease (PD). COMT inhibitors boost central L-DOPA levels by inhibiting its peripheral metabolism in PD patients. COMT is also present in the brain and regulates dopaminergic function there, but the current FDAapproved COMT inhibitors have limited brain penetration. One COMT inhibitor, tolcapone, is able to cross the blood-brain barrier and exploratory, proof of concept studies have indicated that high doses may improve cognition in multiple neurological and psychiatric disorders. However, tolcapone has multiple disadvantages as a long-term treatment option. The brain to plasma ratio for tolcapone is 1:100. Due to a poor pharmacokinetic profile, tolcapone requires three doses a day and long-term use is associated with high risk for fatal liver toxicity. The limitations of tolcapone highlight the need for brain-penetrant COMT inhibitors with improved safety and efficacy profiles to determine if COMT inhibition is a viable strategy for cognitive enhancement. Here we describe novel brain-penetrant COMT inhibitors that are structurally distinct from the nitrocatechol inhibitors (tolcapone, entacapone, opicapone), but produce similar effects on peripheral and central dopamine metabolism. We utilized direct sampling of cerebrospinal fluid (CSF) and microdialysis within the medial prefrontal cortex (mPFC) of rats and our novel COMT inhibitors increased the concentration of 3,4-dihydroxyphenylacetic acid (DOPAC) and decreased the concentration of homovanillic acid (HVA). These changes in DOPAC and HVA indicate that our compounds significantly inhibit central COMT activity. Our compounds also inhibit the degradation of L-DOPA in the periphery, suggesting that these new COMT inhibitors

would also be effective as adjunctive therapies in PD patients. Taken together, these results indicate that the novel brain-penetrant COMT inhibitors may provide the same benefits as peripherally-restricted inhibitors with additional potential in the treatment of cognitive dysfunction and other indications modulated by central dopaminergic neurotransmission.

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Poster

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Program#/Poster#: 341.10/VV1

Topic: H.03. Schizophrenia

Title: Tegmental GABA receptor expression in a model for tardive dyskinesia

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Abstract: We have previously found nigral and tegmental glutamate decarboxylase (GAD) mRNA to be correlated with chronic haloperidol (HAL)-induced vacuous chewing movements (VCM), a rat model for tardive dyskinesia (Bachus et al., Behav. Brain Res. 231:323, 2012). Here we have measured GABA-A alpha-1 mRNA in tegmentum in this cohort, to explore the possibility that altered tegmental GABA receptor expression might mediate the effect of HAL on tegmental GAD activity.

Group housed male Long-Evans rats, with starting body weights of 90-165g, were treated for 24 weeks with HAL (28.5mg/kg/ml, i.m.: n=43) or vehicle (sesame oil: n=21) injections every 3 weeks. VCM were counted for each rat for 2 minutes weekly, for 7 weeks prior to HAL treatment and then throughout HAL treatment, by observers blinded to treatment group. Over the final 2 weeks, 4 samples were rated under both quiet (only ambient air-conditioning) and noisy (constant loud music and key-rattling) conditions. Cryostat-cut sections from fresh-frozen brains, containing the nigrotegmental projection field, were assayed by in situ hybridization histochemistry with oligonucleotide probes complementary to the GABA-A alpha-1 subunit mRNA, or a mis-sense control probe.

There was a non-significant trend toward an increase in tegmental GABA-A alpha-1 expression in HAL treated rats (t=-1.26, p=.21). Tegmental GAD mRNA was highly negatively correlated with tegmental GABA-A alpha-1 mRNA (r=-.59, p<.005) among controls, suggesting that there is normally an inverse relationship between these variables, but not among HAL-treated rats (r=-.23). Nor was tegmental GABA-A alpha-1 expression significantly correlated with VCM among HAL-treated rats. Interestingly, among HAL-treated rats, previously measured nigral tyrosine hydroxylase mRNA was highly correlated with tegmental GABA-A alpha-1 expression (r=.41, p<.006), suggesting that chronic HAL can upregulate tegmental GABA-A alpha-1 expression in HAL-treated rats in which nigral tyrosine hydroxylase is upregulated. However, altered tegmental GABA receptor expression does not appear to mediate the neural changes responsible for chronic HAL-induced VCM.

Disclosures: S.E. Bachus: None.

Poster

341. Pharmacology in Schizophrenia Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: H.03. Schizophrenia

Title: Translational utility of the mismatch negativity: Implementing MMN in rats for drug discovery

Authors: *D. BREGNA¹, D. R. ANDERSON², T. M. PISER², S. C. LEISER¹ ¹Translational EEG, Psychogenics, Tarrytown, NY; ²Cadent Therapeut., Cambridge, MA

Abstract: Mismatch negativity (MMN) is a validated, objective measure of central auditory processing and has become a key clinical biomarker in schizophrenia and holds utility in other neuropsychiatric disorders. MMN is an auditory evoked potential (AEP) elicited clinically by an auditory oddball paradigm in which a different, deviant ('oddball'; DEV) auditory (tonal) stimulus occurs infrequently and unexpectedly within a sequence of repetitive identical tonal stimuli ('standards', STD) and reflects pre-attentive processing dependent upon NMDA receptor function. Here we demonstrate back-translatability of this AEP in Sprague-Dawley (SD) rats and show that NMDA receptor antagonism can impair rat MMN. First, we developed a system whereby we utilized the Data Sciences International (DSI) telemetry system to provide the flexibility and high-throughput nature of wireless recording up to 16 SD rats simultaneously in sound-attenuated chambers with the robust data handling and timestamping capabilities of the Cambridge Electronic Design (CED) micro1401 processor. A custom sequencer file enabled Spike2 software to generate tonal stimuli delivered to all rats simultaneously and timestamp the EEG data with digital precision. Rats were subjected to a standard oddball paradigm using 6kHz and 8kHz tones and assessed 40-60min following MK-801 treatment. Vehicle-treated rats had a significantly larger response to DEV than STD as measured by the N1 peak (between 20-60ms) and the area under the curve (AUC) for N1 (30-60ms) while the N1 and N1-AUC for STD and DEV did not differ after MK-801 (0.3 mg/kg), revealing clearly inhibited AEPs after NMDA antagonism. Further, the Difference Waves (DIFF), generated by subtracting STD from DEV, revealed MK-801 significantly suppressed both N1 and P2 (between 50-150ms) peak amplitudes and AUCs. Showing clear dose-dependency, MK-801 (0.1 mg/kg) had intermediate effects but did not demonstrate as robust an impairment of MMN as 0.3mg/kg. This work further validates

that rats have a MMN correlate to that of the human and that this biomarker is dependent upon NMDA receptor function. Further, we demonstrate the ability to perform this in a higher-throughput manner necessary for drug discovery, which will lead to the further work needed to validate the translatability and predictive validity of the rat MMN.

Disclosures: D. Bregna: None. D.R. Anderson: None. T.M. Piser: None. S.C. Leiser: None.

Poster

341. Pharmacology in Schizophrenia Models

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Topic: H.03. Schizophrenia

Support: Allergan Inc.

Title: Rapastinel, a novel NMDA receptor modulator, produces prolonged rescue of subchronic phencyclidine - induced deficits in episodic memory as well as other beneficial effects on cognitive function in a rapamycin sensitive manner

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Abstract: Rapastinel (GLYX-13), a novel NMDAR modulator with rapidly-acting antidepressant (RAD) properties, lacks the psychotomimetic and cognitive impairing effects that sometimes occur with ketamine, another RAD under development. We have previously reported that rapastinel (i.v.) significantly prevented ketamine-induced deficits in novel object recognition (NOR), a test of episodic memory, in mice (Rajagopal et al., Behav Brain Res. 299:105-110). The antidepressant effects of rapastinel and ketamine are mediated by synaptic effects, suggesting neuroplasticity, which have been reported to be blocked by the mTOR signaling pathway inhibitor, rapamycin. The subchronic phencyclidine treatment produces an enduring deficit in NOR and other cognitive functions in rodents, mediated in part by the effects on GABAergic neurotransmission, which can be reversed both transiently and for several weeks by atypical antipsychotic drugs (AAPDs) and selective ligands which restore synaptic function (e.g., the 5-HT_{1A}R partial agonist, tandospirone and the 5-HT₇R antagonist SB269970). We now report that rapastinel (1 mg/kg sub cut), administered to subchronic PCP-treated C57BL/6J mice 30 min before acquisition trial, can restore NOR transiently, while rapastinel, (1 mg/kg b.i.d sub cut for 3 or 5 days), can restore NOR for 7 and >9 weeks, respectively, the most prolonged rescue by any drug we have observed. A single dose of rapastinel (1 mg/kg), was also able to restore NOR to normal levels following the wearing off of beneficial effects of 3 days of rapastinel b.i.d.
treatment. Rapastinel, tested 30 min, but not 24 h, post-injection restored cognitive flexibility, as measured by reversal learning (RL) in subchronic PCP-treated mice. These effects are comparable to those of AAPDs such as lurasidone. The ability of rapastinel (1 mg/kg sub cut) to restore NOR and RL in subchronic PCP-treated mice was blocked by rapamycin (0.2 nM, given ICV). The effects of rapastinel, PCP, and ketamine on neurotransmitter efflux in mPFC and hippocampus were compared. Rapastinel selectively enhanced DA efflux in the mPFC, while ketamine and PCP produced marked increases in efflux of glutamate and other neurotransmitters in both regions. DA and glutamate efflux were increased in the cortex and hippocampus during NOR in mice treated for 5 days with rapastinel b.i.d. and studied 8-10 weeks post-treatment. These results suggest rapastinel may improve cognitive impairment in mood disorders and that intermittent treatment schedules may be effective for both depression and cognitive impairment.

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Poster

341. Pharmacology in Schizophrenia Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 341.13/VV4

Topic: H.03. Schizophrenia

Title: The effect of inactivation of amygdalar GABAergic neurons on behaviors associated with schizophrenia in rats

Authors: *N. PREM¹, L. T. RAO¹, J. P. JOHN², B. M. KUTTY¹ ¹Dept. Of Neurophysiol., ²Dept. of Psychiatry, Natl. Inst. of Mental Hlth. & Neurosci., Bangalore, India

Abstract: Schizophrenia (SZ) is a complex neuropsychiatric disorder with heterogenous symptoms, characterized by abnormalities in sensory processing and cognition. Several postmortem studies in SZ patients postulates GABAergic transmission dysfunction in the limbic-lobe circuitry, that consists of interconnected brain regions such as dorsolateral prefrontal cortex, hippocampus, basolateral amygdala, anterior cingulate cortex (ACCx) etc. In previous studies, when a noncompetitive GABA_A receptor antagonist, picrotoxin was stereotaxically infused in the basolateral amygdala (BLA) in rats, it resulted in decrease in inhibition in BLA and increase in excitatory output from BLA to hippocampal formation and ACCx. Consequently, a decrease in density of glutamate decarboxylase 65 and 67 immunoreactive terminals was observed in sub-regions of hippocampus, specifically CA3 and CA2 regions in these rats. These changes mimics

postmortem findings and GABA defects implicated in SZ pathophysiology.

In the present study, we used this "partial" rodent model for neural circuitry abnormalities based on postmortem findings in schizophrenia to assess various schizophrenia-like behavior including cognitive dysfunction. Seven days after infusion of picrotoxin into the BLA and postsurgical recovery, the rats were subject to test battery consisting of open field test, social interaction test and pre-pulse inhibition to assess locomotor activity, social withdrawal and sensorimotor gating, respectively. Following assessment in test battery, two separate cohorts were used to assess long term effects of picrotoxin infusion in BLA on cognitive functions in these rats. One cohort was used to assess working memory deficits using radial arm maze task and another cohort was used to assess episodic memory deficits using Morris water maze task.

The rats infused with picrotoxin in BLA did not show locomotor hyperactivity; however, we could demonstrate deficits in social interaction and pre-pulse inhibition. When tested in working memory and episodic memory based tasks such as 8-arm baited radial maze task and Morris water maze task, respectively, these rats did not show cognitive deficits and results were comparable to that of control rats. The neural circuitry abnormalities found in this rodent model may be more specifically responsible for social cognition as we could demonstrate deficits in social interaction. However, episodic memory (spatial learning and memory) and working memory were spared, as this is predominantly hippocampal and prefrontal cortex dependent task, respectively and may not be affected by the changes found in the neural circuitry in this rat model.

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Poster

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Support: DA039991

DA038453

Title: The metabolites clozapine-N-oxide (CNO) and N-desmethylclozapine (NDMC, norclozapine) both share discriminative stimulus properties with the parent compound clozapine in mice and rats

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Abstract: The atypical antipsychotic drug clozapine has two major metabolites: clozapine-Noxide (CNO) and N-desmethylclozapine (NDMC, norclozapine). NDMC is considered to be the "active" metabolite and we have previously demonstrated that it shares discriminative stimulus properties with its parent compound clozapine in both mice (Philibin et al 2009; Wiebelhaus et al 2011, 2012) and rats (Prus et al 2009). In contrast, CNO is considered to be an "inactive" or "inert" metabolite of clozapine and has been utilized in many studies as an inert molecule to activate DREADDs (Designer Receptors Exclusively Activated by Designer Drugs). This has been called into question as MacLaren et al (2016) recently demonstrated that CNO reduced acoustic startle reflex and significantly attenuated d-amphetamine-induced hyperlocomotion in rats that lacked DREADD receptor expression. The present study examined the discriminative stimulus properties of CNO in both mice and rats trained to discriminate 1.25 mg/kg clozapine from vehicle in a two-lever drug discrimination paradigm. After clozapine discrimination was established, substitution tests with CNO (1.0 - 40.0 mg/kg) revealed that CNO partially substituted for the clozapine stimulus in both species. When CNO was tested in combination with low NON-generalizing doses of clozapine, partial to full substitution for clozapine's discriminative cue was obtained. These data replicate previous findings with NDMC and demonstrate that BOTH major metabolites of clozapine share discriminative stimulus properties with the parent drug. Thus, these data demonstrate that CNO is a behaviorally active compound in both mice and rats lacking DREADD receptors trained to discriminate clozapine. These results, along with recent findings by others, emphasize the need for appropriate control groups in studies employing DREADDs.

Disclosures: J.H. Porter: None. D.F. Manvich: 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.; DA039991, DA038453. K.A. Webster: None. S.L. Foster: None. C.J. Herting: None. S.N. Bramlett: None. M.S. Farrell: None. D. Weinshenker: 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; DA039991, DA038453. K.A. Webster: None. S.L. Foster: None. C.J. Herting: None. S.N. Bramlett: None. M.S. Farrell: None. D. Weinshenker: 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.; DA039991, DA038453.

Poster

341. Pharmacology in Schizophrenia Models

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Topic: H.03. Schizophrenia

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Title: A critical role for NF- κ B in the HDAC2-dependent control of synaptic remodelling and antipsychotic-related behaviours

Authors: *D. IBI^{1,2,3}, M. DE LA FUENTE REVENGA², J. GONZÁLEZ-MAESO^{2,3} ¹Chem. Pharmacol., Meijo Univ., Nagoya, Japan; ²Physiol. and Biophysics, Virginia Commonwealth Univ. Sch. of Med., Richmond, VA; ³Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Antipsychotic drugs, including both typical such as haloperidol and atypical such as clozapine and risperidone, were first described in the mid-twentieth century, yet they still remain the current standard of care for schizophrenia. Despite showing relatively tolerable effectiveness in treating hallucinations and delusions, chronic antipsychotic drug exposure leads at present to either persistent or exacerbated cognitive deficits in both clinical studies and preclinical animal models. However, the underlying molecular mechanism of antipsychotics' negative effects on cognition remains enigmatic. Here we identify functional dysregulation of the NF-κB pathway as a principal mediator responsible for deleterious effects of chronic antipsychotic drug treatment along numerous behavioural and physiological traits. Chronic treatment with atypical antipsychotics selectively enhanced NF- κ B (p65) translocation into the nucleus of pyramidal neurons in mouse frontal cortex-trafficking event that was triggered via serotonin 5-HT2A receptor and MAPK/ERK-dependent down-regulation of expression of the NF-kB repressor IκBα. Such up-regulation of NF-κB activity occurred in association with its increased binding at the promoter region of the Histone deacetylase 2 (Hdac2) gene, thereby augmenting Hdac2 transcription. This paradoxical increase in Hdac2 expression was impeded by either pharmacological or genetic inactivation of NF-kB. Notably, selective deletion of HDAC2 function in forebrain pyramidal neurons prevented the unfavourable effects of chronic antipsychotic treatment on cortical synaptic remodelling and cognitive processes. Conversely, virally mediated activation of cortical pyramidal NF-kB-dependent transcriptional activity minimized the formation of mature spine structures, decreased synaptic plasticity, and exacerbated psychosis-related behaviours and cognitive deficits through a signaling mechanism that required up-regulation of HDAC2. Together, our results suggest that activation of the NF-κB pathway by chronic atypical antipsychotic treatment increases HDAC2-dependent negative effects on synaptic plasticity and behaviour. These observations may aid in efforts to develop therapeutic strategies that improve the currently poor outcome in schizophrenia patients.

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Poster

341. Pharmacology in Schizophrenia Models

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Topic: H.03. Schizophrenia

Support: Innovate UK

Title: Gamma frequency oscillations are altered by a novel Kv3 channel modulator in rodent and human neocortical slices

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Abstract: Schizophrenia is a severe psychiatric disorder associated with a range of cognitive deficits. Studies in patients with schizophrenia and preclinical animal models have demonstrated abnormal synchronized high-frequency network activity and a dysfunction of parvalbumincontaining (PV+) GABAergic interneurons, both of which are critical for cognitive processing. Inhibitory fast-spiking PV+ interneurons coordinate synchronized activity (30-80 Hz) by firing at gamma frequencies and entraining large populations of cortical pyramidal cells. The fast spiking properties and temporal fidelity of PV+ interneurons are endowed by the selective expression of Kv3 channels on these cells. Thus, targeting Kv3 channels, and enhancing the activity of PV+ interneurons, has potential as a pharmacological treatment for cognitive symptoms of schizophrenia. Using the sub-chronic phencyclidine (PCP) rodent model of schizophrenia, we examined a range of concentrations of a novel Kv3 modulator (AUT00206) in vitro. Prior to brain slice in vitro studies, animals were behaviourally tested in the novel object recognition task to confirm cognitive deficits in PCP treated animals versus vehicle treated rats. Kainate/carbachol induced gamma oscillations were recorded from the prelimbic (PrL) region of prefrontal slices obtained from both groups of animals. We also examined the effect of AUT00206 in slices of human neocortical tissue. Non-epileptic tissue was obtained during tumour debulking procedures. Gamma frequency oscillations were elicited by the bath application of kainate (400-600 nM). The results show that higher concentrations of AUT00206 (10 and 20 µM) significantly increased the area power of gamma oscillations in PrL region in slices from PCP treated animals (10 μ M: 250 ± 59 μ V² v. 301.7 ± 88 μ V², 21.4 ± 8.9 %, p = 0.02, n = 15; 20 μ M: 148.6 ± 71 μ V²v. 157.6 ± 59 μ V², 27.2 ± 10.2%, p = 0.037, n = 10). Slices from vehicle treated animals showed a significant reduction in gamma area power at 20 µM AUT00206 (209.1 ± 99 μ V² v. 131.7 ± 57 μ V², -29.5 ± 7.3%, p = 0.016, n = 8). In human slices the power of kainate evoked gamma oscillations was unaltered by 10 μ M AUT00206 (-8.5 \pm 4.9

%, n = 9). In slices exposed to kainate and 10 μ M PCP, the power of gamma oscillations was increased by the drug (35.15 ± 10.5 %, p = 0.016, n = 7). Our results suggest that modulation of Kv3 channels by AUT00206 may have the potential to correct aberrant neuronal oscillations in patients suffering from schizophrenia by augmenting gamma frequency oscillations.

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Poster

341. Pharmacology in Schizophrenia Models

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Topic: H.03. Schizophrenia

Support: UK Innovate

Title: Modulation of fast network oscillations in the anterior cingulate cortex (ACC) *In vitro* by NMDA antagonists

Authors: *F. E. LEBEAU¹, J. HOWDEN¹, D. LOPEZ¹, N. MANZANZA¹, C. LARGE², M. CUNNINGHAM¹, T. MODEBADZE¹

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Abstract: The anterior cingulate cortex (ACC) plays a role in remote spatial memory, attention and executive functions. These cognitive functions are associated with network oscillations in the beta (20-30 Hz) and gamma (30-80 Hz) range. We have recently demonstrated that *in vitro* bath application of kainate can generate both beta and/or gamma oscillations either alone, or in combination, in the deep and superficial layers of ACC (Adams et al 2017). Changes in cortical beta and gamma frequency activity occur in patients with schizophrenia which may be linked to changes in NMDA function. We therefore wished to investigate the effects of NMDA modulation on both beta and gamma frequency activity in the ACC.

Following transcardial sucrose perfusion in anaesthetised rats ACC slices 450 uM thick were prepared from normal adult animals. Slices were transferred to an interface chamber for recording. Network oscillations were evoked using bath application of kainate (800 nM) for 1-3 hours until oscillation magnitude stabilised.

Following application of kainate beta and/or gamma frequency oscillations were observed in ACC. In slices in which beta frequency only activity was present acute application of the non-competitive NMDA receptor antagonist phencyclidine (PCP 10 microM) caused a dramatic increase in the power of the beta oscillations with an increase in the power of $110 \pm 42\%$ (n = 5). There was also a significant slowing of the frequency of the oscillation in the beta band from 25

 \pm 1.2 Hz to 20.6 \pm 0.5 Hz (P<0.05, n = 5). In contrast, in slices in which only a gamma frequency oscillation was present, PCP reduced the power of the gamma activity (n = 6). Interestingly the effects of PCP on network oscillations were considerably greater than those seen with either ketamine, MK-801 or DAP-5.

These results suggest that in the ACC NMDA blockade with PCP has different effects on beta and gamma frequency oscillations.

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Poster

341. Pharmacology in Schizophrenia Models

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Topic: H.03. Schizophrenia

Support: R01AG050518

Title: Effects of Intranasal Orexin-A on MK-801-induced attentional deficits

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Abstract: Schizophrenia (SZ) is a debilitating condition wherein those afflicted experience positive symptoms, including hallucinations and delusions, as well as negative cognitive and social symptoms. Antipsychotic pharmacotherapies targeting dopaminergic dysregulation are effective in alleviating positive symptomology of the disorder, but these drugs are unsuccessful in addressing the cognitive deficits. The glutamate hypofunction hypothesis of SZ asserts that the pathology of SZ primarily results from suppression of hippocampal NMDA receptor input, inciting imbalances in neurotransmitter release to striatal and cortical regions pertinent to cognitive processing. Orexin-A (OxA), an excitatory neuromodulator principally involved in wakefulness, has been suggested to potentiate NMDA receptor activity and enhance cognition. In the present study, the effects of OxA on attentional performance were examined in a NMDA receptor antagonist model of SZ. Male Fischer 344 Brown Norway F1 Hybrid rats received both intraperitoneal injections of MK-801 (0, 0.075, 0.125mg/kg) and intranasal administration of OxA (0, 5, 10nM) prior to placement in a sustained attention task requiring differentiation between signal trials (500, 100, and 25ms illumination of a central panel light) and non-signal trials (no light illumination). Following a dose-dependent impairment in both signal and nonsignal trial performance as well as an increase in trial omissions induced by MK-801 administration. OxA modestly improved detection of the 500 and 100 ms signals at the highest

and lowest doses of MK-801, respectively. OxA also reduced trial omissions following the smallest MK-801 concentration. However, at the highest dose of MK-801, OxA administration diminished relative correct rejection rates. Systemic NMDA receptor blockade and OxA are known to elevate cortical acetylcholine, a neurotransmitter that is necessary for performance in this attentional task. Thus, the decrease in correct rejections may be due to overstimulation of cortical cholinergic inputs. Taken together, despite potential overexcitation of prefrontal cortical neurons, OxA may have some beneficial impacts on cognition for individuals with conditions stemming from a hypofunctioning NMDA receptor system.

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Poster

341. Pharmacology in Schizophrenia Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 341.19/VV10

Topic: H.03. Schizophrenia

Support: NMRC/BNIG/2018/2014

NMRC/CG/013/2013 - Core 3 - Neuroscience Phenotyping Core

Title: Finasteride rescues some schizophrenia-like behaviours of the dopamine transporter knockout and MK-801-treated mice

Authors: *P. WONG¹, D. GROENEWOUD², L. MAK¹

¹Dept. of Pharmacol., ²Dept. of Biol. Sci., Natl. Univ. of Singapore, SINGAPORE, Singapore

Abstract: Neuroactive steroids are endogenously synthesized cholesterol-derivatives with multifaceted effects on brain function. Converging evidence implicates neuroactive steroids in the behavioural manifestations of mood disorders and their treatment. Recent research has shown that inhibitors targeting the 5α -reductase enzyme, such as Finasteride, have potential therapeutic efficacy in schizophrenia. $5-\alpha$ reductase is the rate-limiting enzyme of one of the three major metabolic pathways in brain steroidogenesis, and converts progesterone into $5-\alpha$ -dihydroprogesterone. Inhibition of 5α -reductase depletes brain levels of allopregnanolone, whilst increasing brain levels of progesterone and its downstream metabolites. These changes can alter behaviour. Here, we investigate the effects of Finasteride on the abnormal behaviours of two mouse models of schizophrenia; the dopamine transporter knockout (DAT-KO) mouse and the MK-801-treated mouse. The DAT-KO mice exhibit hyperdopaminergic tone due to their inability to reuptake dopamine from the synaptic cleft. These mice exhibit hyperactive and stereotypic behaviours, and recapitulate some schizophrenia-like behaviours, such as psychomotor agitations and sensorimotor gating deficits. MK-801 is a non-competitive

antagonist of the *N*-Methyl-*D*-aspartate receptor. When administered, MK-801 causes schizophrenia-like behaviours in mice, including hyperactivity and disrupted sensorimotor gating.

An single dose of 50mg/kg Finasteride was intraperitoneally administered to DAT-KO mice, 0.1mg/kg MK-801-treated mice, and their respective controls. The animals were then subjected to the open field and prepulse inhibition tests, to examine the effect of Finasteride on locomotor activity and sensorimotor gating, respectively. In the open field test, Finasteride normalized the hyperlocomotion of DAT-KO and MK-801-treated mice. The locomotor activity of control mice were not affected by Finasteride treatment. In the prepulse inhibition test, Finasteride rescued the sensorimotor gating deficits of DAT-KO mice, but only partially rescued the sensorimotor gating deficits of MK-801-treated mice. Finasteride did not affect sensorimotor gating in controls. Our findings join a growing body of literature suggesting that Finasteride has antipsychotic properties. [Supported by funds from NMRC/BNIG/2018/2014 to P.W.]

Disclosures: P. Wong: None. D. Groenewoud: None. L. Mak: None.

Poster

341. Pharmacology in Schizophrenia Models

Location: Halls A-C

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Program#/Poster#: 341.20/VV11

Topic: H.03. Schizophrenia

Support: Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ011582)

Title: Effect of tianeptine on the offspring of adult mice with maternal immune activation

Authors: H. LEE¹, Y.-O. KIM², S. LEE³, I. CHO⁴, S.-W. LEE², H.-K. KIM¹, J.-T. KWON¹, *H.-J. KIM¹

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Abstract: Epidemiological evidence implicates maternal immune activation (MIA) as a risk factor for developing neuropsychiatric disorder in later life, including bipolar disorder, autism, and schizophrenia. We investigated whether tianeptine treatment might function therapeutically on behavioral activity impairment and protein expression in offspring induced by MIA. Polyriboinosinic-polyribocytidilic acid (PolyI:C, 5 mg/kg) was administered intravenously to pregnant dams at gestational day 9. Adolescent offspring received daily injection of tianeptine (10 mg/kg i.p.) and clozapine (5 mg/kg i.p.) for 30 days starting on postnatal day 35. Changes in sensorimotor gating, forced swim test, open field test, and social interaction test and

neurodevelopmental protein expression were measured. Exposure to Poly I:C in early, gestation resulted in transient impairments in social interaction and sensorimotor gating deficits. Several neurodevelopmental proteins were downregulated in medial prefrontal cortex of MIA model. Chronic administration of tianeptine recovered sensorimotor gating deficits in MIA offspring. Additionally, the administration also reversed the downregulations of protein induced by MIA. Our results provide initial evidence that in MIA-induced model of schizophrenia, the disturbances in behavioral patterns and neurodevelopmental protein expressions were affected by the tianeptine administration.

Disclosures: H. Lee: None. Y. Kim: None. S. Lee: None. I. Cho: None. S. Lee: None. H. Kim: None. J. Kwon: None. H. Kim: None.

Poster

341. Pharmacology in Schizophrenia Models

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Topic: H.03. Schizophrenia

Support: NIH T32MH015330

NIH R01MH083728

Title: Region specific effects of astrocyte DISC1 on cognitive behaviors in mice

Authors: *C. TERRILLION¹, J. A. CRAWFORD², A. V. SHEVELKIN², S.-H. KIM³, D. FUKUDOME², A. SAWA⁴, A. KAMIYA⁵, M. V. PLETNIKOV² ¹Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, MD; ³Psychiatry and Behavioral Sci., ⁴Dept. of Psychiatry, ²Johns Hopkins Univ., Baltimore, MD; ⁵Dept Psychiatry and Behavioral Sci., Johns Hopkins Univ. Sch. Med., Baltimore, MD

Abstract: Background: DISC1, which is expressed in both neurons and astrocytes, has been identified as a genetic risk factor associated with major psychiatric disorders, including schizophrenia. Neuronal Disc1 in the hippocampus and the prefrontal cortex has been implicated in cognitive function. However, the role of Disc1 in astrocytes, which have increasingly been shown to play a role in normal cognition, has not been evaluated. We hypothesized that decreased expression of DISC1 in astrocytes in the hippocampus or prefrontal cortex would impair cognitive behavior in mice.

Methods: Mice were injected with the vector AAV1-GFAP::GFP-miR30-DISC1 (Disc1 KD) in the hippocampus or prefrontal cortex (PFC) to knock down DISC1 in astrocytes, or the scrambled control vector AAV1-GFAP::GFP-mir30-Ctrl (Ctrl). 2 weeks following injections, mice were tested in anxiety related measures including elevated plus maze and the open field, as

well as several complex cognitive behaviors, including social interaction, Barnes maze and trace fear conditioning.

Results: Injection of AAV1-GFAP::GFP-miR30-DISC1 led to significant reduction of DISC1 expression compared to mice injected with the control vector. DISC1 KD in hippocampal or prefrontal cortex astrocytes did not alter locomotor activity or anxiety related behavior. DISC1 KD in astrocytes in the hippocampus, however, significantly decreased social preference and reduced preference for a novel mouse in the social interaction test. Additionally, DISC1 KD in hippocampal astrocytes impaired performance in the Barnes maze and reduced cue-dependent freezing in in trace fear conditioning. While DISC1 KD in PFC astrocytes also impaired performance in the Barnes maze, no significant effects of this KD were observed on cue-dependent freezing in trace fear conditioning. We found that DISC1 KD altered astrocyte morphology through increased branch diameter.

Conclusion: Reduction of DISC1 expression in brain astrocytes leads to brain region-dependent deficiencies in complex cognitive tasks. Changes in astrocyte morphology following DISC1 KD suggests that astrocytic dysfunction likely contributes to the cognitive deficits. Further understanding of the role DISC1 has in astrocyte function in relation to cognitive behaviors will allow us to improve treatment of cognitive symptoms in patients with major psychiatric disorders.

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Poster

341. Pharmacology in Schizophrenia Models

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 341.22/VV13

Topic: H.03. Schizophrenia

Title: Oxytocin effects on social behavior are genetically modulated by cortical functioning

Authors: *M. NIGRO¹, S. BRUNI², V. FERRETTI³, F. PAPALEO⁴ ¹Neurosci., Inst. Italiano Di Tecnologia, Genova, Italy; ²Neurosci., Baylor Col. of Med., Houston, TX; ³Inst. Italiano di Tecnologia, Genova, Italy; ⁴Fondazione Inst. Italiano Di Tecnologia, Genova, Italy

Abstract: Social behavior is a complex function mediated by multiple brain regions and neural circuits, which is crucial for animals' survival and development. Neuropsychiatric diseases such as schizophrenia and autism are characterized by social dysfunctions, and there is growing enthusiasm concerning the potential use of intranasal oxytocin (OXT) for the treatment of social deficits. However, clinical and preclinical findings are warning that OXT might not be indiscriminately beneficial, but could even produce detrimental effects in healthy subjects. There

is then a need to understand the brain mechanisms at the base of such variability. Here, we explored the hypothesis that clinically-relevant dysbindin-1 genetics might indicate a subpopulation of subjects positively responding to intranasal OXT. Genetic variations reducing dysbindin-1 in mice results in social deficits and alterations in the OXT system. Following intranasal OXT, we observed improved social responses in dysbindin-1 mice, while in control mice we confirmed the socially detrimental effects previously described. Recent studies from both humans and mice reported that intranasal OXT strongly activates the prefrontal cortex (PFC), a brain region involved in social functioning. Thus, we performed in vivo single-unit electrophysiological recordings in the medial PFC (mPFC) of wild-type and dysbindin-1 heterozygous mice treated with either vehicle or intranasal OXT during free social interactions. We found an increased activity of the mPFC before the onset of social behaviors in wild-type mice treated with vehicle. This effect was abolished after intranasal OXT treatment. Remarkably, dysbindin-1 mice treated with vehicle showed an increased basal activity of the mPFC compared to control mice with no modulation by social contacts. In contrast, intranasal OXT recapitulated a similar pattern of mPFC activation as that observed in wild-type mice treated with vehicle. In summary, we found a divergent effect: in wild-type mice OXT induced alterations in PFC neuronal patterns and social deficits; in dysbindin-1 mice OXT restored PFC neuronal patterns and rescued social deficits. These findings provide initial evidence on how intranasal OXT might tightly regulate social behaviors and associated mPFC activity depending on different genetic backgrounds.

Disclosures: M. Nigro: None. S. Bruni: None. V. Ferretti: None. F. Papaleo: None.

Poster

342. Connectomics: Anatomical Techniques

Location: Halls A-C

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Program#/Poster#: 342.01/VV14

Topic: I.03. Anatomical Methods

Support: Columbia University Startup grant

Title: Light sheet theta microscopy for high-resolution quantitative imaging of large biological systems

Authors: *B. MIGLIORI¹, M. S. DATTA², M. C. APAK³, O. HERMANSON⁴, R. TOMER³ ¹Biol. Sci., Columbia UNIVERSITY, New York, NY; ²Neurobio. and Behavior, Columbia Univ., New York, NY; ³Columbia Univ., new york, NY; ⁴Dept. of Neuroscience, Karolinska Institutet, Stockholm, Sweden

Abstract: Advances in tissue clearing and molecular labelling methods are enabling unprecedented optical access to large intact biological systems. These advances fuel the need for

high-speed microscopy approaches to image large samples quantitatively and at high resolution. The Light Sheet Microscopy (LSM) approach of illuminating a sample with a thin sheet of light, and detecting the emitted signal with an orthogonally arranged detection arm provides two main advantages: minimal energy load and high imaging speed. However, LSM as a general approach has been restricted in the lateral dimension of image volumes because the illumination light sheet enters from the side of the sample, and needs to penetrate the entire sample and the working distance of the illumination objective places a hard physical limit of the imaging volume. In addition, physical tissue expansion approaches for achieving high imaging resolution are producing even larger samples. To address these challenges, we developed a conceptually distinct imaging framework, called Light Sheet Theta Microscopy (LSTM). As for LSM, LSTM is based on planar illumination, but achieves this goal by using non-orthogonally arranged illumination objectives to produce light sheets that intersect the detection plane in a line profile, which is then synchronously (along with line-by-line detection of sCMOS camera) scanned along the detection plane. An immediate advantage of such a configuration is that it alleviates the restrictions on lateral sample dimensions, while providing uniform image quality for achieving true quantitative imaging. We found that a strategy of simultaneous 2-axes illumination (i.e. along and perpendicular to light sheet propagation) provided the best imaging performance. In comparison to LSM, LSTM allows imaging of larger samples such as a ~2 cm wide and ~5mm thick rat brain slice - with high uniform resolution across the entire sample. Moreover, LSTM is uniquely compatible with live imaging of highly motile samples, which undergo non-isomorphic change

Disclosures: B. Migliori: None. **M.S. Datta:** None. **M.C. Apak:** None. **O. Hermanson:** None. **R. Tomer:** None.

Poster

342. Connectomics: Anatomical Techniques

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 342.02/VV15

Topic: I.03. Anatomical Methods

Support: Columbia University Startup grant

Title: Rapid high-resolution mapping of mammalian brain architecture

Authors: *M. S. DATTA¹, B. MIGLIORI³, M. C. APAK², R. TOMER² ¹Neurobio. and Behavior, ²Columbia Univ., New York, NY; ³Karolinska Institutet, Stockholm, Sweden

Abstract: Advances in tissue clearing and molecular labelling techniques are enabling unprecedented optical access to large intact biological systems. The current imaging approaches,

however, are limited in capabilities to quantitatively image large specimens, such as a rat or large human brain slice, with uniform high-resolution and high-speed. To address these limitations, we recently developed a high-speed and high-resolution quantitative microscopy framework, called Light Sheet Theta Microscopy (LSTM). By building upon the principles of Light Sheet Microscopy (LSM), LSTM allows for rapid quantitative high-resolution imaging of large biological systems while maintaining the imaging depth and speed. Building upon this work, we have also developed a highly optimized integrated pipeline, including optimal clearing, imaging and data analytics, for high-resolution and high-content mapping of large biological systems. We validate the pipeline with several large-scale example datasets, including from rodent and human brains. These methods can enable a detailed three-dimensional view of normal and abnormal tissue architecture to facilitate deeper understanding of the structure-function relationships of highly complex organs such as the brain.

Disclosures: M.S. Datta: None. B. Migliori: None. M.C. Apak: None. R. Tomer: None.

Poster

342. Connectomics: Anatomical Techniques

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: I.03. Anatomical Methods

Support: NIH Grant NS091421

Title: Automated neuron tracing with deep learning and random sample consensus

Authors: *S.-L. WANG, S. MOUSAVI KAHAKI, A. STEPANYANTS Physics, Northeastern Univ., Boston, MA

Abstract: Recent advances in genetic engineering and optical microscopy have allowed neuroscientists to label sparse populations of neurons and image their arbors in 3D on the scale of the entire brain. At present, semi-manual tracing is the only reliable way of extracting information about the layout of axonal arbors of individual neurons from such imaging data. However, semi-manual tracing is extremely time consuming, can be prone to user bias, and, therefore, it is unsuitable for high-throughput experiments. In this study we describe an automated algorithm which has the potential to overcome the challenges of semi-manual tracing. This algorithm consists of four major steps. In the first step, a feed forward artificial neural network is trained on optimized, semi-manual traces of expert users to segment neurites. Our result show that one or two hidden layers in the network trained with the stochastic gradient descent method are sufficient to produce satisfactory segmentation results. In the next step, seed points are detected in the segmented image by using the multi-scale Laplacian of Gaussian filter. These points are subsequently connected all-to-all with the minimum cost paths generated with the A* algorithm. In the third step, multiple trees are produced by sampling the seed points and applying the Minimum Spanning Tree algorithm to the matrix of costs associated with connecting the sampled seeds. These trees are then combined with the modified RANdom SAmple Consensus method. Finally, topological errors in the branching pattern of the combined trace are corrected by using a specifically designed Support Vector Machine. The described automated tracing approach produces results comparable to semi-manual traces in terms of accuracy, while providing tremendous improvement in tracing time.

Disclosures: S. Wang: None. S. Mousavi Kahaki: None. A. Stepanyants: None.

Poster

342. Connectomics: Anatomical Techniques

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Topic: I.03. Anatomical Methods

Support: NIH Grant NS091421

Title: Automated registration of light microscopy image stacks designed for whole-brain imaging experiments

Authors: *S. MOUSAVI KAHAKI, S.-L. WANG, A. B. STEPANYANTS Physics, Northeastern Univ., Boston, MA

Abstract: The ability to map neural circuits on the scale of an entire brain is critical for advancing our understanding of brain functions. Circuit mapping can be based on whole-brain imaging of sparsely labeled populations of neurons with 3D confocal or two-photon microscopy. Such an imaging experiment, if applied to the mouse brain, will result in tens of thousands of image stacks, totaling several terabytes of data. Registration of these image stacks is the first step on the way to accurate, automated circuit mapping. Because, imaging is generally done with small overlaps between neighboring stacks, the information contained in the stack overlap region can be used for registration. Here, we describe an automated registration method which consists of the following steps. First, multi-scale Laplacian of Gaussian filter is used to detect small features in all stacks associated with background fluoresce. Second, for all overlapping stack pairs we compute a matrix describing similarity for features detected in the stacks. This similarity matrix is then used as an input to the Hungarian algorithm to establish initial correspondence between the features. Third, Random Sampling Consensus algorithm is employed to eliminate outliers from the initial set of corresponding features. Fourth, global optimization is performed to find the best rigid or non-rigid transformation for simultaneously registering the entire set of stacks. Finally, the registered stacks are resampled into nonoverlapping tiles that can be used for visualization and neuron tracing. Accuracy of the proposed

algorithm was tested by tracing multiple axons in individual stacks and calculating the distances between traces of same axons in the stack overlap regions before and after the registration.

Disclosures: S. Mousavi Kahaki: None. S. Wang: None. A.B. Stepanyants: None.

Poster

342. Connectomics: Anatomical Techniques

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Crick-Clay Fellowship

H. N. Mahabala Chair

NSF Eager 1450957

NIH DA036400

Title: Computational anatomy methods for registration of high resolution mouse brain histology images using multichannel LDDMM

Authors: ***B. LEE**¹, D. J. TWARD², D. D. FERRANTE³, K. RAM⁵, P. P. MITRA⁴, M. I. MILLER¹

²Biomed. Engin., ¹Johns Hopkins Univ., Baltimore, MD; ³Cold Spring Harbor Lab., Cold Spring Harbor, NY; ⁴Cold Spring Harbor Lab., Cold Spg Hbr, NY; ⁵IIT Madras, Chennai, India

Abstract: New developments in histological brain sectioning techniques have enabled histology imaging at sub-micron resolution, creating a need for computational methods for large scale imaging data. This work describes the development of a pipeline for registering histology volumes of different imaging modalities to an atlas and presents an application in mouse brain connectivity analysis.

Brain sections are downsampled and reconstructed into volumes using a variational method which simultaneously optimizes the position of all slices along with a regularizing prior to weight the relationship between slices against global distortions. Bayesian template estimation is used to compute a population average atlas. Mapping of the estimated atlas' coordinate systems onto each reconstructed brain is computed by the multichannel LDDMM algorithm, wherein

multiple high-dimensional matching markers arising from image intensity and the convex hull of brain structures are defined and their sum of squared error minimized, producing the optimal diffeomorphic mapping. Cross modality registration is driven by non-MSE metrics for cases where a corresponding atlas modality may not exist. Due to the smooth, 1-to-1 properties of diffeomorphisms, the resulting transforms can be upsampled back to native resolution where they retain these desirable traits.

The pipeline has been applied in collaboration with the Mouse Brain Architecture Project to register 600+ high resolution mouse brains for connectivity analysis. Brains in this dataset had been injected with fluorescent tracer prior to being sectioned at 20 um. Sections were alternately Nissl stained and fluoro imaged at 0.46 um coronal resolution.

Each of the full resolution reconstructed MBA volumes were mapped onto a common atlas space, with the resulting segmentation viewable online through the MBA Portal's online viewer. The viewer overlays the Nissl and fluorescence image data with the registered atlas segmentation, and provides interactive cell-counting tools for connectivity studies. Workflow and sample results are shown in the figure.



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Poster

342. Connectomics: Anatomical Techniques

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NINDS

CNRM

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Title: Tape-transfer enabled 3D digital histology for whole human brains with MRI coregistration

Authors: *A. S. TOLPYGO¹, Z. LODATO¹, A. GRIFFIN², B. C. LEE³, M. M. MILLER³, L. LATOUR², P. P. MITRA¹

¹Cold Spring Harbor Lab., Cold Spring Harbor, NY; ²Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD; ³Johns Hopkins Univ., Baltimore, MD

Abstract: High-fidelity 3D neuro-histological reconstructions of whole brains with light microscopic resolution have not yet been achieved for human. Our laboratory previously standardized a tape-transfer based, high-throughput method for 3D digital histology in Mouse, Rat, Zebrafinch, and Marmoset. We present an extension of this method for processing hemisphere slabs of human brain that will permit histological processing and imaging of entire coronal sections without tissue blocking. This is the first step in a larger effort for creating a pipeline for whole human brain digital histology.

Processing of neuroanatomical tissue is subject to many forms of distortion. Some are due to the sectioning method, others to the handling of the tissue through embedding, and blocking tissue to fit on traditional histology slides. In most cases, these result in damage to select regions of a given histological section with distortion of the tissue morphology that causes problems in applications that require the co-registration of 2D sections and subsequent 3D reconstruction. A fixed hemisphere slab of brain tissue of a TBI subject imaged at 7T postmortem was processed to capture every section onto large format slides (100x130mm or larger). Brain tissue was sectioned at 20µm using a custom tape-transfer system; mounted sections were stained with H&E and Iron/Prussian blue. A third series was kept for additional stains. All sections are scanned using a whole-slide imager at 0.46µm/pixel. This large-format approach studies the morphology of traumatic vascular injury seen in human post-mortem tissue and adds microscopic details to findings on the in-vivo MRI. Mechanical forces acting on the parenchyma are suspected to cause direct injury to the vasculature; microbleeds can be seen on T2* weighted or SWI MR images of patients. It is suggested that iron accumulates along the track of the vessel and surrounds the neighboring area. Pathology demonstrates iron-laden macrophages in perivascular space, along small and large vessels associated with the damage site along with disruptions in cellular structure and myelination. We create a brain stack by aligning images within a reference frame via 2D rigid transformations. The stack is registered to the MRI by optimizing correspondences between the volumes through a nonlinear transformation. The transformation is computed using the large deformation diffeomorphic metric mapping algorithm with a mutual information cost metric suitable for cross-modality matching, producing smooth and 1-to-1 deformations inherent

to diffeomorphisms. The aligned 3D-stack is further processed to yield reconstructed intensity projections in each plane.

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Poster

342. Connectomics: Anatomical Techniques

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Topic: I.03. Anatomical Methods

Support: CAS XDB02050002

Fundamental Research Funds for the Central Universities WK6030000033

Title: Scalable volumetric imaging at subcellular resolution for high-speed brain mapping

Authors: ***H. WANG**¹, Q.-Y. ZHU², L.-F. DING¹, Y. SHEN¹, Q.-R. YANG¹, C. SHU³, H. HAN³, Z.-W. XIONG¹, J.-N. ZHOU¹, F. WU¹, P.-M. LAU¹, G.-Q. BI¹ ²Integrative Imaging Center, Hefei Natl. Lab. for Physical Sci. at the Microscale, ¹Univ. of Sci. and Technol. of China, Hefei Anhui, China; ³Inst. of Automation Chinese Acad. of Sci., Beijing, China

Abstract: We developed a new, scalable strategy of volumetric imaging with single beam scanned illumination and perpendicular planar detection that achieves $0.5*0.5*5\mu$ m voxel resolution imaging of an entire mouse brain structure within 3 hours. We also developed an optimized pipeline of thick brain slice sample preparation as well as automated 3-D reconstruction and image analysis. The whole procedure from sample preparation to cell counting for activity mapping of multiple mouse brains with antibody-based cell type identification could be completed within 5 days.

Disclosures: H. Wang: None. Q. Zhu: None. L. Ding: None. Y. Shen: None. Q. Yang: None. C. Shu: None. H. Han: None. Z. Xiong: None. J. Zhou: None. F. Wu: None. P. Lau: None. G. Bi: None.

Poster

342. Connectomics: Anatomical Techniques

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Program#/Poster#: 342.08/VV21

Topic: I.03. Anatomical Methods

Support: Pritzker Neuropsychiatric Research Consortium

Title: Hybridization chain reaction based fluorescence *In situ* hybridization in iDISCO cleared brain tissues

Authors: *V. KUMAR¹, D. M. KROLEWSKI¹, B. MARTIN², H. AKIL³, S. J. WATSON, Jr.³ ¹Mol. and Behavioral Neurosci. Inst., ²MBNI, ³Univ. of Michigan, Ann Arbor, MI

Abstract: Fluorescence in situ hybridization (FISH) in intact tissues has potential to provide vital spatiotemporal information for molecular characterization of heterogeneous neuronal populations. At present, mainly two fluorescent methods are available for detecting mRNAprobe hybridized complex: 1) Peroxidase catalyzed tyramide amplification method- using Digoxigenin/biotin/FITC labelled oligo/cRNA probes. This method however fails to give uniform signal across the depth in tissues thicker than 300-500 µm which is mainly due to the rapid deposition of reactive tyramide on the superficial layers. Additionally, this method is not fully compatible with CLARITY or iDISCO clearing methods. 2) The Hybridization chain reaction (HCR) method - where binding of single stranded cDNA probes to the target mRNAs initiate chain reactions in which metastable fluorophore-labeled DNA hairpins self-assemble into tethered fluorescent amplification polymers (Choi et al., 2014). Although HCR method has been shown to work with CLARITY, it remains unexplored with clearing methods like iDISCO or CUBIC which depending upon molecular targets and tissue type can yield better volumetric staining and visualization. Hence, we investigated the compatibility of HCR-FISH with different clearing methods- iDISCO and CUBIC along with CLARITY. Following the tissue processing and FISH, slices were imaged on confocal/COLM systems and quantified for the uniformity of cellular labelling and S/N ratio across the tissue depth and region. Based on our preliminary results, iDISCO showed the most uniform labelling of cells and high S/N ratio for FISH in fresh frozen slices thicker than 2 mm, whereas with CLARITY (<400 µm) or CUBIC (<100 µm) failed to show comparable staining. The combination of two simpler methods-HCR and iDISCO provides the best opportunity for high resolution intact tissue FISH. However, generation of functional 50-mer DNA probes flanked by initiator sequence appears to be the most challenging part of this method.

Disclosures: V. Kumar: None. D.M. Krolewski: None. B. Martin: None. H. Akil: None. S.J. Watson: None.

Poster

342. Connectomics: Anatomical Techniques

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Support: Doctoral school grant from ED3C

LabexLifesenses

Title: Novel variants of adeno-associated virus type 2 provide enhanced anterograde transsynaptic gene delivery

Authors: *A. PLANUL¹, M. DEROSIERS¹, C. NGUYEN², F. MARTI², D. DALKARA¹ ¹Thérapie génique et modèles animaux des maladies neurodégénératives, Inst. De La Vision, Paris, France; ²Neurophysiologie et Comportements, Inst. de Biologie Paris-Seine, Paris, France

Abstract: Viral tracers are widely used tools to study the neural circuitry of the brain. Adenoassociated viruses (AAVs) are particularly versatile vectors as they are non-pathogenic and display low immunogenicity. Despite the success of new AAV variants for retrograde transsynaptic labeling; no such variant exists for anterograde trans-synaptic transport. The only available vectors capable of anterograde axonal spreading are the toxic rabies and HSV1 viruses and to some extent AAV serotypes 1 and 9. However, AAV1 and 9 are not efficient enough and need amplification of the transported transgene to be useful. Thus, we lack an efficient, non toxic vector capable of trans-synaptic anterograde transport. Here we used a high-throughput screening approach, called directed evolution on AAV serotype 2 (AAV2) to find new AAV variants with enhanced trans-synaptic anterograde transport abilities. Because the anterograde trans-synaptic transport capacity depends on the viral capsid, three random DNA libraries of the cap2 gene coding the viral capsid of AAV2 have been created. Then, 3 random viral libraries were made on which we applied 6 consecutive cycles of selection. For each selection cycle, viral libraries transporting their own *cap* gene were injected into the eye and the *cap* genes found into visual regions of the brain were amplified. After 4 cycles, a convergence toward two modified capsids was observed. These variants were able to do efficient trans-synaptic anterograde transport and provide strong reporter gene expression in post-synaptic neurons after delivery in the brain. Our data suggests that we were able to improve the anterograde trans-synaptic transport properties of AAV2. These new variants open many new opportunities to study brain circuits. Currently, we are in the process of evaluating them for functional studies. Indeed, if these variants are capable of sufficient trans-synaptic expression of reporter genes like GCamP, we would be able to measure simultaneously the activity and the connectivity of neuronal networks without toxicity impairing the signal. We anticipate these variants will also be useful in gene therapy approaches where efficient spreading of regenerative factors in connected brain regions is necessary. Transsynaptic gene delivery might limit the number of injection sites in gene therapy approaches for diseases like Parkinson's disease.

Disclosures: A. Planul: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); In process to have a patent, Confidential before SfN's 47th annual meeting, Neuroscience 2017. M. Derosiers: None. C. Nguyen: None. F. Marti: None. D. Dalkara: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); in process to have a patent, Confidential before SfN's 47th annual meeting, Neuroscience 2017.

Poster

342. Connectomics: Anatomical Techniques

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 342.10/VV23

Topic: I.03. Anatomical Methods

Support: NHI Grant U01MH105971

Title: Dendritic anatomy analysis using oblique light sheet tomography

Authors: *A. NARASIMHAN¹, U. SÜMBÜL², K. UMADEVI VENKATARAJU¹, D. F.

ALBEANU¹, P. OSTEN¹ ¹Cold Spring Harbor Lab., Cold Spring Harbor, NY; ²Grossman Ctr. for the Statistics of Mind, Columbia Univ., New York City, NY

Abstract: Oblique Light Sheet Tomography (OLST), which operates on the principle of light sheet fluorescence microscopy, is a whole brain volumetric imaging platform. The oblique orientation, in which the illumination/detection paths are oriented obliquely (45°) with respect to the tissue surface, allows for imaging the surface of the brain upto ~400 µm depth in an x-y raster pattern. Once the raster scan is completed, the brain is translated to an integrated vibratome to section the imaged portion of the tissue. The raster scan and automated sectioning is repeated iteratively to obtain whole brain coverage. Since tissue scattering limits the light penetration depth, we modified and optimized a clearing protocol based on the CUBIC protocol for clearing the brains. Clearing removes the lipids in the tissue, making the brain structurally soft. We developed a novel gelatin based embedding to improve the rigidity of the brain so that it can be sectioned in our integrated vibratome. Based on the current instrument configuration, a cleared adult mouse brain is imaged within ~14 hrs at 0.4 x 0.4 x 2.5 µm voxel resolution with overlapping regions for image registration and reconstruction. We use this custom built OLST to understand the somatodendritic morphology and axonal projections of pyramidal cells in the mouse neo-cortex. This is achieved by sparse labeling of the pyramidal cells (Emx-Cre) using

intra-venous injections of Cre dependent reporter viruses. Manual annotation of neuronal morphology is time consuming and labor intensive. To overcome these problems and to process the large volumes of data that our OLST platform generates, we developed a supervised machine learning-based approach: deep neural networks are trained to enhance binarize the raw microscopy images to and subsequently obtain the skeletons of neuronal arbors automatically. This framework allows us to study branching and positioning patterns of cortical neurons at an unprecedented scale, which is a prerequisite for robust identification of cell types.

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Poster

342. Connectomics: Anatomical Techniques

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Topic: I.03. Anatomical Methods

Support: KAKENHI grants 16K13116, 16H01888

Title: A spherical aberration free microscopy system for live brain imaging

Authors: *Y. UE^{1,2}, H. MONAI^{3,2}, K. HIGUCHI^{1,2}, D. NISHIWAKI^{1,2}, T. TAJIMA^{1,2}, K. OKAZAKI^{1,2}, H. HAMA³, H. HIRASE³, A. MIYAWAKI^{3,2} ¹R&D Group, OLYMPUS Corp., Hachioji-shi / Tokyo, Japan; ²RIKEN BSI-Olympus Collaboration Ctr., Wako, Japan; ³RIKEN BSI, Wako, Japan

Abstract: The high-resolution in vivo imaging of mouse brain for quantitative analysis of fine structures, such as dendritic spines, requires objectives with high numerical apertures (NAs) and long working distances (WDs). However, this imaging approach is often hampered by spherical aberration (SA) that results from the mismatch of refractive indices in the optical path and becomes more severe with increasing depth of target from the brain surface. Whereas a revolving objective correction collar has been designed to compensate SA, its adjustment requires manual operation and is inevitably accompanied by considerable focal shift, making it difficult to acquire the best image of a given fluorescent object. To solve the problem, we have created an objective-attached device and formulated a fast iterative algorithm for the realization of an automatic SA compensation system. The device coordinates the rotation and the z-position of an objective, enabling correction collar adjustment while stably focusing on a target. The algorithm provides the best adjustment on the basis of the calculated contrast of acquired images. Together, they enable the system to compensate SA at a given depth. As proof of concept, we applied the SA compensation system to in vivo two-photon imaging with a 25×water-immersion objective (NA, 1.05; WD, 2 mm). It effectively reduced SA regardless of location, allowing quantitative and

reproducible analysis of fine structures of YFP-labeled neurons throughout the mouse cerebral cortical layers.

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Poster

342. Connectomics: Anatomical Techniques

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Topic: I.03. Anatomical Methods

Support: NRF-2016R1A6A3A01009971

Title: Contributions of lipid extraction, RI-matching and size expansion in tissue clearing

Authors: ***J. KIM**¹, J. CHOI¹, E. LEE^{1,2}, W. SUN^{1,2} ¹Anat., Col. of Medicine, Korea Univ., Seoul, Korea, Republic of; ²Brain Korea 21, Korea Univ., Seoul, Korea, Republic of

Abstract: Tissue clearing techniques, such as CLARITY and ACT (active CLARITY technique), have received great attention for deep (brain) tissue imaging and for the potential application to the diagnosis in recent years. In principle, tissue clearing is achieved by a simple combination of extracting lipids, tissue swelling, and matching refractive indices (RI) between imaging solution and a cleared tissue whose main component is cellular and extracellular matrix (ECM) proteins. However, the contributions of major factors in tissue clearing, lipids extraction, tissue swelling and RI matching, have not been evaluated systematically yet. Interestingly, we found that four organs (brain, liver, kidney, and lung) shows different contribution of three factors during the ACT process in this study. While matching RI which depends on the volume of collagen in the tissue contributes to reducing light scattering, lipid extraction is expected to greatly improve antibody staining in a thick tissue, affecting the diffusion rate of the molecules into the tissues. We estimated the optimal time for the completion of lipid removal by measuring diffusion rate of RI matching solution into the tissue, which could be utilized for the Q/C process during the tissue clearing. Finally, we propose that the changes in the tissue clearing properties can be used as diagnostic measures. Our preliminary proof-of-concept results with traumatic brain injury (TBI) model will be presented.

Disclosures: J. Kim: None. J. Choi: None. E. Lee: None. W. Sun: None.

Poster

342. Connectomics: Anatomical Techniques

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Program#/Poster#: 342.13/VV26

Topic: I.03. Anatomical Methods

Title: Novel modernisation of Golgi-Cox stain and its optimisation of tissue clearing

Authors: *M. KULIGOWSKI¹, M. KASSEM², S. FOK³, K. SMITH⁴, B. BALLEINE² ¹Univ. of Sydney, Camperdown, Australia; ²Sch. of Psychology, ³Biomed. Imaging Facility, Univ. of New South Wales, Sydney, Australia; ⁴Brain and Mind Ctr., The Univ. of Sydney, Sydney, Australia

Abstract: High resolution neuronal information is extraordinarily useful in understanding the brain's functionality. Development of the Golgi-Cox stain allowed observation of the neuron in its entirety with unrivalled detail and remains still one of the best ways to visualise a neuron. However, the Golgi-Cox method is restricted by tissue transparency, so imaging is typically 200µm in depth using confocal microscopes

Within recent years, modern tissue clearing techniques such as CLARITY and CUBIC have been used to great effect, completely clearing tissue. Currently, there is no literature on the combination of Golgi-Cox and modern tissue clearing. The application of a Golgi-Cox stain to cleared brain tissue would allow complete neurons to be 3D rendered within intact biology, offering the most accurate data on neuron morphology.

We will show modernisation the Golgi-Cox stain, by combining with modern clearing techniques, and visualization with multiphoton microscopy, which has the advantage of deeper laser penetration, penetrating up to 2 mm. This will rectify the restrictions currently imposed by the Golgi-Cox stain and lead to even greater neuron resolution, allowing for improved 3D rendering and morphology visualisation.

Disclosures: M. Kuligowski: None. **M. Kassem:** None. **S. Fok:** None. **K. Smith:** None. **B. Balleine:** None.

Poster

342. Connectomics: Anatomical Techniques

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Topic: I.03. Anatomical Methods

Support: Vascular Dementia Research Foundation

Synergy Excellence Cluster Munich (SyNergy)

ERA-Net Neuron 01EW1501A

Title: Improved uDISCO clearing for 3D imaging of the nervous system

Authors: *A. J. PARRA-DAMAS, R. CAI, C. PAN, M. TODOROV, B. FÖRSTERA, A. ERTÜRK

Inst. for Stroke and Dementia Res., Muenchen, Germany

Abstract: Tissue clearing technologies provide substantial advantages over conventional histology and tissue sectioning approaches in terms of preserving structural three-dimensional information as well as increased time and cost efficiency. Imaging transparent specimens with single plane illumination (light-sheet) ultramicroscopy has become a golden standard for system biology level analysis of tissues in entire organs and organisms, including mammalian brains. We recently developed the 'ultimate DISCO' (uDISCO) clearing method, which preserves signal from fluorescent proteins over months while reducing the size of the specimens up to 65%, allowing to image not only whole organs but also entire rodent bodies. Here we present an uDISCO-based approach to simultaneous image neurons, glia and vasculature through the intact skull, thus preserving not only the deep brain vasculature but also meningeal vessels. Importantly, our protocol is also compatible with standard immunolabeling once the brain tissue has been rehydrated after clearing and imaging, expanding the possibilities for further phenotypic characterization of the specimens. Thus, this new clearing method can provide novel insights about the neurovascular environment, which would not be possible using standard histology protocols based on tissue sectioning.

Disclosures: A.J. Parra-Damas: None. R. Cai: None. C. Pan: None. M. Todorov: None. B. Förstera: None. A. Ertürk: None.

Poster

342. Connectomics: Anatomical Techniques

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Topic: I.03. Anatomical Methods

Support: NIH R01 HL126747

NIH R01 HL083048

Title: Lipid-preserving index matching for prolonged imaging depth

Authors: *Y. LIU¹, A. M. ROLLINS¹, M. WATANABE², M. W. JENKINS² ¹Biomed. Engin., ²Pediatrics, Case Western Reserve Univ., Cleveland, OH

Abstract: Light scattering limits the imaging depth in microscopy. Recently, several optical clearing techniques have been developed to reduce scattering and increase imaging depth enabling imaging of large regions of interest. These techniques are especially attractive for 3-D imaging of complex morphology in the nervous system. Here, we introduce a new optical clearing method called lipid-preserving index matching for prolonged imaging depth (LIMPID). Because LIMPID contains no detergent or organic solvent, it maximally preserves the cell's lipid bilayers. In addition, it has a higher clearing capability than most water soluble optical clearing agents due to a high refractive index (1.47). Low viscosity and optimal osmolarity properties reduce tissue processing time and minimize tissue distortion. We have also verified its compatibility with some common fluorescent proteins and antibodies. A significant benefit of LIMPID over other clearing methods is its simplicity. The clearing solution is easy to make and the clearing process only requires one step (immersion of the labeled sample in the clearing agent before imaging). Using LIMPID, we are able to investigate the organization of the peripheral nervous system and the state of innervation in different embryo tissues in 3D with fluorescent confocal microscopy.



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Poster

342. Connectomics: Anatomical Techniques

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Topic: I.03. Anatomical Methods

Support: FAPESP Grant 2015/26777-7

Title: Parafilm assisted microdissection: An alternative and effective technique for the microdissection of the mice nucleus accumbens

Authors: *M. F. PAGLIUSI, JR, *M. F. PAGLIUSI, JR, A. D. BRANDÃO, I. J. M. BONET, C. R. SARTORI, A. S. VIEIRA UNICAMP, Campinas, Brazil

Abstract: The Nucleus Accumbens (NAc) is a fundamental structure for the reward system and, therefore, influences several types of behavior. The literature shows several studies that highlight the importance of NAc in conditions like drug addiction, depression, nociceptive behavior and in conditioned behaviors in general. However, many of these studies fail to deepen into the real importance of NAc in these conditions because they have difficulty in accessing molecular changes that may occur in this structure. A major limitation in accessing such changes is the lack of knowledge and studies that describe how to identify and perform the microdissection of the NAc in a simple and inexpensive way. Therefore, the objective of this work is to describe the parafilm assisted microdissection procedure, a simple and inexpensive technique to microdissection the mouse NAc. We first described the best way to perform the mouse euthanasia and how to remove the brain from the skull without damaging the tissue. Next we describe how to prepare the slides with parafilm that will be used to receive the slices of the brain. In the next step we describe in detail how to position the brain in the object holder of the cryostat, how to align the cerebral hemispheres and how to identify the beginning and the end of the NAc. We also describe how to perform the staining and the dehydration of the slices, a critical step to facilitate the identification of the structure for the microdissection and also to preserve protein, DNA and RNA molecules from the sample. In the final step of this procedure we describe how to identify the anatomical limits of the NAc in each slices made in the cryostat and, finally, we also describe how to perform the microdissection of the structure. The microdissection is performed in a simple way, using a scalpel that the researcher will use to cut the tissue belonging to the NAc. This technique allows the researcher specifically collect the NAc tissue, from which intact RNA and proteins can be extracted to perform real-time PCR and Western Blot as well as others molecular analysis.

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A.D. Brandão: A. Employment/Salary (full or part-time):; CAPES. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); FAPESP. **I.J.M. Bonet:** A. Employment/Salary (full or part-time):; CAPES. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); FAPESP. **C.R. Sartori:** A. Employment/Salary (full or part-time):; UNICAMP. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); FAPESP. **A.S. Vieira:** A. Employment/Salary (full or part-time):; UNICAMP. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); FAPESP. **A.S. Vieira:** A. Employment/Salary (full or part-time):; UNICAMP. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); FAPESP. **A.S. Vieira:** A. Employment/Salary (full or part-time):; UNICAMP. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); FAPESP. **A.S. Vieira:** A. Employment/Salary (full or part-time):; UNICAMP. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); FAPESP.

Poster

342. Connectomics: Anatomical Techniques

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Title: FAST, High-speed serial-sectioning imaging for whole brain analysis with high scalability

Authors: ***A. KASAI**¹, K. SEIRIKI^{1,2}, T. HASHIMOTO⁷, M. NIU¹, S. YAMAGUCHI⁸, Y. NAKA¹, H. IGARASHI¹, M. TANUMA¹, T. NAKAZAWA^{1,3}, K.-I. INOUE⁹, M. TAKADA⁹, K. FUJITA⁴, H. HASHIMOTO^{1,5,6}

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Abstract: Subcellular resolution imaging of the whole brain and subsequent image analysis is prerequisites for understanding anatomical and functional brain networks. We have developed a high-speed imaging apparatus based on automated mechanical sectioning and confocal imaging,

named FAST (block-FAce Serial microscopy Tomography), and image data processing pipeline for 3D image construction without tissue-clearing techniques. FAST acquires submicrometerresolution images of whole mouse brain in a speed range comparable to those of light-sheet fluorescence microscopy. FAST allows unbiased quantitative comparisons between normal and disease model brain based on the cell numbers obtained from 3D image analysis. Moreover, FAST is scalable to the brains of nonhuman primates, which visualizes neuronal projections in the whole brain of an adult marmoset. Thus, FAST provides new opportunities for global approaches to gain a better understanding of brain systems.

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Poster

342. Connectomics: Anatomical Techniques

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Support: NIH Grant U01 MH109062

Title: Brain mapping and stainless staining for computed digital histopathology using vibrational hyperspectral imaging

Authors: N. SPEGAZZINI^{1,2}, S. DEB^{1,2}, *J. W. MITCHELL^{3,1}, K. YEH^{1,2}, S. TIWARI^{1,2}, K. FALAHKHEIRKHAH^{1,2}, C. KAUFMAN^{1,4}, E. K. NEUMANN^{1,5}, T. J. COMI^{1,5}, S. S. RUBAKHIN^{1,5}, J. V. SWEEDLER^{1,5,4}, M. U. GILLETTE^{3,1,4,2}, R. BHARGAVA^{1,2,5} ¹Beckman Inst. for Advanced Sci. and Technol., ²Dept. of Bioengineering, ³Dept. of Cell and Developmental Biol., ⁴Neurosci. Program, ⁵Dept. of Chem., Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: The brain consists of billions of cells that are organized in a large number of distinctive regions which have specific functional properties. In case of mammalian brain, the "cytoarchitecture" assembly is diverse and still remains to be significantly explored. In each brain region many different cells types are present, such as oligodendrocytes, astrocytes, microglia, and neurons along with an extensive blood vessel network. The cells are organized into localized multicellular units, in the vast cortical cell layers, and the "niches" of neural stem cells. Overall, a comprehensive mapping of the whole brain using combined optical microscopy and spectroscopy tools has not yet been achieved, and much remains unknown about the brain's structure illuminated by this technology. Here we report on rat brain chemical maps using vibrational hyperspectral spectroscopic imaging and high-performance computing. We report

two different methods: 1. Fourier Transform Infrared (FTIR), and 2. Stimulated Raman Scattering microspectroscopies. Both methods measure the fundamental vibrational modes of molecules to provide multiplexed chemical information on major classes of molecules (lipids, proteins, etc) and microscopic (micron and submicron) morphological details. The spectroscopic data obtained from these methods are the foundation of developing a chemical brain map based on computational methods and signal processing techniques. Another goal of our research is to develop a digital staining method from the hyperspectral IR data by combining high-performance computing to quantify the cytoarchitecture in the brain and pathophysiological conditions studies in the near future. The chemical map will provide the chemical and molecular details to capture the molecular specificity and structural complexity of brain tissue that are not possible to obtain from other histological imaging methods.

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Poster

342. Connectomics: Anatomical Techniques

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Support: EU Horizon 2020, Human Brain Project, Grant 720270

Norwegian Node of the International Neuroinformatics Coordinating Facility

Title: Determining and documenting the anatomical location of experimental neuroscience data: Best practice recommendations

Authors: *I. E. BJERKE, K. A. ANDERSSON, M. ØVSTHUS, M. A. PUCHADES, J. G. BJAALIE, T. B. LEERGAARD Inst. of Basic Med. Sci., Univ. of Oslo, Oslo, Norway

Abstract: Anatomical location is a key parameter for interpretation and comparison of neuroscientific data. Location is typically determined by looking up diagrams in anatomical reference atlases, communicated using anatomical terms, and shown in representative images. But the documentation provided varies considerably among scientific publications. Essential information about nomenclature and reference atlases, or criteria used to define boundaries of structures is often missing. This lack of accuracy limits the opportunities for comparing and integrating data from different publications, and could lead to failure in replicating scientific

experiments. To clarify and address this challenge, we have investigated current practice for assigning and documenting anatomical location for different categories of experimental neuroscience data reported in > 120 articles investigating the rodent brain. Our findings show that the specificity and accuracy of anatomical documentation in most cases can be considerably improved with relatively simple procedures. We here suggest some general and method-specific recommendations for such improvements, and discuss how these steps may contribute to increase the accuracy of anatomical descriptions and data interpretation. We demonstrate how new three-dimensional rodent brain reference atlases, and associated software tools for spatial registration of brain image data to a common anatomical space, offer new opportunities for efficient integration and comparison of neuroscience data.

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Poster

342. Connectomics: Anatomical Techniques

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Topic: I.03. Anatomical Methods

Title: The visualization of spinal cord glioma using the mitochondrial translocator protein (TSPO)-ligand PET

Authors: *T. YUJI¹, Y. NISHIYAMA¹, O. TSUJI¹, N. NAGOSHI¹, N. NITTA², S. SHIBATA², I. AOKI², T. YAMASAKI², Z. MING-RONG², M. MATSUMOTO¹, Y. FUJIBAYASHI³, M. JINZAKI³, H. OKANO⁴, M. NAKAMURA¹

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Abstract: [Introduction]PET plays a crucial role in evaluating brain glioma, and a number of PET tracers are investigated to validate clinical application. Among these, the mitochondrial translocator protein (TSPO)-ligand is a novel and promising biomarker for brain glioma, but little is known about glioma occurring in spinal cord. The purpose of this study is to evaluate spinal cord glioma in mouse xenograft model using PET imaging with TSPO-ligand, (¹⁸F-FEDAC). [Method]Adult female NOD-SCID mouse received a transplantation of human glioblastoma multiforme cell line U-251 MG (GBM group) or phosphate-buttered saline (PBS group) into the tenth level thoracic spinal cord. U-251 MG cells were cultured and labeled with firefly luciferase (ffLuc) genes via lentiviral transduction. Three weeks after transplantation, gadolinium-enhanced MRI was performed. PET imaging was performed with ¹⁸F-FEDAC in the transplanted

site was measured. Then the animals were immediately sacrificed and the spinal cord was dissected for autoradiography. The correlation between in vivo imaging data and immunohistochemistry was evaluated for the expression of TSPO.[Result]Gadolinium-enhanced MRI showed enhancing lesions in the transplanted site of the GBM group compared with the PBS group. In vivo dynamic TSPO-ligand PET imaging revealed an increase in tracer uptake in the transplanted site compared with the PBS group. These results were consistent with enhancing area of MRI. Autoradiography confirmed the high affinity and specific ¹⁸F-FEDAC bindings in the transplanted site of the GBM group compared with the bindings of the PBS group. Immunohistochemistry showed a high level of TSPO expression in the transplanted site of the GBM group. These cells were human nuclear antigen antibody-positive .[Conclusion]¹⁸F-FEFDAC PET imaging can elucidate the area of increased TSPO expression that can be visualized and quantified in vivo after transplantation. These results suggest that TSPO-ligand PET could be a promising in vivo imaging modality to visualize spinal cord glioma as well as brain glioma. In the future, we will evaluate the FEDAC-PET against spinal cord glioma in clinical settings.

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Poster

342. Connectomics: Anatomical Techniques

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Topic: I.03. Anatomical Methods

Title: The Janelia MouseLight Database: Complete axonal reconstructions from hundreds of individual long-range projecting neurons

Authors: *J. WINNUBST, E. BAS, J. DUDMAN, C. GERFEN, A. HANTMAN, W. KORFF, S. MURPHY, N. SPRUSTON, S. STERNSON, K. SVOBODA, J. CHANDRASHEKAR MouseLight, HHMI Janelia Res. Campus, Ashburn, VA

Abstract: One of the main goals of modern neuroscience is to understand how communication between different brain regions facilitates complex sensory processing and behavior. While much is now known about the gross anatomical connectivity between different brain areas, there is still a lack of information regarding how individual neurons within a brain region differ in their projection targets. Here, we combined a custom tissue clearing technique with serial two-photon tomography to image labeled neurons throughout the entire mouse brain with sub-micron precision. This allowed us to fully reconstruct the neuronal morphology of hundreds of

individual neurons in several key output areas of the brain: motor cortex, thalamus, hypothalamus and subiculum. We found that neurons within a brain area, which would classically be described as belonging to the same projection class, showed significant variation in their projection targets and axonal branch patterning. Reconstructed neurons will be made available in an online database that includes extensive search features and 3d visualization capabilities. By sharing these resources and techniques with the wider neuroscience community we hope to facilitate new insights into the fundamental properties of neuronal connectivity in the mammalian brain.

Disclosures: J. Winnubst: None. E. Bas: None. J. Dudman: None. C. Gerfen: None. A. Hantman: None. W. Korff: None. S. Murphy: None. N. Spruston: None. S. Sternson: None. K. Svoboda: None. J. Chandrashekar: None.

Poster

342. Connectomics: Anatomical Techniques

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 342.22/VV35

Topic: I.03. Anatomical Methods

Support: NYS Office of People with Developmental Disabilities

Title: Reversible clearing of rat brains for interrogation of histopathology using visikol histo approach

Authors: X. E. FLOWERS¹, T. VILLANI², G. GARDNER², M. JOHNSON², N. CRIDER², *J. H. GOODMAN^{3,4}

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Abstract: Since the introduction of the CLARITY tissue clearing technique in 2013, interest in tissue clearing techniques and three-dimensional tissue imaging has increased dramatically. While current tissue clearing techniques (CLARITY, iDISCO, Scale, CUBIC) result in highly transparent samples for 3D imaging, these processes irreversibly alter underlying tissue chemistry (e.g. hyperhydration and denaturation of proteins, extraction of lipids and/or cross-linking to polyacrylamide) and result in tissues that cannot be subsequently analyzed by traditional section-based histological processes. As such, these techniques, while showing great promise in an academic setting, cannot be directly validated against the gold-standard histological approaches, and as such have limited scope beyond basic research. Furthermore, while these techniques have been successfully applied to mouse tissues of all types, difficulty has been reported with rat tissues due to their size and extent of myelination. Many experimental

models of epilepsy, stroke and traumatic brain injury are performed in rats, and as such there exists a need for a histology-preserving tissue-clearing technique suitable for use in rat brains. To this end, the Visikol HISTO clearing technique was employed in conjunction with immunolabeling to obtain three-dimensional image stacks for large sections (>1 mm) and hemispheres of rat tissue highlighting vasculature, neurons, and astrocytes. The distribution of distance to nearest vessel was calculated for neurons and astrocytes. After clearing and imaging, tissues were subsequently un-cleared and sectioned for H&E and Nissl stain, to afford traditional histopathological correlation to 3D datasets.

Disclosures: X.E. Flowers: None. **T. Villani:** A. Employment/Salary (full or part-time):; Visikol Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Visikol Inc. **G. Gardner:** A. Employment/Salary (full or part-time):; Visikol Inc. **M. Johnson:** A. Employment/Salary (full or part-time):; Visikol Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Visikol Inc. **N. Crider:** A. Employment/Salary (full or part-time):; Visikol Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Visikol Inc.. **J.H. Goodman:** None.

Poster

342. Connectomics: Anatomical Techniques

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 342.23/VV36

Topic: I.03. Anatomical Methods

Support: Pennsylvania Department of Health using Tobacco CURE Funds

NIH Grant U01 MH105971

Title: Towards unified mouse brain atlas anatomical segmentation: Paxinos label on Allen common coordinate framework

Authors: *U. CHON¹, Y. KIM²

¹Dept. of NBS, Pennsylvania State Univ. Col. of Med., Hershey, PA; ²Dept. of NBS, Penn State Univ. Col. of Med., Hershey, PA

Abstract: Anatomical brain atlas is essential to study anatomical and functional organization of the brain. For mice, most widely used atlases include Paxinos and Franklin's The Mouse Brain in Sterotaxic Coordinates (Paxinos), and Allen Institute for Brain Science's the Allen Mouse Brain Atlas (Allen). By utilizing Nissl staining for a single mouse brain, Paxinos and Franklin created 120 micrometer spaced 2D atlas with unique abbreviations and boundaries. In addition, the Allen

Institute recently created 10 micrometer spaced 3D digital atlas with another set of labels and boundaries. With two different atlases used, labeled regions and their boundaries are inconsistent with one another. Moreover, nomenclatures of brain regions differ significantly between the two atlases. This caused confusion and misinterpretation of brain regions in many studies. As an initial step to reconcile the two different atlases, Paxinos labels were manually imported into latest Allen common coordinate framework within matching coronal *z* planes. Thus, we created a digital adult mouse brain atlas with 2 independent labels merged into a single atlas framework. We compared the anatomical borders and names of brain regions, and identified significant discrepancy between the two atlases on the same 2D plane. We envision that the converged atlas will provide an important bioinformatical platform for future neuroanatomical studies.

Disclosures: U. Chon: None. Y. Kim: None.

Poster

342. Connectomics: Anatomical Techniques

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 342.24/VV37

Topic: I.03. Anatomical Methods

Title: Identification of specific brain regions by triple fluorescent images and Voronoi tessellation

Authors: *S. INOUE, K. HOTTA, K. OKA Keio Univ., Yokohama, Japan

Abstract: Brain has many anatomically identified regions with specific functions. For the comparative studies of brains from different animals, brain draft map plays an important role. However, in previous studies, specific brain regions are generally identified by the cytoarchitectures visualized by several staining methods, and their borders have been arbitrarily determined. In this study, we propose a new method for identifying specific brain regions and generating the draft of brain map rationally and automatically by using the triple florescent imaging and Voronoi tessellation. This new method enables us to divide a brain preparation into sub-regions (Voronoi polygons) by vertical bisectors among cell nuclei. The advantage of Voronoi tessellation is that it divides the brain preparation into Voronoi polygons uniquely. Furthermore, their area and distribution of Voronoi polygons should reflect anatomical information for the density of cells.

We applied this method to the brain of zebra finch (Taeniopygia guttata). The whole brain was coronally sliced as more than 200 sections. Cell nuclei, myelin and cell bodies of neurons were fluorescent stained by DAPI, fluoro-myelin and fluoro-nissl (NeuroTrace), respectively, by the method used for visualization of brain regions in previous studies (Karten et al., 2014). The coordinates of cell nuclei were determined from DAPI images, and Voronoi tessellation was
applied to them in each brain section. Each section was divided into about tens of thousands of Voronoi polygons. The fluorescent intensities of myelin and nissl were assigned to each Voronoi polygon to integrate multiple information of brain cytoarchitecture. Furthermore, we visualized relationship between the mean fluorescent intensities of myelin and nissl in each Voronoi polygon by the scatter plot. Each Voronoi polygon was grouped into three clusters: nissl-rich, myelin-rich and the others. This method could identify both nissl-rich and myelin-rich brain regions rationally and automatically. We succeeded in identifying brain regions of the zebra finch by multiple brain tissues and Voronoi tessellation.

Disclosures: S. Inoue: None. K. Hotta: None. K. Oka: None.

Poster

342. Connectomics: Anatomical Techniques

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 342.25/VV38

Topic: I.03. Anatomical Methods

Support: NIH U01

IARPA Microns

Title: Multi-scale volumetric imaging of whole mouse brains using correlated x-ray microtromography, magnetic ressonance imaging and electron microscopy

Authors: *R. VESCOVI¹, V. DE ANDRADE⁵, S. FOXLEY², M. DU⁶, K. FEZZAA⁵, H. LI³, P. LA RIVIERE², D. GURSOY⁵, S. MIKULA⁷, C. JACOBSEN⁶, N. B. KASTHURI⁴ ¹Biol. Sci. Dept., ³Neurosci., ⁴Neurobio., ²Univ. of Chicago, Chicago, IL; ⁵Argonne Natl. Lab., Chicago, IL; ⁶Northwestern Univ., Chicago, IL; ⁷Electrons - Photons - Neurons, Max-Planck Inst. For Neurobio., Martinsried, Germany

Abstract: The mammalian brain has the most complex cellular architecture of any known biological tissue. Neurons can span great distances (several centimeters in the human brain) but connect with each other at the nanoscale. Fully characterizing this architecture and the web of directional connections would allow for an hierarchial understanding of the nervous system and its potential pathologies. However, no existing imaging approach can span the six orders of length-scale magnitude required to bridge those levels of understanding. At low resolution scales (e.g. mm voxels), magnetic resonance imaging (MRI) techniques allow for in vivo or ex vivo mapping of neuronal tracts through a combination of diffusion-weighted imaging techniques and post-imaging computational tractography. While powerful, they cannot achieve the nanometer-scale resolution required to identify neuronal connections. Moreover, they have never been thoroughly validated against ground truth high-resolution data. At the highest resolution scale

(e.g. nm voxels), recent efforts at automated electron microcopy (EM) provide synapse-level resolution (3 nm) but are currently limited to brain volumes of the size of a grain of sand and face serious computational challenges scaling to even 2 pixels of MRI data at 1 mm resolution (i.e. almost 2 petabytes of EM data). This unresolved mismatch between these imaging modalities (and others) partitions our understanding of the brain into 'silos' divided by resolution scales with little to no cross validation. The strengths of each modality are not leveraged for a more comprehensive understanding of the brain. This problem is only exacerbated since brains potentially operate at multiple scales in parallel (from communication via individual connections between neurons to communication between brain regions). Multi-resolution multi-modal brain maps are critically necessary for a more complete understanding of the brain. We propose to use synchrotron-based micro-CT (uCT) to fill this gap, bridging the resolution divide between MRI and EM by providing intermediate resolution (e.g. micron voxels) over entire brains and with sample preparation conditions compatible with MRI and automated EM on the same brains. We propose to use uCT as a 'Rosetta Stone' enabling mapping of complete neuronal paths, allowing, for the first time, validation of dtMRI, and identifying areas of interest in both coarser resolution modalities for subsequent nanometer reconstructions with automated serial EM.

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Poster

342. Connectomics: Anatomical Techniques

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 342.26/VV39

Topic: I.03. Anatomical Methods

Support: The Danish Medical Research Council

Lundbeck Foundation

Aarhus University

Title: An internet based histological atlas of the Göttingen minipig brain

Authors: *D. ORLOWSKI¹, A. N. GLUD², N. PALOMERO-GALAGHER³, J. SORENSEN⁴, C. R. BJARKAM⁵

¹The Dept. of Neurosurg., Dept. of Clin. Medicine, Aarhus Univ., Aarhus C, Denmark; ²Ctr. for Exptl. Neurosci. (CENSE), Inst. of Clin. Medicine, Aarhus Univ., Aarhus C, Denmark; ³Inst. of Neurosci. and Med., Res. Ctr. Julich, Julich, Germany; ⁴Aarhus Univ. Hospital, Head-Neuro Ctr., Aarhus C, Denmark; ⁵Dept. of Neurosurg., Aalborg Univ. Hosp., Aalborg, Denmark

Abstract: In recent years, pigs have become popular as large animal models in translational neuroscience as the feasible substitute for non-human primates. However, the anatomy of the pig brain is not well described. Minipigs are often used in such research and to our knowledge, there are no published cytoarchitectonic atlases describing the Göttingen minipig brain. We present accordingly an Internet based atlas describing cytoarchitecture of the Göttingen minipig telencephalon. The Atlas was built using 24 consecutive Nissl stained sections with depictions of the cortical and deep brain structures. Sections used in the atlas were derived from female Göttingen minipigs aged 10-14 months as approved by the Danish Animal Experiments Inspectorate and following the "Principles of laboratory animal care" (NIH). The animals were 4% PFA perfused and the brains were removed. To obtain sections presented in the atlas, one brain was paraffin embedded in toto, sectioned into serial 15 µm thick coronal sections and stained with Nissl or myelin (autometallographic) staining. Additional brains (n=17) were frozen and sectioned on cryostat into 40 µm (coronal) or 100 µm (horizontal or sagittal) thick sections followed by Nissl, myelin or DARPP32 staining. Sections from both frozen and paraffin embedded brains were used for preparation of the atlas descriptions. The atlas web page is accessible on http://www.cense.dk/minipig atlas/. It was constructed using HTML and CSS, ensuring easy transferring to the server as well as off-line work. To enable a magnified view of the sections, the JQuery library and a freeware Image Zoom plugin was used. The atlas was built using two pictures of every section, full sized one (6836 x 8223 pixels) for magnified view, and a small one (650 x 782 pixels) for presenting on web page. We tried to keep navigation through atlas simple. The section can be selected either using the thumbnails, or on the drawing presenting sagittal cross-section of the brain with embedded sectioning planes. For every atlas section, its own list of abbreviations can be shown. Additional full lists of all abbreviations, sorted in alphabetical order or grouped according to the brain anatomy, include the links to the first atlas page where the structure appears. The unique feature of our atlas is the presentation of the approximate borders between various cortical areas, which may serve as a great help in planning animal experiments. The structure of the atlas web page is open and allows subsequent addition of detailed information about various brains structures. Likewise, the analysis of the brain connections using DTI and addition of the MRI pictures to the atlas are in progress.

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Poster

342. Connectomics: Anatomical Techniques

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 342.27/VV40

Topic: F.04. Stress and the Brain

Title: Methods and applications for magnetic resonance imaging in hamsters: Characterization of sex differences through DTI and T2-weighted imaging

Authors: *T. R. MORRISON¹, S. C. IRIAH¹, X. CAI², P. P. KULKARNI³, C. F. FERRIS⁴ ¹Psychology, ²Northeastern Univ., Boston, MA; ³Psychology, Northeastern Univ. Dept. of Psychology, Boston, MA; ⁴Psychology, Northeastern University, Ctr. for Translational NeuroImaging, Boston, MA

Abstract: The Syrian hamster is a research model most widely used in studies focusing on sleep, aggression, and hormone regulation. Most recently, the Syrian hamster has been developed as a model of Ebola and Marburg hemorrhagic fever due to its superiority over other rodent models regarding its many similarities to human clinical signs and pathology. Despite its usefulness, the Syrian hamster remains an underutilized model that possesses a highly translatable neuroanatomy and behavioral repertoire that parallels (and in some cases supersedes) that of mice and rats. Magnetic resonance imaging (MRI) techniques are a virtual toolbox of analytic modalities that allow for the imaging of changes in the brain and identification of novel biomarkers associated with genetic variation, disease states, and the dynamic effects of pharmacotherapeutics. With these methods, it is possible to repeatedly and reliably scan an individual animal throughout its lifetime to follow changes in neurobiology and physiology brought on by any number of genetic and/or environmental insults. Using images from highresolution T2-weighted scans we developed 3D segmented and annotated MRI atlases for both male and female hamsters with over 140 brain regions in each. When data from different imaging protocols e.g. BOLD, functional connectivity, manganese enhanced MRI, from multiple animals are registered to the atlas it is possible to reconstruct distributed and integrated neural circuits that constitute virtual "fingerprints" of brain activity. In this example, we applied diffusion weighted imaging (DWI) and quantitative morphometry to identify differences in grey matter microarchitecture and region specific differences in brain volumes between sexes. The tools and data we have generated are of great value to pre-clinical researchers that currently use, or are interested in working with the Syrian hamster model. Moreover, to our knowledge, this is the first imaging study using the Syrian hamster thus highlighting an important addition to the wider pre-clinical animal imaging field.

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Poster

343. Electrophysiological Techniques

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 343.01/VV41

Topic: I.04. Physiological Methods

Support: LDRD 17-SI-002

Title: Development and characterization of primary neuronal-glia networks

Authors: *H. A. ENRIGHT, D. LAM, S. K. G. PETERS, J. OSBURN, C. HUANG, D. SOSCIA, A. SALES, K. KULP, E. K. WHEELER, N. O. FISCHER Lawrence Livermore Natl. Lab., Livermore, CA

Abstract: Organ-on-a-chip systems are designed to recapitulate the microenvironment of human tissue and organ systems, and hold promise in evaluating new drugs, characterizing toxicants, and to aid in elucidation of disease mechanisms. Multi-electrode arrays (MEAs) provide a means of non-invasively interrogating neuronal health and function, and are traditionally used to assess cultured networks of purified primary neurons. While these primary cultures can provide key insights into neuronal response and function, they fail to recapitulate the cellular complexity in vivo as they lack supporting glia including astrocytes, microglia, and oligodendrocytes. In this study, we have developed a complex in vitro system which incorporates these other cell types using primary rat cortical neurons and glial cells on a MEA device. Flow cytometry was used to identify cell-type specific populations. Immunocytochemistry was used to identify each cell type, evaluate cell morphology, and assess the phenotypic state of supporting microglia, oligodendrocytes, and astrocytes. Electrophysiology measurements were compared between complex and simple neuronal cultures over several weeks in vitro. Significant differences in electrophysiology responses were observed as neuronal complexity increased, including an earlier firing response and increased burst rate. These results suggest that complex neuronal/glial cultures may provide additional insight and relevance when evaluating the effects of new drugs and toxicants on primary neurons. This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344 through LDRD award 17-SI-002.

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Poster

343. Electrophysiological Techniques

Location: Halls A-C

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Program#/Poster#: 343.02/VV42

Topic: I.04. Physiological Methods

Support: BMBF Grant FKZ031L0059A

Title: *Ex vivo* electrical imaging of retinal and hippocampal layers using CMOS-based highdensity microelectrode arrays

Authors: ***G. ZECK**¹, F. JETTER¹, M.-J. LEE¹, L. CHANNAPPA¹, T. HERRMANN¹, F. HELMHOLD²

¹NMI at the Univ. Tübingen, Reutlingen, Germany; ²Inst. of Med. Psychology and Behavioural Neurobiology, Eberhard Karls Univ. Tübingen, Tübingen, Germany

Abstract: Electrical imaging refers to the mapping of electrical activity in neural networks at high spatial (~ 10 μ m) and temporal (~ 20 kHz) resolution. The technique has been used to evaluate (i) signal propagation in dissociated cultured networks, (ii) the functional diversity in the ganglion cell layer of mammalian retinas and (iii) the interplay of field potentials and single unit activity in brain slices. Two conceptually different microelectrode techniques are currently in use, one employing metal-based electrodes, the second technique using capacitive electrodes insulated by a thin dielectric.

Here, we first compare the recording performance of the two techniques by analyzing the signalto-noise (SNR) ratio from identified ganglion cells in the mouse retina under equivalent experimental conditions. For the two techniques signal amplitude and recording noise differ by about an order of magnitude; however the SNR ratio are undistinguishable. Secondly, we apply CMOS-based MEAs with capacitive electrodes to electrically image signal propagation in brain slices (hippocampus and retina) over a wide temporal range. Spectral analysis of the hippocampal recordings reveals spatially segregated signal power in frequency bands ranging from the alpha regime up to sharp-wave ripples (200 - 500 Hz). Electrical imaging in retinal slices reveals signal propagation across layers from outer nuclear layer to ganglion cells but also horizontal signal spreading within layers.

The presented experiments demonstrate the power of electrical imaging using high-density microelectrode arrays for the investigation of signal propagation across different neural layers and reconcile the two approaches of electrical imaging using metal-based or capacitive electrodes.

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Poster

343. Electrophysiological Techniques

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 343.03/VV43

Topic: I.04. Physiological Methods

Title: Human Cerebrospinal fluid promotes long-term neuronal viability and network function in human neocortical organotypic brain slice cultures

Authors: *H. KOCH¹, N. SCHWARZ¹, U. HEDRICH¹, F. BEDOGNI², H. SCHWARZ¹, N. DAMMEIER¹, E. AUFENBERG¹, H. LERCHE¹, H. PA¹, J. B. HONEGGER¹, T. V. WUTTKE¹ ¹Univ. Tübingen, Tübingen, Germany; ²San Raffaele Res. Inst., Milan, Italy

Abstract: Pathophysiological investigation of CNS-related disease, such as epilepsy or neurodegenerative disorders, largely relies on histological studies on human post mortem tissue, tissue obtained by biopsy or resective surgery and on studies using disease models including animal models, heterologous expression systems or cell culture based approaches. However, in general it remains elusive to what extent results obtained in model systems can be directly translated to the human brain, calling for strategies allowing validation or even primary investigation in live human CNS tissue. In the work reported here, we prepared human organotypic slice cultures from access tissue of resective epilepsy surgery. Employing different culture conditions, we systematically compared artificial culturing media versus human cerbrospinal fluid (hCSF), obtained from patients with normal pressure hydrocephalus (NPH). Presented data demonstrates sustained cortical neuronal survival including not only maintenance of typical cellular electrophysiological properties and activity, such as robust action potential generation and synaptic connectivity, but also preservation of tonic and phasic network activity up to several weeks in vitro. As clearly delineated by immunocytochemistry, single cell patch clamp- and extracellular recordings, we find that in contrast to artificial culturing media, hCSF significantly enhances neuron viability and maintenance of network activity.

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Poster

343. Electrophysiological Techniques

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 343.04/VV44

Topic: I.04. Physiological Methods

Support: Military Operational Medicine, JPC-5

Title: Feasibility of brain slice electrophysiology in the swine

Authors: *J. G. ROHAN¹, S. M. MCINTURF¹, M. K. MIKLASEVICH^{1,2}, N. M. GARGAS^{1,2}, P. M. SHERMAN³, K. L. ARMSTRONG⁴, K. L. MUMY⁵ ¹Naval Med. Res. Unit Dayton, Wpafb, OH; ²Henry Jackson Fndn. for Military Res., Bethesda, MD; ³59 MDW - USAFSAM/FH, San Antonio, TX; ⁴AFMC 711 HPW/RHDV, Wpafb, OH; ⁵Naval Med. Researh Unit Dayton, Wpafb, OH **Abstract:** The swine model is becoming a popular alternative to nonhuman primates in translational neuroscience research mainly because of the reduced cost and ethical liability, especially when performing large cohort studies. Unlike brains from small animals such as rodents, swine brains are gyrencephalic and are believed to better represent the human brain. There has been a recent surge in neuroscience-related studies in swine that utilized non-invasive imaging technologies such as positron emission tomography (PET) and magnetic resonance imaging (MRI), as well as behavioral testing to characterize neurological impairments. However, to our knowledge, there has been no documentation reporting electrophysiology recordings on viable brain slices from swine. Electrophysiological (EP) recordings provide critical information pertaining to the integrity of synapses throughout the brain. Its use in neuroscience research is vital not only in understanding the mechanisms of various neurological disorders but also in developing therapeutics that target various ion channels, receptors, enzymes and other synaptic proteins. A major difficulty in performing electrophysiology work in swine is obtaining rapid access to the brain while maintaining neuronal viability. Unlike rodents, swine possess a very thick skull that is extremely difficult to penetrate. Here, we describe how to quickly harvest the brain from the swine while maintaining neuronal viability and how to prepare functional hippocampal slices. Two different strains of female minipigs at different ages were used. The first is the Yucatan strain, ages 9-11 months weighing 41-57 kg. The second is the Sinclair strain, ages 3-4 months, weighing approximately 15 kg. We were able to obtain signals from a majority of intact slices, and recordings can be made for hours following brain harvest. From these slices, we were able to collect spontaneous spiking data, input/output relationship plots, and short term plasticity assessments using paired pulse stimulation protocol.

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Poster

343. Electrophysiological Techniques

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Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 343.05/VV45

Topic: I.04. Physiological Methods

Title: Simultaneous multiwell optogenetic stimulation and microelectrode array recording for neural electrophysiology assays

Authors: *M. BEAUMONT^{1,2}, H. B. HAYES², A. M. NICOLINI², C. A. ARROWOOD², I. P. CLEMENTS², D. C. MILLARD² ¹Inst. for Genomic Med., Columbia Univ. Med. Ctr., New York, NY; ²Axion BioSystems, Atlanta, GA

Abstract: Microelectrode arrays (MEAs) monitor and manipulate cultured cell activity *in vitro*, providing insight into neuronal network interactions to inform "disease-in-a-dish" models, stem cell characterization, toxicology screening, and drug safety and development. Recentlydeveloped multiwell MEA systems, such as the Maestro, enable high-throughput assessment of functional endpoints at greatly reduced time and cost. While network activity may be monitored under spontaneous conditions, stimulation of neural activity allows evaluation of evoked activity measures, reduces variability across wells, reduces assay duration by increasing activity levels, and enables creation of application specific protocols to assess features of network connectivity. Optogenetics integrates fast, light-activated channels (opsins) that allow targeted, precise manipulation of cellular activity, and provides advantages such as targeting of specific cell types, the ability to suppress activity, minimal stimulus artifact, and uniform stimulus delivery across a culture. Here, we demonstrate the application of Lumos, a commercial multiwell optical stimulation system, to characterize the use of opsins for in vitro neurophysiology assays. ChR2, Chronos, Chrimson, and Jaws were evaluated at multiple light intensities across four wavelengths (470nm, 530nm, 612nm, 655nm) to assess spectral separation in the neural response. As expected, ChR2 exhibited a greater evoked response for 470nm light than other wavelengths, whereas Chronos was slightly green-shifted, with consistent evoked response for 470nm and 530nm wavelengths. Chrimson had the broadest activity across wavelengths, but the peak response occurred for the 612nm wavelength. Additionally, stimulation frequency was varied to quantify the effect of opsin kinetics on neural response, with Chronos exhibiting the highest precision. Finally, measures of evoked neural response were used to quantify changes in activity induced by pharmacological manipulation with carbamazepine and picrotoxin, which produced significant decrease and increase, respectively, in the evoked response. These findings demonstrate the potential of optically-integrated multiwell MEA systems to improve assessment of neuropharmacological effects.

Disclosures: M. Beaumont: 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. I.P. Clements: A. Employment/Salary (full or part-time):; Axion BioSystems. D.C. Millard: A. Employment/Salary (full or part-time):; Axion BioSystems.

Poster

343. Electrophysiological Techniques

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Topic: I.04. Physiological Methods

Support: Department of Energy DE-AC52-07NA27344

LDRD award 17-SI-002

Title: Investigation of primary rat neuronal-glial networks on hydrogel scaffolds using a 2D cell culture system

Authors: *D. LAM¹, H. A. ENRIGHT¹, J. OSBURN¹, S. K. G. PETERS¹, D. A. SOSCIA², K. KULP¹, E. K. WHEELER², N. O. FISCHER¹ ¹Physical and Life Sci. Directorate, ²Engin. Directorate, Lawrence Livermore Natl. Lab., Livermore, CA

Abstract: Multi-Electrode Array (MEA) devices present a noninvasive experimental approach for long-term interrogation of neuronal networks. Often, a monolayer of purified primary neurons is used to form these spontaneously active networks in vitro, in the absence of neuroglia. In this regard, it is not fully understood how supporting glial cells influence the neuronal network and bioelectrical activity in a more complex culture system. We have recently developed a complex culture system using primary rat neurons and glial cells (i.e. astrocytes, oligodendrocyte precursor cells, and microglia) on a poly-d-lysine (PDL)-coated MEA device. Preliminary work has shown an earlier firing response (e.g. firing rate, burst events) with complex cultures versus neuronal cultures alone. However, this system lacks tissue-like features. Hydrogels have been used to mimic, to some extent, the extracellular matrix found in the brain. In this study, we used electrophysiology and imaging techniques to investigate the effects of natural and synthetic hydrogels on primary rat mixed neuron and glial cells for long term cultures in vitro. We compared electrical activity of the neuronal-glial networks grown on the 2D scaffold interfaced to a PDL-coated MEA device to the PDL-MEA device alone. Immunocytochemistry was used to identify the cell-type specific populations, examine cell morphology, and characterize the phenotypic state of astrocytes and microglia. Preliminary results show that the 2D construct represents a reproducible and reliable method to study the complex neuronal-glial network, and can be further enhanced to recreate a 3D tissue model relevant to the brain.

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Poster

343. Electrophysiological Techniques

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 343.07/VV47

Topic: I.04. Physiological Methods

Support: NSF INSPIRE (CBET-134193)

Title: Multi-region recordings from the hippocampus of free-moving rats with a Parylene-based multi-electrode array

Authors: *H. XU^{1,2}, A. HIRSCHBERG², K. SCHOLTEN², E. MENG², T. W. BERGER², D. SONG² ²Biomed. Engin., ¹USC, Los Angeles, CA

Abstract: The hippocampus is crucial for the formation of long-term declarative memories. Unitary activities recorded from the hippocampus of free-moving animals can provide valuable insights into understanding how short-term memories are transformed into output signals for long-term storage by hippocampal sub-regions. In order to target different regions of the

hippocampus simultaneously a multi-electrode array with a complex layout and high spatial resolution is necessary. In this work, we developed a 64-channel, flexible, penetrating electrode array which uses Parylene-C, a polymer with the highest USP biocompatibility rating, as its structural material. The layout of the electrode array was designed to conform to the cell body layer of the rat hippocampus. Temporarily reducing the effective length of the Parylene probe with a dissolvable polyethylene glycol (PEG) brace increases the probe buckling threshold and enables the successful implantation of the probe into the desired hippocampal region. Immunohistochemistry (IHC) staining performed one month post-implantation verified the depth of insertion (>4 mm). No severe immune response was observed around the Parylene probe. Neural activities were monitored both during the implantation and after the animal recovered. Unitary activities with amplitudes greater than 200 µV were recorded from both the CA1 and CA3 region of the rat hippocampus during the implantation. After the animal fully recovered, spike activities from the CA1 and CA3 region were recorded while the animal ran freely in an open field. Complex spikes with maximum signal to noise ratios (SNR) of up to 12 were recorded with the Parylene probe. Units with an average SNR of 3 to 4 were successfully recorded with the Parylene probe over the period of one month after implantation.

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Poster

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Topic: I.04. Physiological Methods

Support: NIH 1U01NS094190

Title: A 512 channels CMOS-based neural probe for acute high-density in-vivo intracortical recordings

Authors: *L. BERDONDINI¹, G. ANGOTZI², F. BOI², M. MALERBA², E. MIELE², G. MANDELBAUM⁴, A. CASILE⁵, S. ZUCCA³, T. FELLIN⁶, J. ASSAD⁷, B. L. SABATINI⁷ ¹Neurosci. and Brain Technologies, Fondazione Inst. Italiano Di Tecnologia, Genova, Italy; ²Neurosci. and Brain Technologies, ³Inst. Italiano Di Tecnologia, Genova, Italy; ⁴neurobiology, Harvard Med. Sch., Boston, MA; ⁵Dept. of Neurobio., Inst. Italiano di Tecnologia and Harvard Medica, Boston, MA; ⁶Inst. Italiano di Tecnologia, Genova, Italy; ⁷Neurobio., Harvard Med. Sch. Dept. of Neurobio., Boston, MA

Abstract: Over the last decade, significant advancements in CMOS electrode array devices permitted neuroscientists to start monitoring with cellular and sub-millisecond resolutions the electrical activity of large neuronal networks and brain circuits in-vitro. These devices counterpart recent developments in optogenetics and biotechnology (such as iPS cells, cellular reprogramming, organoids), thus enabling to face fundamental questions in neuroscience and brain diseases with innovative experimental designs exploiting large-scale neuronal activity recordings. An ongoing challenge consists in advancing this CMOS-MEA technology to realize implantable devices capable of large-scale neuronal activity recordings in-vivo. With this aim, we are developing a novel generation of micro-structured microelectronic neural probes integrating hundreds of densely integrated microelectrodes on single-shafts. Here we will present the design of the current platform and acute recordings obtained from anesthetized and behaving mice. Current probes were designed in a standard CMOS technology based on the active pixel sensor concept. They provide low-power circuits for 512 active sensing electrodes, including inpixel circuits for amplification underneath each electrode site and on-chip circuits for reading out the whole-array at 20 kHz/electrode using time division multiplexing. The whole sensing area occupies an area of 96 μ m \times 6 mm (W x L) and it provides an electrode density >1000 electrodes/mm² (electrode size of 15 μ m × 15 μ m, centre-to-centre pitch of 29 μ m). For implantation, silicon micromachining techniques were used to shape the CMOS devices and to reduce their thickness from 500 µm down to 40-50 µm. To manage potential issues that might arise from power dissipation and heating we also implemented a dynamic power scheduling solution and we achieved a power consumption of < 3 mW, well below the limit of 40 mW reported in the literature. Electrodes were post-processed by electrodepositing platinum, gold or PEDOT and the resulting impedances (< 1 M Ω at 1.1 kHz) and noise performances were assessed with electrical testing. To operate these devices we realized a complete hardware and software platform. Preliminary in-vivo results confirmed that these devices are able to resolve biological signals in a frequency band including both LFPs and spikes (amplitudes ranging from 100uV up to 400uV) and that these CMOS-probes provide adequate mechanical properties for multiple implantations and recordings.

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Poster

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Topic: I.04. Physiological Methods

Support: FRM Grant ARF20150934124

Title: In situ recordings of brain activity using organic electrochemical transistor-depth probes

Authors: *L. KERGOAT¹, M. DONAHUE², P. QUILICHINI¹, A. GHESTEM¹, A. WILLIAMSON¹, I. UGUZ², G. MALLIARAS², C. BERNARD¹ ¹Inst. de Neurosciences des Systèmes, Aix-Marseille Univ., Marseille, France; ²Lab. de bioélectronique, Ecole des Mines de Saint-Etienne, Gardanne, France

Abstract: A better understanding of the nervous system requires accurate electrophysiological recordings necessary for diagnostic purposes and for brain-machine interfaces. State-of-the-art recordings are currently performed with microfabricated arrays of metal electrodes (silicon probes, Utah arrays and tetrodes), which capture the local field potentials (LFPs). Such probes are also being used in the clinic to improve diagnosis and treatments. A key parameter for such electrodes is the impedance, which should be as low as possible in order to achieve high signal to noise ratio. Organic electronic devices constitute a promising class of devices because of their mixed ionic/electronic conductivity, mechanical flexibility and biocompatibility, making them ideal to interface with brain tissues. The use of conducting polymer-coated metallic electrodes have shown to decrease the impedance of metallic electrodes by one order of magnitude, leading to a higher resolution signal. To further improve the resolution of the signal, one can use organic electrochemical transistors (OECTs). In the same way than a transistor, OECTs are devices with an inherent amplification property, leading to higher intensity signal. It has been shown than using an ECOG made of OECTs lead to higher signal to noise ratio compared to an ECOG made of conducting polymer coated metallic electrodes. The quality of the signal achieved was of the same magnitude than the one obtained using state-of-the-art depth silicon probes. However, to further improve the accuracy of the recordings, one needs to go deeper into the brain. We developed a depth probe made of OECTs to achieve high signal to noise ratio recordings of the brain activity. We fabricated OECTs on a flexible parylene substrate, which was later on attached on a laser-cut glass capillary (25 um x 115 um). Passive conductive polymer electrodes were also fabricated and located near the OECTs to compare the performance of both kinds of electrodes. The sharp tip obtained thanks to the laser cutting insured smooth implantation in the brain. The electrophysiological signals were first recorded from the hippocampus of anesthetized rats. We also investigated the stability of the signal over time in chronic experiments. The quality of the signals obtained, both for LFPs and single-unit recordings, with the OECTs, makes them promising candidates for accurate electrophysiological recordings.

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Poster

343. Electrophysiological Techniques

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Topic: I.04. Physiological Methods

Support: ONR YIP

Title: Laser-printed porous graphene microelectrodes with high charge injection capacity for cortical stimulation

Authors: *D. KUZUM¹, Y. LU¹, A. G. RICHARDSON², T. H. LUCAS, JR² ¹Univ. of California San Diego, La Jolla, CA; ²Neurosurg., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Neuroscience research and clinical brain-machine interfaces require safe and efficient neural sensing and stimulation. To-date, most of the neural stimulation systems have relied on sharp metal microelectrodes with poor electrochemical properties that induce extensive damage to the tissue and significantly degrade the long-term stability of implantable systems. Therefore, efficient and durable electrophysiological sensing and stimulation without penetrating brain tissue is of great research interest. Here, we demonstrate a flexible cortical microelectrode array based on porous graphene. Microelectrodes based on porous graphene show low impedance and high charge injection capacity, which is critical for high efficiency cortical sensing and stimulation. We demonstrate that porous graphene microelectrodes exhibit no physical delamination or degradation after 1 million biphasic stimulation cycles, confirming long-term endurance. Moreover, porous graphene is easy to fabricate with direct laser pyrolysis, providing great potential for large area fabrication and commercialization. In *in vivo* experiments with rodents, same porous graphene array is used to sense brain activity patterns with high spatiotemporal resolution and to control leg muscles with high precision electrical stimulation from the cortical surface. Flexible porous graphene array offers a minimally invasive but high efficiency neuromodulation scheme with potential applications in cortical mapping, brain-computer interfaces, treatment of neurological disorders, where high resolution and simultaneous recording and stimulation of neural activity are crucial.

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Poster

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Topic: I.04. Physiological Methods

Support: 1U01NS094375-01

Title: Fabrication and validation of a low pitch and high channel count carbon fiber electrode array using a minimal and stackable silicon support structure

Authors: *P. R. PATEL¹, D. EGERT², E. J. WELLE¹, J. D. BERKE², C. A. CHESTEK¹ ¹Biomed. Engin., Univ. of Michigan, Ann Arbor, MI; ²Neurol., UCSF, San Francisco, CA

Abstract: Our group has shown that arrays of ~8µm carbon fiber electrodes can record chronically from rodent motor cortex for at least 5 months and cause minimal damage (Patel 2016), essential properties for future implanted microelectrodes in humans. Those arrays were built by manually securing bare fibers to exposed gold traces on a printed circuit board (PCB) using silver epoxy, then encasing the fibers in poly(ethylene-glycol) to facilitate insertion. However this approach has limited capacity for further enhancements in electrode density, length, and channel count above 16. To improve on the existing arrays, we have developed a silicon support structure that allows for more accurate assembly, range of target depths, and ease of insertion.

Fabrication of silicon supports starts with a bare 4" silicon wafer. The wafer undergoes a deep reactive-ion etching (DRIE), creating two trenches. The first is a narrow trench (10 x 11um (h x w)), designed to hold the carbon fiber, with length depending on the target brain region. Connected to the backend of this trench is a larger trench (11 x 100 x 290µm (h x w x l)) for the silver epoxy. Next, the wafer undergoes a deep boron diffusion step followed by insulating layers of silicon oxide (1585Å) - nitride (487Å) - oxide (1506Å). Then, a stack of chrome/gold (300/3000Å) is deposited and patterned, creating a connection from the larger trench, with the silver epoxy - carbon fiber bond, to a bond pad at the device periphery. A final DRIE defines the shape of the device including shank length (1.5-9mm) and width, which starts at 25µm at the base of the device and tapers to 15µm for the last 300µm of the shank. The devices are released from the wafer using a wet etch.

We have successfully fabricated a 4-layer cortical version of this device with 16 1.5mm long shanks (pitch= 80μ m) per layer. Each shank was populated with a carbon fiber that extended past the device by 500um and stayed well aligned. Two preliminary non-functional 64-channel devices were inserted 1.5mm into rodent motor cortex. To our knowledge this is the highest density array able to self-insert without a shuttle. To perform a preliminary functionality test, a single fiber was insulated with parylene-c, the tip re-exposed with a laser, and then wire bonded

to the PCB. This device was implanted in motor cortex and able to detect activity from two units with amplitudes of $162\mu V \& 77\mu V$. While placement of the silver epoxy and carbon fibers is still done manually and will require further refinement, the support structure vastly improves the scalability, accurate placement, and ease of insertion of our carbon fiber electrodes.

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Poster

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Topic: I.04. Physiological Methods

Support: Welch Foundation Grant C-1668

Title: A bilayer insulation scheme for carbon nanotube fiber neural microelectrodes

Authors: *S. PAMULAPATI¹, F. VITALE³, R. G. BRYANT⁴, C. KEMERE², M. PASQUALI¹ ¹Dept. of Chem. & Biomolecular Engin., ²Dept. of Electrical & Computer Engin., Rice Univ., Houston, TX; ³Ctr. for Neuroengineering and Therapeut., Univ. of Pennsylvania, Philadelphia, PA; ⁴Advanced Materials and Processing Br., NASA Langley Res. Ctr., Hampton, VA

Abstract: Neural electrode implants hold great potential for the diagnosis and treatment of neurological disorders such as Parkinson's disease and epilepsy. Recently, high performance carbon nanotube fibers (CNTfs) were employed as multifunctional neural electrodes ^[1,2]. However, the longevity and consistent performance of these CNTf electrodes heavily relies on the properties of insulation material used in the design. An ideal insulation is one that is conformal, stable, and biocompatible with good dielectric and impermeable barrier properties. Commonly used insulation materials like parylene, silicon nitride, and silicon carbide either tend to show a dramatic degradation and water penetration under in vivo and in vitro aging conditions or require high deposition temperatures [3, 4]. Here, we present a hermetic insulation scheme for CNTf microelectrodes using a bilayer encapsulation of polyimide (PI) over aluminum oxide (Al₂O₃), which is highly conformal and biocompatible with superior dielectric, chemical and moisture resistance properties. The inner Al₂O₃ layer serves a powerful moisture barrier, while the flexible outer layer of new solution-based PI acts as an excellent external water and ion barrier in addition to providing overall structural stability ^[5,6]. Individual single filament CNTfs were insulated with Al₂O₃ using atomic layer deposition (ALD) and then PI by dip coating technique at low deposition temperatures. The electrochemical properties and integrity of the bilayer insulation and individual layers were assessed using electron microscopy, leakage current and impedance measurements. Further, we evaluated the long-term stability of bilayer coated

CNTf electrodes using longitudinal impedance analysis under *in vitro* electrode aging tests against commercial platinum-iridum (PtIr) metal electrodes. The impedance of PtIr electrodes at 1 kHz decreased by 45% and 77% after second and ninth weeks respectively. However, impedance of bilayer-CNTf electrodes at 1 kHz increased by 10% after first week and remained consistent suggesting a reliable and stable electrode performance. High coating conformality, low leakage current and stable long-term insulation impedance prove the bilayer encapsulation as a promising insulation scheme for chronic implantable neural electrodes. References

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Poster

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Support: NIH Grant NS76462

Title: Nanofabricated fMRI probes for the detection of neuronal electrical signaling

Authors: *A. HAI¹, E. A. LIMA², B. P. WEISS², Y. LIN³, A. JASANOFF¹ ¹Biol. Engin., ²Earth, Atmospheric and Planetary Sci., ³Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Different functional neuroimaging modalities are currently used for in-vivo studies of brain function and dysfunction. While blood-oxygen- level-dependent functional magnetic resonance imaging (BOLD-fMRI) currently provides neuroscientists with the highest quality, noninvasive whole-brain readout in animal models as well as humans, it can only give information about areas of changing blood flow in the brain, and leaves substantial guesswork as to the underlying electrical neural activity patterns that constitute the communication mechanism of the nervous system. Novel methods to perform direct whole-brain readouts of neural activity would address this limitation, and allow for the elucidation of the neural basis of neuropathological disorders, as well as have an enormous effect in basic neuroscience. Here, we

present a novel strategy by which nanofabricated electrical components can be used as fMRI contrast agents that are specifically coupled to neural activity. We describe the synthesis of voltage sensitive probes that convert electrical neural activity to detectable magnetic field perturbations that can be measured using MRI. This strategy will ultimately allow for the non-invasive detection of neural signals and provide an unprecedented tool for the study of brain function and dysfunction in neurodevelopmental disorders, as well as normal cognition and behavior.

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Poster

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Support: MEXT Regional Innovation Strategy Support Program

MEXT COI Tohoku

Title: Conductive polymer based silk electrode for bio-activity measurement

Authors: *K. TORIMITSU, Y. TAKIZAWA, K. MIURA, H. TAKAHASHI Tohoku Univ., Sendai, Japan

Abstract: Development of a bio-flexible electrode for neural measurement is one of our interest. We reported previously usage of a conductive polymer, poly(3,4-ethylenedioxythiophene) based compound improved the biocompatibility and the electrical characteristic of the electrode. We used this type of electrode for biological activity measurement, such as neural development. Here we report the flexible silk electrodes using as for a wearable/ implantable electrode. The conductive polymer modified silk allowed us for a higher biocompatibility and longer measurement capability. Neural activities and/or muscle activities of mouse and chick were measured. Stable signal detection has been achieved. Detected activities were used for controlling machinery movement. As the conductive silk electrode is a flexible and stable measurement, activity-based interaction could be achieved for a primary evaluation of activity - dependent interface.

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Poster

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Support: NIAAA 2 R44 AA02067

Title: A distributed real time multiple processor system to capture neural data from 40,000 channels or 300 imager devices over long times from behaving animals

Authors: *D. J. WOODWARD¹, J. CHANG²

¹Neurosci. Res. Inst. of North Carolina, Winston Salem, NC; ²Neurophysiol., Neurosci Res. Inst. North Carolina, Winston Salem, NC

Abstract: A goal of modern neural instrumentation is to capture streams of time stamped data from enough sites within interconnected circuits so that ongoing logic operations can be determined. A key desired feature is the need to expand the traditional computer workstation to control capture of analog and digital signals from a very large number of multiple sensor types. Our aim has been to establish the framework for a parallel acquisition system on a local network that can grow and accommodate use of new high site density recording probes and other devices. The new system has three major components. A first is a configuration service based on Microsoft .net framework that allows members of a cluster to be recognized by a master control CPU and assign functions to each. Separate machines on a network can operate for control, data acquisition, or storage and real time analysis. A second feature is an advanced use of precision time protocol that allows all acquisition processors to share a common high precision clock. A third feature is use of a real time state machine software module installed on all cpus in the cluster to pace data collection, time output to stimulation devices, and control behavioral protocols. This system is linked to a cascade of parallel field programmable gate arrays (FPGAs) with internal ARM processors. These units process bit streams or frames from groups of 24 ADs, 16 bit ADs, SPI interfaces (from IntanTechnologies VLSI recording interface), or other DIGIO devices. Interfaces include ability to capture data flow with signals processing from most available imager devices. We are interfacing this system with one or more experimental chambers to collect data over many days in behaving rats from multiple imagers for light field, mini-microscopes, multiple compressed video streams, light-field, tissue voltages from microwire arrays, arrays of carbon fibers, or other probes with modified surfaces as chemical sensors for tissue oxygen, dopamine, etc. Real time control and timing is provided for output of timing pulses, waveform generation, or HD video displays. This enhanced workstation provides a platform for very high throughput behavioral neurophysiology. NIAAA 2 R44 AA02067

Disclosures: D.J. Woodward: A. Employment/Salary (full or part-time):; Biographics Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biographics Inc. **J. Chang:** A. Employment/Salary (full or part-time):; Biographics Inc.

Poster

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American Heart Association 15CSA24460004

Dan Duncan Foundation Neuroengineering Fellowship

Title: Microfluidic actuation of flexible microelectrodes for neural recording and stimulation

Authors: *A. V. RODRIGUEZ¹, D. G. VERCOSA², F. VITALE⁷, E. M. LEWIS³, S. PAMULAPATI⁴, J. S. YAN⁵, M. PASQUALI⁶, C. KEMERE¹, J. T. ROBINSON³ ²Applied Physics Program, ³Electrical and Computer Engin., ⁴Dept. of Chem. & Biomolecular Engin., ⁵Bioengineering, ⁶Dept. of Chem. and Biomed. Engin., ¹Rice Univ., Houston, TX; ⁷Ctr. for Neuroengineering and Therapeut., Univ. of Pennsylvania, Philadelphia, PA

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. Despite tremendous advances, technologies for high-resolution electrical recording and modulation of neural activity at the cellular level still rely on rigid metal or silicon components which poorly match soft brain tissue and cause extensive acute and chronic injury.

Flexible electrodes and ultra-small microwires have been shown to significantly reduce neural damage during chronic implantation and increase the quality and longevity of neural recordings. However, flexible electrodes require stiffening agents to overcome the buckling force upon implantation. These agents increase the device footprint and potentially cause additional damage to the brain during implantation.

To overcome this challenge, we have developed a novel technology to precisely actuate and

implant high-performance soft carbon nanotube fiber (CNTf) microelectrodes without using stiffening agents. Instead, our technology uses fluid flow in a microfluidic device to drive electrodes into tissue. The device's hydraulic design with embedded valves enables precise control of the CNTf position with minimum fluid output. In vitro experiments in brain phantoms show that microfluidic-actuated CNTf electrodes can be implanted up to a 4 mm depth with 30 μ m precision, while keeping the total volume of fluid injected below 0.5 μ L. To demonstrate the feasibility of this technology as a tool for recording neural signals in vivo, we performed acute anesthetized recordings in rats. We used the microfluidic drive to insert CNTf electrodes into the left parietal cortex to a depth ranging from 0.5 to 4 mm. After insertion, the microdrive can be retracted, leaving the CNTf inserted in the brain. Following device retraction and electrical connection, we were able to record spike waveforms from neurons in the cortex and deeper brain regions such as hippocampus. These waveforms were stable across minutes of recording, with firing patterns that varied with the depth of the probe. Future work will focus on chronic stability of electrical recordings in comparison to standard insertion strategies of flexible electrodes.

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Poster

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Title: Electrode designs for reducing the edge effect in transcranial electical stimulation (TES)

Authors: *M. P. WEISEND¹, L. F. BURCH², C. L. DUCKETT², D. P. MARSHALL², M. J. VILARDO²

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Abstract: Transcranial Electrical Stimulation (tES) targets brain networks and shows promise in the treatment of multiple neurological disorders and in cognitive enhancement. Application of a current during tES produces an edge effect at the electrode-skin interface (ESI). This edge effect is a high current density at the edge of the ESI with a low concentration in the center of the contact surface. The edge effect arises from a difference between the conductivity of the electrode and the skin. Common complications in tES include unpleasant skin sensations and

redness. Partially or fully mitigating this edge effect may reduce these complications. We investigated the edge effect with Finite Element Analysis (FEA) using Solidworks, Simpleware ScanIP, and COMSOL. Our model included a Ag-AgCl electrode, electrode gel to serve as an electrically conductive medium between the electrode and skin, and an acrylonitrile butadiene styrene (ABS) plastic housing to hold the electrode and gel. The conductivity of the electrode was set to 654.50 S/m, skin 0.45 S/m, Signagel 4.00 S/m, and ABS Plastic 1.00*10⁻¹⁶ S/m. Our baseline housing was circular with a diameter of 20.32 mm, height of 8.64 mm, a right angle at the ESI, and a sparse internal structure of three ridges to hold the Ag-AgCl electrode in place. The team made modifications to the internal geometry of the ABS electrode housing to alter the path of current through the electrode assembly to reduce edge effect during tES. Seventy variations of the baseline housing were created. FEA of the baseline housing showed the peak current density (PCD) was 7.41 A/m^2 at the edge of the ESI. Our designs ranged from greater than 30 A/m^2 to 5.04 A/m^2 as the PCD at the edge of the ESI. We observed that small holes and sharp angles at the ESI increased PCD. The creation of an obtuse angle at the base of the electrode housing lowered the PCD at the edge of the ESI. Varying the diameter of the gel column immediately under the Ag-AgCl electrode also decreased the PCD. The most effective design, 5.04 A/m^2 , at reducing edge effect consisted of a 6.00 mm central hole ending 1.00 mm above the ESI. The cavity between the ESI and the bottom of the central hole had a lofted cut 0.88 mm high and 4.42 mm in length with a 6.00 mm radius fillet at the edge of the ESI. Variations in the internal geometry can alter the PCD at the ESI. Our best design improved the overall PCD by 32% at the edge of the ESI. However, some designs increased the PCD by over 400%. Our results indicate exotic electrode shapes should be used with caution. Future human testing of the housings will determine if decreased PCD at the ESI reduces tES complications.

Disclosures: M.P. Weisend: A. Employment/Salary (full or part-time):; Rio Grande Neurosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Rio Grande Neurosciences. L.F. Burch: None. C.L. Duckett: None. D.P. Marshall: None. M.J. Vilardo: None.

Poster

343. Electrophysiological Techniques

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 343.18/VV58

Topic: I.04. Physiological Methods

Support: NSF Grant IOS 1120291

Title: Novel algorithm to discriminate bursts and tonic activity in spike trains: Extended Hill-Valley method

Authors: ***B.** CHUNG¹, D. H. EDWARDS²

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Abstract: As the size of neural networks in simulations and experiments increases, analysis algorithms need to consistently and efficiently identify a range of activity patterns including tonic spiking and bursting across large datasets. Visual classification can be time consuming and subjective as baseline firing rates change. Algorithmic approaches focus mainly on detecting bursts and classify activity using inter-spike interval (ISI) thresholds based on the ISI distribution. Depending on the mixture of neural activity, however, ISI distributions of a spike train vary. Consequently, visual inspection and burst detection algorithms yield variable results across datasets that contain different activity patterns. The Extended Hill-Valley (EHV) method builds off of concepts used in the Hill-Valley analysis technique. EHV detects both bursting and bouts of tonic spiking using a smoothed, history-dependent analysis signal derived from a spike train. Because events are classified based on the height-to-width ratio of peaks and troughs, EHV can accurately classify activity across a large dataset consisting of a range of activity patterns without recalibration. The performance of EHV was compared to two other methods, including the Poisson Surprise method, based on events identified by visual inspection. EHV outperformed both algorithms across a range of activity patterns after an initial calibration. EHV can also be used to identify hierarchical features, such as phasotonic bursts that occur when a neuron fires a burst and then spikes at a lower, constant frequency. EHV objectively identified bursts and bouts of tonic spiking across a range of neural activity and can be used to qualitatively classify the activity of individual neurons or networks of neurons in large datasets.

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Poster

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McKnight Foundation

NINDS (U01 NS090454-01)

Title: Characterization of carbon fiber electrode coatings using a complex impedance model

Authors: *E. J. WELLE¹, P. R. PATEL¹, F. POURDANESH¹, F. DEKU³, D. EGERT², A. GHAZAVI³, A. JOSHI-IMRE⁴, J. D. WEILAND¹, J. D. BERKE⁵, C. A. CHESTEK¹ ¹Biomed. Engin., ²Dept. of Electrical Engin. and Computer Sci., Univ. of Michigan, Ann Arbor, MI; ³Bioengineering, ⁴Ctr. for Scientific Innovation, Univ. of Texas at Dallas, Richardson, TX; ⁵Neurol., Univ. of California, San Francisco, San Francisco, CA

Abstract: Brain-computer interfaces rely on recordings from a large number of neurons consistently for long durations. Carbon fiber electrodes have a diameter of ~8µm and have been shown to cause minimal scarring in the brain and record high fidelity signals when coated with PEDOT (Patel *et al.*, 2015). However, PEDOT-coated carbon fiber electrodes often only record single units for several months after implant (Patel *et al.*, 2016). One possible explanation for this decrease in recording ability is PEDOT degradation over time. In a previous study from our lab, PEDOT-coated carbon fibers were soak tested in 1x PBS at 60°C for 35 days, and it was found that the 1kHz impedance values significantly increased over time by 151% (p < 0.001, n=16) indicating PEDOT degradation.

To better analyze coating integrity, a model of the complex impedance behavior of these electrodes was developed using nonlinear least squares fitting. This model was used to determine the resistivity of the Warburg-like element of the electrode, where parameter α denotes a how resistive or capacitive the element is in the range of 0-1, with the aim of a more resistive recording electrode (Otto *et al.*, 2006). This model was applied to PEDOT-coated carbon fibers and α values were found to significantly increase over time by 11.1% (p < 0.001, n=16) indicating PEDOT degradation and an increase in capacitive behavior.

Therefore we have begun exploring other more robust surface treatments and coatings, beginning with verifying single unit recording ability acutely *in vivo*. We first attempted to roughen the surface of the carbon recording site using oxygen plasma treatment after laser removal of 50-100µm of parylene. This process reduced impedance, but increased recording surface area. High α values of 0.87 (n=26) for oxygen plasma treated probes indicated that the electrodes were acting in a capacitive manner, although single units were still detectable. We also tried two additional coatings: platinum iridium (Pt-Ir) and electrodeposited iridium oxide (EIROF) (Petrossians *et al.*, 2011, Meyer *et al.*, 2001). The impedance of these two coatings at recording sites of ~40µm² were 182 kOhm (n=16) and 2.11MOhm (n=15) respectively. For comparison, the impedance of PEDOT was 140kOhm (n=83). The α values of both coatings, 0.640 and 0.635 respectively, were similar to that of PEDOT at 0.609.

All electrodes achieved functional recording of single units at depths of 1.2mm, 1.3mm, and 1.5mm in rat M1 with average amplitudes of $173\mu V_{p-p}$ for oxygen plasma (n=15 units, 7 fibers), $308\mu V_{p-p}$ for Pt-Ir (n=5 units, 1 fiber), $193\mu V_{p-p}$ for EIROF (n=8 units, 2 fibers), and $243\mu V_{p-p}$ for PEDOT (n=53 units, 14 fibers). Soak tests are currently on going.

Disclosures: E.J. Welle: None. **P.R. Patel:** None. **F. Pourdanesh:** None. **F. Deku:** None. **D. Egert:** None. **A. Ghazavi:** None. **A. Joshi-Imre:** None. **J.D. Weiland:** A. Employment/Salary (full or part-time):; Platinum Group Coatings, LLC. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Platinum Group Coatings, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder,

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Poster

343. Electrophysiological Techniques

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Topic: I.04. Physiological Methods

Title: Nearly equidistant spacing of EEG electrodes on a scalp surface coverage area

Authors: *M. E. PFLIEGER^{1,3}, A. M. THIRAKUL², L. T. SMITH²

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Abstract: Optimal sampling of EEG scalp potential fields typically calls for bilateral symmetry and uniform spatial density, i.e., fixed spacing within a coverage area. The adequacy of particular spatial densities for a given purpose (such as source estimation) needs more study, which needs, in turn, a method to generate uniform arrays with arbitrary inter-electrode spacing. The International 10-20 electrode system (Jasper, 1958; Klem et al., 1999)--a proportional landmark-based nomenclature that accommodates 74 locations--was extended to a 5% system that accommodates 345 locations (Oostenveld and Praamstra, 2001). Using combinatorial optimization, we sought nearly uniform subsets of the 5% locations for specific numbers of locations, but found this approach to be infeasible. An alternative approach based on icosahedral geometry was designed expressly for uniform sampling of a sphere (Tucker, 1993); but arbitrary spacing is not supported because each refinement of triangles cuts the spacing by one half. We devised an algorithm that generates nearly uniform arrays with any specified target spacing (see rows of the figure) on a given scalp wireframe (here, MNI average head) for a defined coverage area (here, we digitized the boundary of a cap designed to extend coverage on the back and sides of the head). From a central seed point (here, 12 mm anterior to FCz), sagittal midline points at the target spacing are generated to the boundaries. Parasagittal rows have either hexagonal or orthogonal spacing relative to the midline. A parallelogram rule generates a new point from three existing points; a triangle rule may generate one more point near the boundary from two existing points; and a skip rule keeps spacing within a percentage (here, 80%) of the target. The parallelogram rule supports intermediate arrangements between hexagonal and orthogonal.

Electrode arrays of various densities may used for both modeling and experimental studies of optimal spatial sampling. Of note, the algorithm can generate custom layouts for developmental (e.g., infant) research and for highly-studied individuals.



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Poster

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Topic: I.04. Physiological Methods

Support: NIH Grant U01NS090454

Title: Evaluation of amorphous silicon carbide multielectrode arrays in rodent motor cortex

Authors: *F. DEKU, E. SHIH, A. KANNEGANTI, A. JOSHI-IMRE, J. J. PANCRAZIO, S. F. COGAN Dept. of Bioengineering, Univ. of Texas At Dallas, Richardson, TX

Abstract: Microscale electrode arrays with small cross-sectional dimensions provide potential alternatives to avoid adverse tissue reactions over time, thereby improving their ability to reliably record single-unit and multi-unit extracellular potentials. These arrays also permit recording of neural spikes with high spatial resolution and present the potential of using novel current steering patterns for stimulating neural activity using subthreshold current densities. Along these lines, previously reported sub-cellular scale carbon fiber multielectrode arrays (MEAs) with cross-sectional area less than 50 μ m² have provided chronically stable and high fidelity neural recordings with reduced immune response overtime. However, carbon fiber assembly are not

amenable to large scale manufacturing processes thereby impeding design flexibility, scaling and control of the geometric surface area (GSA). To this end, we investigated the development of MEAs based on photopatternable thin-film amorphous silicon carbide (a-SiC) with shank crosssectional area similar to carbon fiber. We demonstrated functionality of a 16 channel, 150 μ m² Pt electrodes by recording decoupled neural signals in zebra finches basal ganglia. Translation and feasibility in mammalian animal models is yet to be evaluated. Here, we investigated similar electrode arrays with GSA of 50 μ m² in the motor cortex of rat brain for their chronic reliability. The MEAs were fabricated using plasma enhanced chemical vapor deposited a-SiC as the sole substrate and insulator to conformably encapsulate a thin layer of Au on both sides. Electrode sites and bond pads were defined in the top a-SiC layer using SF₆ reactive ion etching techniques. Additionally, we sputter-deposited iridium oxide films on the electrode sites to reduce the electrode impedance and improve the charge storage and charge injection capacities of the electrode. The arrays were then evaluated in motor cortex for recording and stimulation properties. Our preliminary acute testing showed successful implantation into rat motor cortex using PEG 2000 stabilized tips. We also observed a 56% yield for recording single/multi-unit activity under anesthetized preparations. We are currently investigating the reproducibility in a larger number of animals. Moreover, we also plan to evaluate the chronic performance of such electrode arrays for rodent cortical interfacing over a period of 4 months by periodically assessing the in-vivo recording stability, electrochemical properties and charge injection limits. In summary, this study aims to address the implementation of ultra-microscale a-SiC MEAs for chronic rodent cortical interfacing.

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Poster

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Topic: I.04. Physiological Methods

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DoD Grant W81XWH-16-1-0580

Title: Chronic tracking of neuronal clusters using ultraflexible electrode arrays

Authors: ***H. ZHU**¹, X. LI¹, Z. ZHAO¹, X. WEI¹, L. LUAN^{2,3}, C. XIE¹ ¹Biomed. Engin., ²Dept. of Physics, The Univ. of Texas At Austin, Austin, TX; ³Biomed. Engin., The Univ. of Texas at Austin, Austin, TX **Abstract:** The ability to reliably record and track an ensemble of neighboring neurons over months and longer are of great importance to basic and clinical neuroscience, as a large number of brain functions are realized by coordinated activation of a local neuronal population. Assorted Multi-site dense electrode arrays have been designed based on conventional structures, but their chronic recording capacity is often compromised by the instability of the tissue-probe interface. Ultraflexible, cellular-dimensioned neural electrodes have recently demonstrated seamless integration with brain tissue and reliable recording of individual neurons for several months. Here we design dense electrode arrays with oversampling capacity on the ultra-thin (1 µm) and flexible architect to achieve chronic tracking of neuronal clusters. Recordings were performed on head-constrained awake behaving mice who received intracortical implantation in somatosensory or motor cortices for over three months. The oversampling electrodes maintained stable impedance, single-unit yield, and single-unit amplitude during the experiment period. Klusta, a multi-channel spike sorting software was used to detect and isolate unit activities. After extracting the mean waveform of every unit, a semiautomatic program that evaluated the mean square error in the pair-wise comparison between longitudinal recording sessions was used to track the same unit. Multiple units were repeatedly identified and tracked over three months as shown in Figure 1. Repeated in vivo two photon imaging was performed to verify the minimal relative displacements between electrodes and neurons. These results show that the novel electrode design improves from previous studies for the simultaneous recording of closely spaced neuronal populations with minimal tissue damage and stable long-term recording performance.



Figure 1. Left: Representative Tracking results from one animal showing that 10 units were repeatedly detected and tracked using the ultraflexible oversampling electrodes. Right: Representative waveforms from detected units in one week's recording overlaid on the schematic diagram of the 4x8 electrode arrary(electrode dimension: 25 x 25 µm; inter eletrode spacing: 5 µm. A Cross marks the 2D projected location of the neuron from triangulation. Scale bar is 50µV.

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Title: Probing brain function with a novel combination of optogenetics and neuroimaging

Authors: *E. ROGERS^{1,2}, G. ALBERTO¹, D. KLORIG¹, J. R. STAPLETON-KOTLOSKI², V. POPLI², C. CONSTANTINIDIS¹, J. B. DAUNAIS³, D. W. GODWIN^{1,2,3} ¹Dept. of Neurobio. & Anat., ²Dept. of Neurol., ³Dept. Physiol. & Pharmacol., Wake Forest Sch. of Med., Winston Salem, NC

Abstract: Magnetoencephalography (MEG) is a unique, non-invasive technology that allows for studying biomagnetic signals. A major criticism of MEG is centered on the assumption that biomagnetic signals recorded from the brain are distorted due to the complexity of the brain's cytoarchitecture. Convolutions in the cortex are thought to distort dipoles, nuclei are assumed to be very difficult to localize due to irregular distribution of axons, and signals from deep sources with distance from the MEG sensors. Prior work has suggested that we are able to accurately induce oscillatory current into a non-human primate brain using optogenetics ("OptoMEG"). We stereotaxically injected 5µL AAV10-CaMKIIa-ChR2-eYFP into CA3 of hippocampus and separately into the arcuate sulcus in the prefrontal area of a vervet monkey. After 3 weeks of recovery, we stimulated optically through a 400µm diameter implanted fiber optic while simultaneously performing MEG scans. Analyses revealed that we were able to localize both superficial and deep sources of activity in a spatially- and temporally- precise manner using the beamforming technique synthetic aperture magnetometry (SAM). Optical pulse stimuli, pulse trains, and sinusoidal modulation produce distinct and reproducible oscillatory MEG activity consistent with the stimulus type. Our ability to localize both superficial and deep brain regions provides evidence that SAM can aid in detecting relevant sources of magnetic flux. To further understand the contribution of complex oscillatory activity, we performed dual-site stimulation of CA3 and arcuate sulcus simultaneously in an anesthetized preparation while recording MEG. Stimulation consisted of fast and slow modulation of sinusoidal optical input as well as ongoing sinusoidal stimulation in hippocampus with pulsatile stimulation in cortex. We were able to detect temporal and oscillatory components at both stimulation sites using magnetic source imaging. OptoMEG permits a novel kind of functional brain mapping that relies on whole brain recording of experimenter-determined signals introduced into discrete areas of the brain - these may include oscillatory activity within physiological ranges (e.g. beta, gamma, etc) or pathological patterns of activity (e.g. interictal spike-like discharges). This method allows the stimulation and recording of coordinated brain activity to study the complexity of brain networks, with possible extension to pathological oscillations that underlie seizures and epilepsy. Disclosures: E. Rogers: None. G. Alberto: None. D. Klorig: None. J.R. Stapleton-Kotloski: None. V. Popli: None. C. Constantinidis: None. J.B. Daunais: None. D.W. Godwin: None.

Poster

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Title: Large-scale microelectrode array for closed-loop cellular-resolution electrophysiology of the retina

Authors: *D. TSAI¹, D. SAWYER², A. BRADD¹, R. YUSTE², K. L. SHEPARD¹ ¹Electrical Engin., ²Columbia Univ., New York, NY

Abstract: The ability to observe and manipulate, with single-cell precision, the activities of large neuronal populations is an essential step toward better understanding of the nervous system. However, conventional approaches in electrophysiology is fundamentally unable to achieve simultaneous, cellular-resolution recordings at scale, while providing sufficient signal-to-noise-ratio (SNR). This is due, in particular, to the need for low-pass anti-aliasing filters at each recording channel front-end, where at-scale electrophysiological systems experience the highest density constraints. In the simplest form, these filters are RC-networks, consisting of a capacitor and resistor. We show that, with high-quality, foundry-grade capacitors in today's advanced microelectronic processes, these RC-filters cannot be made smaller than approximately 20-times the size of the average mammalian neuronal soma, thus limiting the scalability of electrophysiology.

We present an acquisition paradigm, based on compressed sensing, that obviates the need for these spatially inefficient, per-channel filters. To prevent aliasing, the spectral content contributed by the under-sampled thermal noise is digitally constructed, and removed, from the sparsely-sampled channel data. We built an extracellular electrophysiology platform with 65,536 simultaneously recording and stimulation electrodes, with cellular-resolution electrode pitch of $25.5 \,\mu$ m. The platform is able to record with 10 uV rms input referred noise over the spike waveform bandwidth, providing greater than 2- to 5-fold SNR improvements over existing high-channel count systems. When recording from the whole-mount mouse retina, the system senses

single-unit activities from more than 34,000 electrodes - 10-fold more than the best existing attempts.

In conjunction with a high-performance processing pipeline, built from GPU-based streaming multiprocessors, the platform automatically sorts and classifies in excess of 1700 retinal ganglion cells (RGC) following visual stimulation over a 1-mm spot in the mouse retina, including automated identification of rare RGC types.

Using the voltage-based, capacitive stimulation capability built into each recording front-end, we show that the system is able to evoke activities from single neurons, while providing the ability to simultaneously track activities across the entire retina. This capability opens the possibility to examine the relationship between stimulus strength and focal activation, at a higher spatiotemporal resolution than previously achievable.

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Topic: I.04. Physiological Methods

Support: N66001-15-C-4019

Title: Design and validation of a 0.75 cc networked implantable neuromodulation device with 32 channels of sensing and stimulation

Authors: *J. J. WHEELER¹, J. R. LACHAPELLE², C. K. BJUNE¹, C. A. SEGURA¹ ¹Draper Lab., Cambridge, MA; ²Draper laboratory, Cambridge, MA

Abstract: An implantable neuromodulation device is described which provides on-the-fly reconfiguration of sensing and stimulation across 32 electrodes in a highly miniaturized sub- 1cc hermetic package. While useful for a wide variety of neuromodulation applications, this poster describes the use of the system to control and deliver sensory feedback from a sensorized neuroprosthetic limb in amputees. Each implantable module is 14mm in diameter and less than 5mm in height, allowing surgical placement of multiple systems within the arm adjacent to electrodes. Close proximity to electrodes enables high-resolution recordings with noise <1uV RMS, sampling rates up to 100ksps, and selectable referencing. The system can deliver stimulation ranging from 200nA to 100mA with 1us waveform resolution. Complete ISO-14708 human-implant safety is maintained through custom ASIC safety circuits. A percutaneous version of the system is wired to an EIC60601-clinic-capable controller through flexible helical

cables for durability. Results from benchtop, non-human primate, and human studies will be presented.

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Poster

343. Electrophysiological Techniques

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HFSP (RGP0048/2013)

Andreas Schaefer is a Wellcome Trust Investigator (110174/Z/15/Z)

Title: jULIEs: ultra-low impedance neural probes for minimally invasive in-vivo recordings in the mouse olfactory bulb

Authors: *R. R. RÁCZ, M. KOLLO, W. WRAY, G. RACZ, A. T. SCHAEFER Neurophysiol., The Francis Crick Inst., London, United Kingdom

Abstract: Simultaneous, stable, scalable and minimally invasive interrogation and interaction with electrogenic cell populations of behaving animals is of fundamental importance for the advancement of our understanding of how different neural systems interact. Extracellular recordings using electrical charge sensing probes are considered the most powerful techniques capable of addressing this issue. Technical challenges in neural probe size, recording capability, channel count have limited our understanding of brain function. To overcome these challenges, we introduce a modular, flexible, insulator-ensheathed metal microwire platform with ultra-low interfacial impedance recording sites compatible with industry standard read-out electronics. At the center of this approach are glass-ensheathed cast microwires with outer diameter of $25\mu m \pm 2\mu m$ and inner diameters of $1\mu m \pm 0.2\mu m$, resulting in minimal stray capacitance (<0.1pF/mm length). These wires have been semi-automatically polished at an angle of <30° to facilitate brain

insertion and penetration. Interfacial impedance was reduced by modification of the recording sites with nanostructured iridium oxide (IrOx) to achieve coupling capacitances above $1pF/\mu m^2$ with the extracellular space. Bundles containing 16 individual microwires have been assembled into DIL connectors through a low-ohmic connection $(100\Omega \pm 2\Omega)$. Site impedances have been determined using electrochemical impedance spectroscopy between 1Hz and 200kHz by applying a 10mV since-wave around the open circuit potential in 150mM PBS. Advantages of jULIEs are their recording sites which are up to 50x smaller than in conventional electrodes $(1.3\mu m^2 \pm 0.05\mu m^2)$ designed to result in reduced tissue displacement and damage as well as in highly localized, high signal-to-noise recordings. To test jULIE properties in-vivo we performed recordings in the olfactory bulb (OB) of anaesthetized mice (4-6 weeks old) using conventional amplifiers. Action potentials were reliably recorded with amplitudes of up to 1.6mV. When jULIEs were lowered several mm into the brain and returned to a superficial recording position, extracellular units were reliably obtained throughout the OB. Confirming the predicted minimal damage to the tissue, jULIEs were found to be exceptionally suited for recording both large amplitudes (300-1500µV), well isolated units (20-30µm) and multicellular activity throughout the travelled vertical distance. We conclude, that the combination of glass-ensheathed metal wires with nanostructured surface modifications provides a versatile platform for minimally invasive, highly localized neural recordings.

Disclosures: R.R. Rácz: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CyberDeal S.r.L. **M. Kollo:** None. **W. Wray:** None. **G. Racz:** None. **A.T. Schaefer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Paradromics.

Poster

343. Electrophysiological Techniques

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Topic: I.03. Anatomical Methods

Support: FWF P 23102-N22

Title: Isotropic recording of whole mouse brains by light sheet microscopy breaking the diffraction limit

Authors: *H.-U. DODT^{1,2}, S. SAGHAFI¹, K. BECKER^{1,2}, M. PENDE^{1,2}, C. HAHN^{1,2}, I. SABDYUHSEVA-LITSCHAUER^{1,2}, M. WANIS¹ ¹Tech. Univ. Vienna, Vienna, Austria; ²Med. Univ. Vienna, Vienna, Austria **Abstract:** Visualization of complete neuronal networks in the brain is an important goal of neuroscience. To this end chemical clearing and recording of mouse brains with light sheet microscopy has become increasingly popular in recent years. Several microscopic setups have been developed for light sheet microscopy but normally there is a trade-off between speed and resolution of the recording.

This is due to the fact that Gaussian light sheets have either a thin beam waist then with a short Rayleigh range or a long Rayleigh range but with a thick beam waist. If one wants to record the brain at high resolution this necessitates the recording of many small tiles with the thin part of the light sheet and subsequent cumbersome aligning and stitching of the single recorded tiles. We therefore developed new optics creating a light sheet with submicron thickness and a vastly extended Rayleigh range. The diffraction limit according to Abbe's formula is normally cited for illumination with high numerical aperture (NA). It applies however also to low NA beams. As our new long light sheet is already rather thin when it leaves the last lens of the light sheet generator our results indicate that we broke the diffraction limit by a large factor. With the new light sheet we were able to image whole mouse brains quickly with one single stack of images giving isotropic resolution.

For recording we used commercial low magnification air objectives with a dipping cone which we optically corrected for imaging in clearing solutions. Apart from brains we also imaged adult GFP labelled drosophilae and cm large pieces of human cancers in autofluorescence. We expect that this breakthrough in optics will become pivotal for the recording of large samples with highest resolution.

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Poster

343. Electrophysiological Techniques

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Topic: I.04. Physiological Methods

Title: SEURAT: A semi-automated pipeline to localize and visualize intracranial electrodes

Authors: *Y. PATHAK¹, T. G. DYSTER¹, J. OH², E. H. SMITH¹, S. SRINIVASAN¹, N. FELDSTEIN¹, C. A. SCHEVON¹, G. MCKHANN, II¹, S. A. SHETH¹ ¹Columbia Univ., New York City, NY; ²Robert Wood Johnson Med. Sch., New Brunswick, NJ

Abstract: Intracranial electrode implantations for deep brain stimulation and seizure localization are common functional neurosurgical procedures. In order to refine therapeutic targets and meaningfully interpret electrophysiological data, intracranial electrodes need to be accurately localized. There is a demand for efficient methods to localize implanted electrodes as the volume

of these procedures continues to increase. In this study, we aimed to build a semi-automated, user-friendly pipeline (SEURAT) to determine electrode locations.

The pipeline integrates MATLAB and FSL to determine the centroids of potential electrode contacts. If all contacts are not localized through the automated algorithm, users have the ability to interpolate contacts on depth electrodes and to manually choose to add or remove contacts. These contacts are localized in patient-CT space, but can be mapped on to the patient-MRI space and standard-MNI space in order to draw relevant insights.

We tested SEURAT on imaging data from epilepsy patients (n=16) who underwent electrode implantation. Without using the manual option in the pipeline, we localized 1427 contacts out of 1520 implanted. We attribute this discrepancy to low image-quality or to some contacts being outside the brain; the toolbox defines a search area based on patient-specific brain extraction. Within-patient accuracy for the number of contacts localized was 94.5% (Figure 1). Visual inspection, validated with clinical implant maps, demonstrated that contacts were localized and mapped on to correct brain regions.

SEURAT is an accurate and user-friendly pipeline that will be useful to clinicians who need to identify the anatomical location of intracranial electrodes, and for basic science investigations that require an understanding of the relationship between contact location and physiology. Compared to similar open access methods, this pipeline requires minimal user input, which reduces time and error. This pipeline allows for seamless analysis of surgical targets with the potential to inform image-guided surgical protocols.



Figure 1: A) Snapshot from MATLAB with localized contacts superimposed on an axial CT slice. B) All contacts for the example patient mapped and visualized on a standard-MNI brain

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Poster

344. Large-Scale, Deep, and High-Speed Functional Light Microscopy

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Topic: I.04. Physiological Methods

Support: U01 NS094286-01

Title: Miniscope.org: An open-source imaging platform and online resource focused on developing the next generation of miniature fluorescence microscopes

Authors: *D. AHARONI¹, T. SHUMAN⁴, D. J. CAI², C. LEE³, B. S. KHAKH⁵, A. SILVA⁶, P. GOLSHANI⁷

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Abstract: One of the biggest challenges in neuroscience is to understand how neural circuits in the brain process, encode, store, and retrieve information. Meeting this challenge requires tools capable of recording and manipulating the activity of intact neural networks in freely behaving animals. Head-mounted miniature fluorescence microscopes are among the most promising of these tools. Taking advantage of the past decade of advancements in fluorescent neural activity reports and miniaturization of high quality imaging sensors, these microscopes use wide-field single photon excitation to image activity across large populations of neurons in freely behaving animals. They are capable of imaging the same neural population across months and in a wide range of different brain regions.

Here we present the current status of our open-source miniature fluorescence microscope project, Miniscope.org. This project aims at accelerating innovation of miniature microscope technology while also extending access to this technology to the entire neuroscience community. Through online guides and in-person workshops, over 250 labs across 18 countries have begun building and using the Miniscope system. Functioning as a robust imaging system as well as a miniature microscope development platform, labs are able to building on the open-source design, extending its use to new areas of interest. We have built and tested a wire-free Miniscope powered by a LiPo battery that is capable of recording for over 20 minutes in large behavioral assays. Our current work ranges from adding optogenetic stimulation and wire-free operation to simultaneous optical and electrophysiology recording. With a fundamental redesign of the Miniscope system, we are adding additional imaging modalities to the platform while making the entire system smaller, lighter, and more flexible. Through continued optimization and
innovation, miniature microscopes will likely play a critical role in extending the reach of neuroscience research and creating new avenues of scientific inquiry.

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Poster

344. Large-Scale, Deep, and High-Speed Functional Light Microscopy

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Topic: I.04. Physiological Methods

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Title: Overlapping retrosplenial cortical ensembles encode memories close in time

Authors: *M. SEHGAL¹, S. HUANG², S. MARTIN¹, A. LAVI¹, D. J. CAI³, A. SILVA⁴ ¹Neurobio., Univ. of California Los Angeles, Los Angeles, CA; ³Neurobio., ²UCLA, Los Angeles, CA; ⁴Dept Neurobiol, UCLA Med. Ctr., Los Angeles, CA

Abstract: Memories are dynamic in nature. A cohesive representation of the world requires memories to be altered over time, linked with other memories and eventually integrated into a larger framework of sematic knowledge. In an attempt to unravel these questions, our laboratory has recently demonstrated that two contextual memories encoded close in time are stored by overlapping hippocampal CA1 neuronal ensembles and the recall of one can lead to the recall of another, i.e. the two memories are linked (Cai et al. 2016). Although, learning- and CREBdependent changes in intrinsic neuronal excitability most likely facilitate the co-allocation of memories in overlapping ensembles, very little is known regarding how this plasticity biases neurons to be preferentially recruited for memory formation. Similarly, we do not know whether memories are linked due to overlap in neuronal ensembles in certain key brain regions, such as hippocampus for contextual memories, or the entire neural circuit involved in contextual memory formation displays this neuronal overlap. We addressed this question by investigating the overlap in neuronal ensembles encoding contextual memories at varying time intervals within the retrosplenial cortex, a brain structure necessary for spatial and contextual information processing. Using head-mounted miniature microscopes (UCLA Miniscopes), we imaged GCaMP6f-meadiated calcium dynamics in retrosplenial cortical neurons of mice that explored distinct contexts. We found greater overlap in the neuronal ensemble activated in response to two distinct contexts when the contexts were explored 5h vs. 7d apart. These data indicate that retrosplenial cortex can mediate temporal memory linking by recruiting a shared neuronal ensemble for memories encoded within the day. In addition to time, we also wanted to investigate the role of context similarity in neuronal ensemble overlap. In order to do this, we

measured calcium dynamics while mice explored the same context 5h or 7d apart. As before, we found greater overlap between the neuronal ensembles activated when the contexts were explored 5h vs. 7d apart. We are currently investigating whether neuronal overlap is greater when the same, or a distinct context is explored 7d apart. Our data indicate that co-allocation of neuronal ensembles encoding temporally proximate contextual memories may be a generalized mechanism, and memory linking may be mediated by many brain structures.

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Poster

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Title: Breakdown of spatial coding and neural synchronization in epileptic mice

Authors: *T. SHUMAN¹, D. AHARONI³, D. J. CAI⁴, C. LEE³, I. TAXIDIS⁵, S. E. FLORES⁵, K. CHENG⁵, M. JAVAHERIAN⁵, J. LOU⁵, C. C. KABA⁵, M. SHTRAHMAN⁶, M. A. HOWARD⁷, K. BAKHURIN², S. C. BARABAN⁸, S. C. MASMANIDIS⁴, B. S. KHAKH⁹, A. SILVA¹⁰, P. GOLSHANI¹¹ ²Neurobio., ¹Univ. of California Los Angeles, Los Angeles, CA; ³Dept. of Neurol., ⁴Neurobio., ⁵UCLA, Los Angeles, CA; ⁶Dept. of Neurosciences, UCSD Sch. of Med., La Jolla, CA; ⁷Neuroscience, Ctr. for Learning and Memory, Univ. of Texas At Austin, Austin, TX; ⁸Dept Neurolog Surgery, Univ. California San Francisco, San Francisco, CA; ⁹Dept Physiol, Univ. of California Los Angeles, CA; ¹⁰Dept Neurobiol, UCLA Med. Ctr.,

Los Angeles, CA; ¹¹UCLA Dept. of Neurol., Los Angeles, CA

Abstract: Epilepsy causes significant cognitive deficits in both humans and rodent models yet the circuit mechanisms leading to cognitive dysfunction remain unknown. We first developed a wireless miniature microscope (Miniscope) for *in vivo* calcium imaging to examine the spatial representation of freely behaving epileptic and control mice running on a linear track. Following pilocarpine-induced status epilepticus, chronically epileptic mice had reduced precision of CA1 place fields which completely remapped across days. Next, using silicon probes to record hippocampal interneurons during head-fixed virtual navigation, we found that epileptic mice had profound deficits in theta and gamma power and coherence, and altered phase preferences to ongoing theta oscillations. In particular, dentate hilar interneurons had a similar magnitude of theta phase modulation of their firing rate, but the preferred phase of these cells as a group was highly dispersed and shifted to a later phase of theta. Finally, we transplanted embryonic interneuron precursors from the medial ganglionic eminence into the hippocampus of epileptic mice and found a partial rescue of theta power. Together, these results support the role of circuit dysfunction in poor spatial processing in epileptic mice.

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Poster

344. Large-Scale, Deep, and High-Speed Functional Light Microscopy

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Topic: I.04. Physiological Methods

Support: Stanford CNC Program

HHMI Allen Institute for Brain Science NINDS DARPA NEI NIMH

Title: The Crystal Skull: A chronic mouse preparation providing long-term optical access to neurons across the dorsal neocortex

Authors: *T. KIM¹, Y. ZHANG¹, J. LECOQ², J. JUNG¹, J. LI¹, H. ZENG², C. M. NIELL³, M. SCHNITZER¹ ¹Stanford Univ., Stanford, CA; ²Allen Inst., Seattle, WA; ³Inst. of Neurosci., Univ. of Oregon, Eugene, OR

Abstract: A major technological goal in neuroscience is to enable the interrogation of individual cells across the live brain. By creating a curved glass replacement to the dorsal cranium and surgical methods for its installation, we developed a chronic mouse preparation, termed the 'Crystal Skull', that provides optical access to an estimated 800,000-1,100,000 individual neurons across the dorsal surface of neocortex. Post-surgical histological studies revealed comparable glial activation as in control mice. In behaving mice expressing a Ca²⁺ indicator in cortical pyramidal neurons, we performed Ca²⁺imaging across neocortex using an epi-fluorescence macroscope and estimated that 25,000-50,000 individual neurons were accessible per mouse across multiple focal planes. Two-photon microscopy revealed dendritic morphologies throughout neocortex, allowed time-lapse imaging of individual cells, and yielded estimates of >1 million accessible neurons per mouse by serial tiling. The Crystal Skull preparation supports a broad range of imaging and optogenetic methods, is compatible with many different behavioral assays for head-fixed mice, and enables studies of cells across >30 neocortical areas. Thus, we expect that this preparation will facilitate a wide variety of experiments probing the interactions between multiple brain areas in awake behaving mice.

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Poster

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Support: UMII MnDRIVE (Minnesota's Discovery, Research, and InnoVation Economy) Graduate Assistantship

MnDRIVE (Minnesota's Discovery, Research, and InnoVation Economy) RSAM

McGovern Institute Neurotechnology (MINT) fund

Title: Cortex wide, multi-modal, cellular resolution neural interfacing via digitally generated skeletal prostheses

Authors: *L. GHANBARI^{1,2}, R. E. CARTER³, G. JOHNSON², M. RYNES⁴, L. HALTOM², J. J. HU⁴, G. M. SHULL⁴, M. LAROQUE², T. J. EBNER³, S. B. KODANDARAMAIAH^{2,4}

²Mechanical Engin., ³Dept. of Neurosci., ⁴Dept. of Biomed. Engin., ¹Univ. of Minnesota, Minneapolis, MN

Abstract: The brain mediates our interaction with the external world by performing complex computations undertaken by highly interconnected brain regions spread across several centimeters. There is a need for tools to measure and manipulate the activities of these widespread brain regions at the single cell resolution, and at multiple timescales. We are engineering a suite of technologies that enable wide-field, cellular resolution neural interfacing with the rodent cortex. We have implemented a precise computer numerically controlled (CNC) surgery robot to excise very accurately and precisely, arbitrarily shaped skull sections for replacement with a morphological conformant cranial implant or 'brain window'. The brain windows allow optical access to the surface of the whole dorsal cortex and can be implanted for long durations (>100 days). Preliminary histology experiments indicate little or no immune response to chronic implantation. We have established an easy to configure design methodology that allows the brain windows to be adapted for both high resolution imaging using two photon (2P) microscope in head fixed animals, as well as mesoscale cortical activity mapping in head fixed and freely moving mice. We have performed cellular resolution 2P imaging through the windows implanted on transgenic mice expressing the calcium indicator GCaMP6f, in the somatosensory, visual and granulate cortices. Building upon the brain windows we are developing a suite of technologies that enable wide field activity mapping in awake, freely behaving animals. These devices are easy to fabricate using readily available laboratory tools. Finally, the flexibility of the device architecture allows numerous modalities to be incorporated within the windows for multi-modal, bidirectional, brain wide neural interfacing.

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Poster

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Topic: I.04. Physiological Methods

Support: MnDRIVE

McGovern Institute Neurotechnology (MINT) Grant

Title: Open source computer numerical control neurosurgery platform for automated craniotomies in small animals

Authors: *G. JOHNSON¹, L. GHANBARI¹, J. HU², M. LAROQUE¹, G. SHULL², J. DOMINGUEZ¹, M. RYNES², S. KODANDARAMAIAH^{1,2} ¹Mechanical Engin., ²Biomed. Engin., Univ. of Minnesota, Minneapolis, MN

Abstract: Many in vivo neuroscience techniques require access to the mouse brain through the skull, which is often obtained by performing craniotomies by a skilled experimenter. With the advent of optogenetics and wide field calcium imaging using 2-photon microscopy, scientists are moving towards excising increasingly large sections of the skull for such experiments. Such operations are challenging to perform and great care must be taken to ensure the underlying dura and brain tissue are not perturbed unnecessarily. Accordingly, progress has been made in automating the process of opening craniotomies. Pak and colleagues (Pak et al J Neurophysiology 2014) recently reported on a closed-loop robotic system for craniotomies using impedance sensing demonstrating the utility of using robotics for performing precise surgical operations in mice. A potential limitation of the impedance sensing approach is the detection of false positives due to the presence of vasculature in some parts of skull. Here, we demonstrate an alternative, generalizable and robust approach using surface profiling and iterative depth milling that enables craniotomies to be opened throughout the mouse dorsal cortex. We first automatically profile the outer surface of the skull in three dimensions using a simple mechanical surface probe mounted on a robotic three-axis stereotaxic robot, a technique common in industrial coordinate inspection tools. Our initial characterization demonstrates that our profiling technique achieves accuracy on the order of single micrometers, which is well within the requirements for ultra-precise, large area craniotomies in mice. Using this 3D profile of the skull, we next map the desired craniotomy contour to the individually measured 3D geometry of the mouse. Finally, we use the three-axis stereotaxic robot to mill the craniotomy contour at precisely controlled iterative depths until the skull fragment can be excised by the experimenter. All of this can be accomplished with a sub-\$1000 lab-built stereotaxic robot. The robot is fully open source, utilizing inexpensive stepper motor electronics and control software with active online development communities. The setup is easy to build, requiring minor technical skills. We are currently working on including robotic manipulators for soft tissue manipulation, perfusion apparatus and computer vision guidance to fully automate the rodent surgery procedure.

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Poster

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Program#/Poster#: 344.07/VV75

Topic: I.04. Physiological Methods

Title: Miniature fluorescence imaging microscope for the study of spinal cord sensory processing

Authors: *I. LECKER¹, Y. SOUDAGAR², R. P. BONIN¹ ¹Univ. of Toronto, Toronto, ON, Canada; ²Neurescence Inc., Toronto, ON, Canada

Abstract: An estimated 20% of the general population suffers from chronic pain. The mechanisms underlying this highly debilitating condition are not fully understood and the most effective therapeutic options, such as opioids and analgesic, simply numb the pain without addressing its root causes. There is therefore a strong need to understand the molecular mechanisms that give rise to pathological pain in order to identify novel approaches for treating or reversing it. The study of pain pathways in the spinal cord has been limited, in part due to a lack of in vivo strategies for studying sensory processing at the cellular level in freely-moving animals. We have recently developed a new miniature microscope which improves upon existing in vivo imagining technology by enabling multi-site imaging to facilitate the study of neuronal activity across receptive fields of the spinal cord dorsal horn. The miniature imagining microscope uses flexible, customized endoscopic fibers and implantable micro-objectives to enable chronic measurement of fluorescence activity in freely-behaving mice. This design of the microscope does not impair animal mobility, enables the animal to participate in normal behaviors, and does not impede experimental conditions. The goal of this project was to implement the novel miniature fluorescence microscope to study the circuitry of sensory plasticity as it relates to pathological pain in the spinal cord of freely behaving mice.

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Poster

344. Large-Scale, Deep, and High-Speed Functional Light Microscopy

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Support: NSF CBET-1631912

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NIH HD041697

Title: Three-dimensional imaging of neural activity in freely-behaving mice by a head-mounted two-photon fiber-coupled microscope with electrically tunable focus

Authors: *B. OZBAY¹, G. L. FUTIA¹, M. MA², E. G. HUGHES², D. RESTREPO², E. A. GIBSON¹

¹Bioengineering, ²Cell & Developmental Biol., Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: Two-photon microscopy is increasingly used for imaging brain activity in head-fixed animals due to the benefits of higher imaging depth compared with single-photon widefield imaging. At the same time, there is a desire to image neuronal activity in freely-behaving animals to expand the types of behavior studies that can be done. Head-mounted single-photon microscopes are commercially available for freely-moving studies, however they are unable to resolve true three-dimensional information. Two-photon head-mounted microscopes have also been described but typically do not incorporate active focusing. We present a miniature twophoton fiber-coupled microscope (TP-FCM) for neuronal imaging in freely-behaving mice with active axial focusing enabled with a miniature tunable electrowetting lens. A high-density imaging fiber-bundle is used to achieve lateral scanning when coupled to a laser-scanning twophoton microscope system. Axial scanning is performed by changing the voltage applied to the electrowetting lens incorporated in the miniature microscope distal to the fiber-bundle. The headmounted miniature microscope is made using a precision 3D-printed housing with a total weight less than 4 g. The working distance of the microscope is 450 µm with an additional 200 µm of active axial focusing. The resolution is $\sim 1.5 \,\mu m$ laterally and $\sim 10 \,\mu m$ axially with a field-of-view of 220 µm. For imaging, the microscope is attached to a baseplate that is permanently affixed to the mouse above a cranial window. After the imaging session, the microscope is detached. The reproducibility for repeated imaging was measured to be stable over four weeks, enabling repeated imaging of the same brain region over time. We acquired multiphoton images of GCaMP6s fluorescence showing calcium transients in layer 2/3 neurons in the motor cortex while the mouse was freely-behaving. We recorded distinct neuronal activity from multiple focal planes in the same imaging session. Our TP-FCM demonstrates true three-dimensional twophoton neuronal imaging enabled for freely-behaving animals.

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Poster

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Support: Howard Hughes Medical Institute (HHMI)

JST-CREST

AMED-Brain/MINDS

KAKENHI 16K13015

Title: Ultrafast two-photon microscopy for high-speed brain imaging in awake mice

Authors: *O. HERNANDEZ¹, R. CHRAPKIEWICZ¹, T. ZHANG¹, A. S. SHAI¹, M. J. WAGNER¹, Y. ZHANG¹, C.-H. WU¹, J. ZHONG LI¹, Y. GONG¹, M. INOUE², H. BITO², M. J. SCHNITZER¹ ¹Stanford Univ., Palo Alto, CA; ²The Univ. of Tokyo, Tokyo, Japan

Abstract: Laser-scanning two-photon microscopy is a mainstay technique for optical brain imaging and normally provides frame-acquisition rates of ~10-30 Hz. However, it has often been challenging to track fast biological events by two-photon microscopy with millisecond-scale temporal resolution, due to the limitations of the existing mechanisms for laser-scanning. Considerable past research has sought faster two-photon microscopes, and much of this has focused on developing faster scanning mechanisms; however, attaining kilohertz-scale image frame acquisition rates requires not just fast optical scanners but also sufficient fluorescence photons to support high-speed imaging. Here we present a two-photon microscope based on 400 illumination beams that can sample 45,000-135,000 μ m² specimen areas at rates up to 1 kHz. Using this microscope, we visualized microcirculatory flow, fast bridging vein constrictions, and Ca²⁺ spiking by neocortical pyramidal and cerebellar Purkinje neurons with millisecond-scale timing resolution in the brains of awake mice. When combined with state-of-the-art neural activity indicators, the superior temporal resolution of our two-photon microscope offers new means of probing the fine temporal features of neural coding by identified cell types.

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Poster

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Wellcome Seed Award (AJF)

Royal Academy of Engineering Research Fellowship (AJF)

EPSRC grant EP/L016737/1

Title: Multifocal two-photon microscopy for high speed functional circuit imaging

Authors: *P. QUICKE, A. J. FOUST, T. KNOPFEL, M. NEIL, S. R. SCHULTZ Imperial Col., London, United Kingdom

Abstract: We present our implementation of a multifocal two-photon microscope for high temporal resolution functional imaging of neural activity. Understanding neural microcircuit function depends on monitoring large numbers of neurons with high temporal and spatial resolution. Two-photon point scanning (TPPS) systems spatially resolve signals deep in scattering tissue but are limited in temporal resolution due to the serial nature of each frame's acquisition. Monitoring large numbers of neurons with TPPS is achieved through the use of slower calcium sensors that allow lower sampling rates. In order to capture neural circuits' full dynamics, faster calcium or voltage indicators need to be used and better imaging methodologies developed to fully exploit their potential. Single point scanning strategies, such as traveling salesman scanning or fast acousto-optic targeting require *a priori* knowledge of signal location and sacrifice full coverage of the field of view to increase acquisition rates. Signals of interest from calcium and especially voltage indicators are distributed throughout neurons' dendritic trees and are often not fully sampled. Parallelising two-photon microscopy by using multiple excitation foci allows increased frame rates and dense spatial sampling without sacrifice of emission photons necessary to resolve small and fast functional signals. This comes at the loss of depth penetration due to the need to image the resulting fluorescence onto a spatially resolved detector, which is vulnerable to image degradation due to scattering. We have implemented a spatially sparse multifocal two-photon microscope using a micro lens array and a commonly used TPPS laser, the Mai Tai HP. Our implementation resolves cellular-resolution calcium transients at 200Hz with a field of view of $110 \times 200 \,\mu$ m in Cal-520 AM loaded mouse cortical slices. We present our implementation and assess its performance, relative merits and disadvantages compared to single point scanning systems for use with a mouse brain slice preparation. We compare achievable signal to noise ratio and scattering resistance for different excitation foci and scanning patterns. We also discuss the factors limiting the achievable imaging depth, which will determine the accessibility and adaptability of this technique to other users and systems. This technique could prove particularly useful for cell-resolved two photon voltage imaging and we will assess the outlook for this application.

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Poster

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Support: NIH MH100024 and an NSF MRI 1430040 to DEB

Title: A head-mounted two-photon fiberscope for high-resolution imaging in freely-moving mice

Authors: *Y.-T. A. GAU, W. LIANG, X. LI, D. E. BERGLES Johns Hopkins Univ., Baltimore, MD

Abstract: Our perception of the world is shaped by our behavioral states. Such context-specific sensory processing is achieved through integration of external inputs and internal states. Determining how this process occurs at the level of cells and synapses in defined circuits remains a central goal in neuroscience. Recent in vivo two photon imaging studies in mice that were engineered to express genetically encoded Ca^{2+} indicators specifically within astrocytes have revealed that cortical astrocytes are significantly activated by neuromodulators such as norepinephrine (NE) and acetylcholine (ACh). As a single astrocyte contacts tens of thousands of synapses and neighboring astrocytes are extensively coupled through gap junctions, astrocytes could serve as an amplifier through which sparse neuromodulatory inputs convey internal behavioral states to shape local neural output. However, restraining mice beneath a microscope restricts behavior and increases stress, which may profoundly alter internal states. Head mounted microscopes have been developed to enable visualization of neuronal and glial cell activity in freely moving animals; however, the low resolution of epifluorescence-based systems limits presents significant challenges for analyzing subcellular activity. To address these challenges, we developed a high-resolution fiber optic endoscope that allows two-photon imaging in freely moving mice. This system consists of: (i) a Ti:Sapphire laser pre-chirped by a single mode fiber plus a grating pair, (ii) a piezoelectric actuating tube that enables spiral laser scanning, (iii) a noise-reduced double-clad fiber to enhance light collection, (iv) a compound lens probe with a working distance of 280 µm, and (v) a head-mounted three- axis manipulator for micropositioning. This fiberscope achieved a lateral resolution of $<1 \mu m$ and an axial resolution of \sim 7 µm. Moreover, the working distance is sufficient to resolve individual axons, dendrites and glial cell processes in Layer I and II/III through a 50 µm-thick cranial window. The spatial and temporal resolution of the fiberscope was sufficient to detect dynamic activity of individual noradrenergic and cholinergic axons (induced to express membrane anchored GCaMP6s) in mice during locomotion. Neuronal dendritic and astrocytic microdomain Ca²⁺ signals were also clearly resolved in the upper layers of the visual cortex. This novel high- resolution imaging platform,

when combined with cell specific manipulations, will help assess the intricate interplay between long-range neuromodulatory projections and local neuron-astrocyte communication in state-dependent cortical processing.

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Poster

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Topic: I.04. Physiological Methods

Support: NIMH intramural research program ZIA MH002897

Title: High yield cranial window technique for imaging cortical neuron activity with headmounted miniaturized microscopes

Authors: *X. LI¹, V. CAO², W. ZHANG¹, S. MASTWAL¹, Q. LIU¹, S. OTTE², K. WANG¹ ¹NIH, Bethesda, MD; ²Inscopix Inc.,, Palo Alto, CA

Abstract: Cranial windows and viral reporters are commonly used to image cortical neuronal activity through two-photon microscopes in head-fixed animals. Recently, head-mounted miniaturized one-photon microscopes have been developed to image neuronal activity in freely behaving animals. However, dura membrane overgrowth and inflammation beneath the cranial window often decrease the optical clarity of chronic windows, and variation in viral spread and damage by intracortical injection add further challenges for imaging cortical neurons with headmounted microscopes. Here, we describe an improved cranial window technique to enhance the yield of successful imaging experiments. We used a glass pipette with large tip diameter to attach to the brain surface and injected virally encoded calcium indicator AAV-GCaMP6 into the cortex without pipette penetration. Immediate after virus injection and window implantation, we sutured the scalp skin over the implanted window to facilitate postoperative recovery. After a couple of weeks, we removed the suture and attached a miniature microscope to image cortical neuronal activity in freely behaving mice. We found that cortical surface virus injection efficiently and selectively labeled neurons in superficial cortical layers. Compared to traditional open-scalp surgical recovery method, skin suturing decreased the tissue growth underneath the optical window and improved its clarity. Together, these improvements in labeling and surgery procedures significantly increased the success rate of cortical neuron imaging by head-mounted microscopes in freely moving animals. Our high-yield cranial window technique may be also applicable to other in vivo optical techniques such as two-photon imaging and optogenetic perturbation.

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Poster

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Title: Rapid whole brain imaging of neural activities in freely behaving larval zebrafish

Authors: K. WANG¹, L. CONG¹, Z. WANG², Y. CHAI², W. HANG¹, C. SHANG¹, W. YANG², L. BAI¹, J. DU¹, *Q. WEN² ¹Inst. of Neurosci., Shanghai, China; ²Univ. of Sci. and Technol. of China, Anhui, China

Abstract: Understanding how collective neural activities give rise to complex behaviors has

Abstract: Onderstanding how conective neural activities give rise to complex behaviors has long been a central question in systems neuroscience. Larval zebrafish has emerged as a powerful vertebrate model system for investigating the neural basis of sensorimotor behaviors due to its small size, optical transparency and genetic tractability. In particular, whole brain calcium imaging of head fixed larval zebrafish in virtual reality [1], where visual sensory feedback can be accurately controlled, has provided significant insight into motor learning and optomotor behaviors.

On the other hand, freeing the fish from the *Matrix* while monitoring neuronal activity would be an ideal strategy for investigating more sophisticated behaviors, when the animal is interacting in a more meaningful way with its natural environment and when *all* sensory feedback loops are intact and active. However, imaging whole brain activity with sufficient spatiotemporal resolution in a speeding brain is very difficult.

Here we have made a step towards this goal, by developing a novel volume imaging and 3D tracking technique that performs whole brain imaging of neural activities in a freely swimming larval zebrafish. Whole brain neuronal activities over a volume of 800 μ m × 800 μ m × 200 μ m can be simultaneously monitored at ~ 3.4 μ m × 3.4 μ m × 5 μ m spatial resolution and at 77 Hz volume rate, a technique we call eXtended field of view Light Field Microscope (XLFM). Armed with XLFM tracking microscope, we were able to capture, for the first time, whole brain neural activities during the entire prey capture process in larval zebrafish. Our technique provides a new window into the brain dynamics during animal's natural behaviors at unprecedented resolution.

[1] Ahrens, M.B., et al., *Brain-wide neuronal dynamics during motor adaptation in zebrafish*. Nature, 2012. **485**(7399): p. 471-7.

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Poster

344. Large-Scale, Deep, and High-Speed Functional Light Microscopy

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: I.04. Physiological Methods

Support: German Research Foundation

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Alfried Krupp von Bohlen und Halbach Foundation

Title: *In vivo* scattering compensation with increased FOV using conjugate F-SHARP microscopy

Authors: I. N. PAPADOPOULOS¹, D. I. KAPLAN², M. E. LARKUM³, *B. JUDKEWITZ¹ ¹Einstein Ctr. for Neurosci., Charite Berlin / Humboldt Univ., Berlin, Germany; ²NeuroCure Cluster of Excellence, Charité Univ. Hosp. Berlin, Berlin, Germany; ³Humboldt Univ. of Berlin, Berlin, Germany

Abstract: Optical techniques have become indispensable tools for neuroscience, but their depth reach is limited by scattering. Aberration correction and scattering compensation techniques can deliver improved image quality, but only within a certain field of view (FOV). This limitation arises from the spatial variation of the inhomogeneities that light encounters while scanned around the focus location. The corrected FOV area can be increased considerably by conjugating the wavefront-shaping device to the dominant scattering layer rather than the back pupil of the microscope objective. Although the conjugation of the wavefront-shaping element to the dominant scattering layer can be implemented by a physical movement of the wavefront-shaping device within the optical path of the microscope, such an implementation is limited only to one particular layer. Here, we overcome this limitation by developing and demonstrating a method for dynamically conjugating the wavefront-shaping element to different depths inside the inhomegeneous medium without the need for the physical movement of the wavefront shaper inside the optical path. Our method, conjugate F-SHARP microscopy, allows us to change the conjugation distance between the wavefront shaper and the imaging depth in real time. We demonstrate the power of conjugate F-SHARP microscopy by performing volumetric imaging with an increased FOV through the thinned mouse skull in vivo.

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DARPA TNT CAN

McKnight Brain Institute

Title: Characterization and application of a quadruple labelled mouse line

Authors: *J. GAIRE¹, H. LEE², R. WARD¹, S. CURRLIN¹, E. W. ATKINSON¹, A. WOOLLEY³, J. E. COLEMAN¹, K. J. OTTO¹ ¹Univ. of Florida, Gainesville, FL; ²Univ. of Texas Southwestern Med. Ctr., Dallas, TX; ³Symic Bio, Inc, Emeryville, CA

Abstract: The advancements in the field of microscopy has been instrumental in three dimensional imaging of thick tissues and whole organs circumventing the need to mechanically section, stain, image and re-construct the 3-D image. This has been facilitated by the ever growing list of clearing techniques that renders the sample's refractive index to match with the mounting medium and optics. Regardless of the clearing techniques used, the sample has to be immunostained with the antibodies of interest for fluorescent imaging. Immunostaining, especially in thick tissues, has many issues including prolonged incubation, limited penetration depth of antibodies and the need for exposure to high temperature and/or strong detergent concentrations. To address this, mainly focusing on the brain, we created a transgenic mouse model that express distinct fluorophores in different cell types. Here we present the application of this mouse model in thick tissue imaging utilizing previously developed clearing methods and studying cellular response to an injury model of cortical implant. For clearing studies, tissues were processed with slight modifications from previously developed clearing methods. Brains were cut into thick sections of varying thickness and imaged using various imaging modalities. We were able to detect endogenous fluorophores in thick samples cleared using the CLARITY protocol. Currently, we are investigating if the fluorophores will be retained using other clearing techniques. For implant studies, silicon microelectrodes were implanted in the somatosensory cortex and sacrificed at 3 different time points. We observed an increase in microglial cells around the implant at earlier time points and a subsided response a later time point whereas the astrocytic response was progressive. Both of these observations are in line with previous studies

that were carried out in wild type mouse and rat models. Together, these data highlight the compatibility of our mouse model with recently developed volumetric imaging modalities. This mouse model, in addition with emerging microscopy, helps to increase efficiency of experiments both in normal and injury models (cortical impact, stroke, etc.) where neuroinflammation cascade is perturbed.

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Poster

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Title: Two-photon calcium imaging of medial prefrontal cortex and hippocampus without cortical invasion

Authors: *M. KONDO^{1,2}, K. KOBAYASHI³, J. NAKAI⁴, M. OHKURA⁴, M. MATSUZAKI^{1,2} ¹Grad. Sch. of Med., The Univ. of Tokyo, Tokyo, Japan; ²Div. of Brain Circuits, Natl. Inst. for Basic Biol., Okazaki, Japan; ³Natl. Inst. For Physiological Sci., Okazaki, Japan; ⁴Saitama Univ., Brain Sci. Inst., Saitama, Japan

Abstract: In vivo two-photon calcium imaging currently allows us to observe the activity of multiple neurons up to ~900 μ m below the cortical surface without cortical invasion. However, many other important brain areas are located deeper than this. Here, we used a 1100 nm laser, which underfilled the back aperture of the objective, and red genetically encoded calcium indicators to establish two-photon calcium imaging of the intact mouse brain and detect neural activity up to 1200 μ m from the cortical surface. This imaging was obtained from the medial prefrontal cortex (the prelimbic area) and the hippocampal CA1 region. We found that the neural activity related to reward timing is higher in the prelimbic area than in layer 2/3 of the secondary motor area, while it is negligible in the hippocampal CA1 region. Reducing the invasiveness of imaging is an important strategy to reveal the brain processes active in cognition and memory.

Disclosures: M. Kondo: None. K. Kobayashi: None. J. Nakai: None. M. Ohkura: None. M. Matsuzaki: None.

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Topic: I.04. Physiological Methods

Title: Optical coherence tomography reveals depth-resolved responses during functional cerebral activation by infrared neural stimulation

Authors: *P. LI¹, P. LI³, W. XI⁵, E. AKSHAY⁴, A. W. ROE²

¹Dept. of Optical Engin., ²ONPRC-NEUROSCIENCE, Zhejiang Univ., Zhejiang, China; ⁴ZIINT, ³zhejiang university, Hangzhou, China; ⁵Zhejiang Univ., ZIINT, Hangzhou, China

Abstract: Infrared neural stimulation (INS) has been reported as a promising modality for evoking neural response during cerebral activation. Optical imaging is an effective tool for measuring the activated dynamics and responses. In contrast to conventional optical intrinsic signal imaging (OISI) modality that provides two-dimensional, depth-integrated maps of stimulus-evoked dynamic activation, we used optical coherence tomography (OCT) to provide depth-resolved, cross-sectional images of functional cerebral activation by INS. In this study, 0.5s-lasting pulsed infrared light (central wavelength 1.875 μ m, pulse width 250 μ s and fiber diameter 200 μ m) was utilized to stimulate the cortex of anesthetized rats with different radiant exposure (0~1 J/cm²) and different repetition rates (50~200 Hz), respectively. Temporal fractional OCT signal change was calculated to evaluate INS efficacy. It revealed statistically significant depth-resolved signals, comprised of both positive and negative responses. These responses were detectable at cortical depths of >1 mm during functional activation. This suggests that OCT is a promising in vivo imaging method for mapping functional neural coupling in neuroscience research.

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Title: Multimodal two-photon random access pointing and scanning to any 3D position or direction at high speed in awake animals using a compact Acousto-Optic Lens (AOL)

Authors: V. GRIFFITHS, *T. FERNANDEZ-ALFONSO, G. KONSTANTINOU, N. NADELLA, P. KIRKBY, A. M. VALERA, H. ROSS, F. LANORE, A. SILVER Univ. Col. London, London, United Kingdom

Abstract: To understand brain function it is essential to identify how information is represented by the firing patterns of large neuronal populations and establish how synaptic inputs are integrated and transformed by individual neurons within microcircuits. This requires monitoring activity in large numbers of neuronal compartments distributed in 3D space at high spatiotemporal resolution in awake animals. In order to meet these requirements, we (and others) have previously developed 3D random access acousto-optic lens (AOL) two-photon microscope technology that can focus to any arbitrary position and scan a laser beam within the imaging volume within 12-40 microseconds. Our AOL design consists of 4 acousto-optic crystals, wave plates and polarizers arranged in a compact unit (12cm x 12cm x 22cm). To simplify its integration into existing and new microscopes, we have developed a new opto-mechanical design for easier alignment and new controller box with state-of-the-art FPGA and custom DAC boards. The FPGA-based AOL control system, which generates the radio frequency (RF) signals for the AOD transducers, supports 5 different imaging modes. High-speed raster scan volume imaging (Z stacks), multiple subvolume imaging, multiplane x-y imaging, multiple x-y patch, and serial pointing mode. All these imaging modalities are achieved with linearly ramped RF waves fed into the acousto-optic crystals. In experiments on awake animals, tissue brain movement can be problematic. The high speed random access functionality enables our system to image reference objects allowing brain movement to be tracked. By feeding back this information to the AOL controller we have previously developed a real time 2D movement correction system. However, the use of linear ramps limits the raster scanning to a single x-y plane, precluding fast tracking of axial movement and thus correction for movement in 3D. Here, we describe and demonstrate the use of non-linear ultrasound ramps to achieve both axial scanning and real-time movement

correction in 3D, and new imaging modalities based on raster scanning in arbitrary 3D angles (e.g., 'skeleton scanning'). This technology enables movement-stabilized, high-speed random access pointing and line scanning along fine neuronal structures *in vivo*. In conclusion, our prototype compact AOL module not only adds real-time movement correction in 3D and axial scanning, but it is also smaller, more adaptable and can be fabricated at a relatively low cost.

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P. Kirkby: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on patents owned by UCLB. A.M. Valera: None. H. Ross: None. F. Lanore: None. A. Silver: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on patents owned by UCLB.

Poster

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WWTF VRG10-11

Title: Large volume functional imaging in the mouse brain

Authors: *S. WEISENBURGER¹, R. PREVEDEL², A. VAZIRI¹ ¹Lab. of Neurotechnology and Biophysics, The Rockefeller Univ., New York, NY; ²European Mol. Biol. Lab. (EMBL), Heidelberg, Germany

Abstract: Unlocking the mystery of how the complex dynamics of neurons in the brain give rise to cognition, perception and behavior is not only of paramount importance to science but may also affect the treatment of neurological diseases impacting hundreds of millions of people around the world. Seminal breakthroughs like multiphoton microscopy, fluorescent indicators and optogenetics have positioned optical microscopy as a key technology in modern brain research. The next frontier in neuroscience is to interrogate neuronal dynamics of large populations in awake, behaving animals in real time in order to unravel the complex interactions

of neurons that underlie cognitive function and behavior. Here, we will introduce a technique termed light sculpting microscopy which was pioneered in our laboratory. It is based on temporal focusing which allows an effective decoupling of the axial and lateral confinement of the excitation volume combined with the advantage of high penetration depth and low scattering of two-photon excitation. The ability to tailor the three-dimensional spatial distribution of light within biological samples allows to maintain optical sectioning while introducing parallelization of excitation and thereby obtain high-speed acquisition rates. We will present different in vivo applications of this method including large-scale calcium imaging of neuronal networks in mouse brain using our latest approach termed scanned temporal focusing (s-TeFo). By optimizing the signal-to-noise ratio using a one-pulse-per-voxel excitation and synchronized detection scheme, we demonstrate unbiased high-speed single plane recordings of mouse posterior parietal cortex (PPC) expressing GCaMP6m of $> 0.5 \times 0.5$ mm field of view at > 200Hz frame rate. We also demonstrate dual-plane calcium imaging from two $> 0.5 \times 0.5$ mm field of views at different depths and at > 10 Hz frame rate. Furthermore, we show volumetric calcium imaging of large volumes (> 0.5 x 0.5 x 0.5 mm) at single-cell resolution and fast volume rates (>4 - 6 Hz) from mouse hippocampus CA1 and across cortical layers 1-5 in PPC with GCaMP6m/s and jRGECKO1a labeling. These advances allow for near-simultaneous in-vivo recording of the calcium dynamics from several thousand active neurons across cortical layers and in the hippocampus of awake behaving mice. We will discuss the challenges, future developments and applications of our technique in fundamental neuroscience questions.

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Poster

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Title: High-content functional imaging reveals differential effects of inflammation on two distinct populations of human nociceptive neurons

Authors: ***A. GHETTI**¹, Y. MIRON¹, J. ZHANG¹, G. PAGE¹, C. GHETTI¹, B. A. COPITS², S. DAVIDSON³, R. W. GEREAU, IV², P. E. MILLER¹ ¹Anabios Corp., San Diego, CA; ²Pain Ctr. and Dept. of Anesthesiol., Washington University, Sch. of Med., St. Louis, MO; ³Pain Res. Center, Dept. of Anesthesiology,, Univ. of Cincinnati, Cincinnati, OH

Abstract: The etiology of chronic neuropathic and inflammatory pain remains incompletely understood, creating serious challenges to the development of new, non-addictive medications. Pain drug discovery has had very limited success in translation from animal models to humans. Some have proposed that animal models have limited ability to predict analgesia in humans, and there has been a call for new approaches that better predict clinical analgesic efficacy. We have developed a novel preclinical discovery strategy, which relies on human sensory neurons isolated from organ donors. In the current study, we describe the high content imaging-based profiling of human sensory neurons under normal as well as inflammatory conditions. Human DRG neurons isolated from organ donors were loaded with Fluo-8-AM and the intracellular calcium dynamics elicited by electrical and chemical stimuli were investigated. By sequentially stimulating the cells with electrical fields, capsaicin, allyl isothiocyanate (AITC), and cold, we were able to define, for each individual sensory neuron tested, the array of functional expression of voltage gated sodium channel subtypes, as well as different TRP channels. When cells were activated with carefully calibrated electric field stimulations (EFS) delivered at two different voltage intensities and frequencies, all responses were blocked by lidocaine, indicating that EFS-induced activities were purely dependent on voltage gated sodium channels (VGSC) but not voltage gated calcium channels (VGCC). The observed intracellular calcium dynamics were therefore the result of EFS-induced activation of VGSC, which led to cell depolarization and consequent activation of VGCC and calcium release from internal stores. Using these EFS protocols we were able to identify two major classes of cells based on: 1) the EFS activation threshold and, 2) the maximal firing frequency. Interestingly the two classes of cells also exhibited differential sensitivity to TTX. We also observed that the inflammatory agents bradykinin and prostaglandin E2 (PGE2) exerted different effects on the two cell classes, whereby the firing frequency was increased in one class of cells, while the other showed increased expression of TTX-R VGSC. The strategy described in the present work has the potential to significantly advance preclinical drug profiling by allowing the human-specific profiling of new drug candidates' selectivity for functionally defined sub-populations of cells therefore improving the ability to select humanactive drugs for clinical trials.

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collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH R21 AR068012. **R.W. Gereau:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH R01 grant NS042595. **P.E. Miller:** A. Employment/Salary (full or part-time):; Employee of AnaBios Corp..

Poster

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Title: Three-dimensional (3D) volumetric Ca^{2+} imaging of astrocytes reveals novel properties of brain networks

Authors: A. VOLTERRA, *I. A. SAVTCHOUK, E. BINDOCCI, N. LIAUDET, D. BECKER, G. CARRIERO

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Abstract: Astrocytes sense neuronal inputs and in turn modulate synapses and blood vessels via intracellular Ca^{2+} signaling. Despite their importance, the properties of astrocytic Ca^{2+} signals are not well understood.

Most of the astrocytic structure presents functional barriers to intracellular Ca^{2+} propagation, with the result that Ca^{2+} events are highly compartmentalized throughout the cell, and exhibit large variability in amplitude and duration, as well as spatial spread. It is difficult to reliably study Ca^{2+} events because only an absolute minority, at best ~5%, of the total astrocyte lies within a given 2-photon focal plane.

We report here our recent success in performing fast 3D volumetric imaging, monitoring entire individual astrocytes in adult hippocampal and cortical slices, and *in vivo*. Morphology of individual astrocytes was visualized using SR101 dye uptake, while genetically-encoded indicator GCaMP6f was used to monitor Ca^{2+} elevations. Both optically resolved (core) structure

of the astrocyte, and optically sub-resolved fine processes (gliapil) were analyzed using two independent strategies.

By visualizing the whole astrocyte, we were able to correctly capture and describe the full gamut of Ca^{2+} events and their properties, including duration, kinetics, and spread volume. Majority of the observed events were three-dimensional, and would not be otherwise properly captured in 2D. Moreover, we were able to simultaneously compare activity across all individual processes on the same cell: something previously impossible due to the limitations of 2D microscopy. We found that most of the activity lies outside the soma, and is concentrated in the processes and endfeet. A large portion of this activity is highly compartmentalized and asynchronous. As a further practical application of the 3D method, we were able to study functional interactions between astrocytes and axons, and between endfeet and blood vessels, as well as astrocytic population activity *in vivo*.

Our volumetric imaging and analysis method has important implications outside the glial Ca^{2+} field. While independently surveilling tens of thousands of um3 per cell, we identified the presence of multiple "hot" and "cold spots," and observed a large diversity of highly compartmentalized Ca^{2+} signals happening within a single astrocyte, as well as functional "coupling" between some but not other "voxels". This is methodologically comparable to monitoring and analyzing the connections between thousands of neuronal cell bodies within a brain circuit/network. We believe our methods and strategies will therefore be useful also for neuronal network Ca^{2+} analysis.

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Title: Purkinje cell ensemble dynamics in freely moving mice

Authors: *M. KISLIN^{1,2}, B.-C. CHO^{1,2}, J. MANLEY^{3,4}, J. W. SHAEVITZ^{3,4}, S. S.-H. WANG^{1,2}

¹Neurosci. Inst., ²Dept. of Mol. Biol., ³Dept. of Physics, ⁴Lewis-Sigler Inst. for Integrative Genomics, Princeton Univ., Princeton, NJ

Abstract: Traditionally, the cerebellum has long been known to coordinate movement on short time scales. In addition, recent evidence suggests that it may also modulate flexible and social behavior. To monitor cerebellar activity during complex behavior, we monitored calcium and delivered optogenetic perturbations in Purkinje cell (PC) dendrites in freely-moving mice using a Doric head-mounted miniature microscope to perform wide-field fluorescence microscopy and deliver light to optogenetic probes. In Pcp2-Cre mice, which express Cre in PCs, we injected cerebellar vermal lobule VI with Cre-inducible adeno-associated virus (AAV2/1-CAG-DIO) coding genetically encoded calcium indicator (GCaMP6f) or optogenetics probes (ChR2 or ArchT). Identified regions of interest showed rapid transients with rise times of 91 ± 4 ms and half-decay times of 189 ± 7 ms, and occurring at a rate of 0.7 ± 0.1 Hz, consistent with prior studies of PCs using two-photon microscopy in awake head-fixed mice (Ozden et al. 2012, PLoS ONE 7(8):e42650). Simultaneous with optical measurements and perturbation, animal behavior was recorded with two cameras, a low-speed, low-resolution, depth-sensitive 3D camera and a high-speed, high-resolution 2D camera, which together can be used to capture poses of the head, body, limbs, and tail. We identified behavioral correlates of PC activity using unsupervised computational methods to classify animal poses and movements. Together, pose classification and imaging with miniature microscope allow us to explore the fine details of behavior in a more natural setting on subsecond time scales.



Top, GCaMP6f signals from Purkinje cell dendrites. *Bottom*, sample ethogram of six identified features of behavioral activity.

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Poster

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Title: Non-degenerate 2-photon excitation for increased fluorophore brightness and deep tissue imaging

Authors: M.-H. YANG¹, C. G. L. FERRI², M. THUNEMANN³, P. SAISAN⁴, E. A. RODRIGUEZ⁵, S. R. ADAMS⁵, S. VINOGRADOV⁷, Y. FAINMAN¹, *A. DEVOR^{6,8} ¹Electrical and Computer Engin., ²Neurosciences, ³Radiology, ⁵Dept. of Pharmacol., ⁴UCSD, La Jolla, CA; ⁶Neurosciences and Radiology, UCSD, LA Jolla, CA; ⁷Dept. of Biochem. and Biophysics, Univ. of Pennsylvania, Philadelphia, PA; ⁸Martinos Ctr. for Biomed. Imaging, MGH, Harvard Med. Sch., Charlestown, MA

Abstract: In conventional 2-photon microscopy, a fluorophore is excited by the simultaneous absorption of two photons of the same energy within the near infrared (NIR) spectrum, derived from the same pulsed laser beam. This is called "degenerate" 2-photon excitation, D-2PE. Alternatively, the same energy needed for the transition to the excited state can be delivered via absorption of two photons of different energy (i.e., different color) [1]. In this "non-degenerate" 2-photon excitation (ND-2PE) regime, the first laser beam can be tuned within the standard NIR range of excitation wavelengths used in conventional 2-photon microscopy, placing the second laser beam within the infrared (IR) wavelengths range for excitation of visible emission fluorophores. Recently, we experimentally demonstrated that, under ND-2PE, an increase in the IR power can be used to compensate for the scattering losses of NIR power, achieving deeper excitation in scattering media [2]. Here, we provide additional data from phantoms and biological samples to support this conclusion and demonstrate another advantage of ND-2PE: an

increase in the excitation cross section leading to more efficient fluorophore excitation. We used both D-2PE and ND-2PE to characterize the 2-photon absorption cross sections of several fluorophores widely used for brain imaging, including eGFP, eYFP, mCerulean, mCitrine and fluorescein. When compared to the optimal D-2PE case, up to 3x enhancement in brightness was observed for this selection of fluorophores over an excitation wavelength range of 740-870nm, for the NIR beam, and 1000-1400nm, for the IR beam (see figure). The enhanced fluorescence brightness combined with a reduction in laser attenuation positions ND-2PE microscopy as a competitive alternative to conventional 2-photon microscopy and, possibly, 3photon microscopy, for deep imaging applications.

[1] P. Mahou, et al., Nat. Methods, vol. 9, no. 8, pp. 815-818, Jul. 2012.

[2] M. Yang, et al., Opt. Express, vol. 24, no. 26, p. 30173, Dec. 2016.



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Poster

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Title: A novel multisite confocal system for rapid Ca^{2+} imaging from submicron structures in brain slices

Authors: *M. CANEPARI¹, L. FILIPIS¹, K. AIT OUARES¹, P. MOREAU¹, D. TANESE², V. ZAMPINI², V. EMILIANI²

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Abstract: In brain slices, resolving Ca²⁺ fluorescence signals from submicron structures, such as spines, terminals or small dendritic and axonal branches, is typically achieved using two-photon or confocal microscopy by scanning the recording region of interest. Yet, the condition for a signal to be above the photon noise sets a limit to the number of recording sites at a given acquisition rate, since the required number of collected photons is inversely proportional to the number of scanned points. Here, we present a novel multiplexing confocal system based on a fast spinning disk and a rapid high-resolution CMOS camera that overcomes the limitations of scanning confocal microscopy. The spinning disk, running at 20,000 rpm, has a custom-designed spiral pattern that maximizes light collection while rejecting out-of-focus fluorescence to discriminate the smallest neuronal compartment. The spinning disk is fed with a 1W multimode laser providing a laser line at 470 nm. Using a 60X objective and no magnification, the novel CMOS camera permits acquisitions of tens of thousands of pixels at the virtual resolution of 240 nm per pixels in the kHz range with a digital depth of 14 bits. By focussing the laser onto a 60 µm diameter spot, we demonstrated the ability of our system to resolve fluorescence signals of the order of 1% from submicron structures positioned 20-40 µm from the slice surface, using the low affinity Ca²⁺ indicator Oregon Green BAPTA-5N. In particular, we monitored Ca²⁺ transients at 0.25-1.25 kHz in single trials or through averages of less than ten recordings from dendritic spines and small parent dendrites in cerebellar Purkinje neurons, following either the stimulation of the climbing fibre or of parallel fibre inputs. Altogether, the unprecedented high spatiotemporal resolution of these Ca^{2+} recordings is obtained with a relatively simple and economical implementation. Thus, we expect that the present approach will be adopted in several laboratories for projects where multisite monitoring of physiological signals from submicron structures is required.

Disclosures: M. Canepari: None. L. Filipis: None. K. Ait Ouares: None. P. Moreau: None. D. Tanese: None. V. Zampini: None. V. Emiliani: None.

Poster

344. Large-Scale, Deep, and High-Speed Functional Light Microscopy

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 344.25/WW1

Topic: I.04. Physiological Methods

Support: AHA grant 13SDG16990083

Title: Correcting micro-positioning artifacts for 3D microscopy

Authors: *J. L. LIN¹, M. A. NAVARRO¹, J. V. K. HIBBARD², L. S. MILESCU¹ ¹Div. of Biol. Sci., Univ. of Missouri, Columbia, MO; ²Mol. Biosci., Univ. of Texas, Austin, TX

Abstract: Precise 3D neuronal targeting and data mapping in electrophysiology and imaging experiments requires the use of motorized micro-positioners. Most commercially available positioning devices have sub-micron resolution, but they are subject to a variety of positioning artifacts and errors. One particularly important aspect is the ability to consistently reach the same 3D position. We found that, with some equipment, the errors in this regard can be up to 10 microns, which may be unacceptable for certain studies. While normal backlash is corrected by the hardware controller, this re-positioning error is not. We have mathematically modeled this error and added correcting code to our QuB software for 3D data mapping and real-time experimental control. With this correction, the same 3D target can be repeatedly reached with improved resolution.

Disclosures: J.L. Lin: None. M.A. Navarro: None. J.V.K. Hibbard: None. L.S. Milescu: None.

Poster

344. Large-Scale, Deep, and High-Speed Functional Light Microscopy

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 344.26/DP14/WW2 (Dynamic Poster)

Topic: I.04. Physiological Methods

Support: NIH grant U01NS099689

Wellcome Trust

ERC

Title: Skeleton scanning: A method for imaging 3D dendritic trees at high speed

Authors: *A. M. VALERA, G. EVANS, P. KIRKBY, G. KONSTANTINOU, V. GRIFFITHS, B. MARIN, S. K. M. N. NADELLA, A. R. SILVER Dept. of Neuroscience, Physiol. & Pharmacol., Univ. Col. London, London, United Kingdom

Abstract: Understanding how synaptic inputs are integrated within complex 3D dendritic trees requires fast recordings of patterns of activity across many dendritic branches. While recordings from individual dendritic branches projecting in the x-y plane is straightforward with conventional galvanometer-based 2-photon technologies, accessing the complete 3D dendritic tree is much more challenging. Acousto-Optic Lens (AOL) 3D two-photon microscopes can

randomly access up to 40000 points or lines per second in a 3D space, with an inertia-free focusing system. However, the linear acoustic drives typically used to date only permits linescans in the x-y plane. Here we show how non-linear drive of the AOL can be used to create line-scans in any arbitrary direction, enabling us to rapidly image the complex 3D branching structures of dendritic trees unprecedented speed. We have developed a software tool chain to image, trace and segment the morphology of a neuron during the experiment. This enabled us to generate a 3D 'skeleton' representation of the neuronal morphology and to calculate the nonlinear AOL drives required to image it with a series of line-scans. This 'skeleton scanning' approach allows the 3D dendritic tree of a neuron to be imaged at high rates. We have tested the performance of skeleton scanning by imaging L2/3 cortical pyramidal cells in acute slices patch loaded with Alexa594 and Fluo4. Our preliminary experiments show that skeleton scanning can be used to monitor $[Ca^{2+}]$ across the dendritic tree at frequencies between 100 and 150 Hz. Local, spontaneously occurring $[Ca^{2+}]$ transients could be detected, as well as more widespread increases induced by backpropagating action potentials triggered by somatic current injection. The 'Skeleton Scan' can be extended to multiple contiguous lines allowing both dendrites and spines to be monitored, confirming that the technology has the capacity to measure both the activity of dendritic branches and spines at high temporal resolution. We are currently working on integrating this technology with real-time movement correction in order to image GCaMP6f expressing-L2/3 pyramidal neurons in visual cortex of behaving animals.

Disclosures: A.M. Valera: None. **G. Evans:** None. **P. Kirkby:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on patents owned by UCLB. **G. Konstantinou:** None. **V. Griffiths:** None. **B. Marin:** None. **S.K.M.N. Nadella:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on patents owned by UCLB. **A.R. Silver:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on patents owned by UCLB. **A.R. Silver:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on patents owned by UCLB.

Poster

344. Large-Scale, Deep, and High-Speed Functional Light Microscopy

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 344.27/WW3

Topic: I.04. Physiological Methods

Support: National Eye Institute grant NEI EY016203

Medical Student Research Grant from Frank Netter School of Medicine at Quinnipiac University

Title: Quantifying wide-field maps of the human corneal subbasal nerve plexus

Authors: *Y. VAISHNAV¹, S. RUCKER², K. SAHARIA³, N. MCNAMARA¹ ¹UCSF Proctor Eye Fndn., San Francisco, CA; ²Phillips Exeter Acad., Exeter, NH; ³TechLab Educ., Saratoga, CA

Abstract: A significant proportion of corneal sensory nerves lie beneath the anterior corneal epithelium within the subbasal nerve plexus (SBP). The morphology of these nerves is altered by keratopathy and refractive surgery. Corneal Confocal Microscopy (CCM) is an imaging modality used to image and quantify corneal nerves. There are two barriers that currently limit the use of CCM as a diagnostic modality: the small-scale field of view given by individual CCM images and the time required to quantify nerves in collected images. We present here a modified OpenCV image stitcher and a vector quantification method to map and obtain measurements of SBP nerve morphology. The stitcher uses feature detection to stitch CCM frames into wide-field maps of the SBP. Our method takes 3-5 minutes to stitch and quantify 40-100 CCM frames on an Amazon EC2 Micro instance. The vector quantification method is a novel, proof-of-concept algorithm that can quantify montages of SBP nerves. The method is more rapid and user-friendly than previous iterations of similar corneal nerve mosaicking software and proposes a new method to quantify CCM morphological parameters.

Disclosures: Y. Vaishnav: None. S. Rucker: None. K. Saharia: None. N. McNamara: None.

Poster

345. Methods: Physiology and Circuitry I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 345.01/WW4

Topic: I.04. Physiological Methods

Support: NEI and NIMH 1-U01-MH106027-01

NIH Single Cell Grant 1 R01 EY023173

Title: Image-guided automated patch-clamp electrophysiology In vitro

Authors: *I. KOLB¹, J. LEE², A. FELOUZIS¹, W. A. STOY¹, E. S. BOYDEN⁴, C. J. ROZELL², C. R. FOREST³

¹Wallace H. Coulter Dept. of Biomed. Engin., ²Sch. of Electrical and Computer Engin., ³Dept. of Mechanical Engin., Georgia Inst. of Technol., Atlanta, GA; ⁴MIT, Cambridge, MA

Abstract: For decades, patch-clamp recording has been the gold-standard technique for highquality electrophysiological measurements and morphology reconstruction of single cells. However, it is a highly manual, laborious technique that requires extensive training to master. Here we present the patcherBot, a robotic system that permits walk-away automation of in-vitro patch-clamp recording. This is enabled by two key innovations: (1) pipette cleaning which enables immediate reuse of the pipette and (2) machine vision for accurate pipette positioning. The patcherBot is the first system that can patch-clamp multiple cells sequentially without human intervention, enabling multi-hour user-free operation. The system was tested in cultured cells and in brain tissue slices. In cultured Human Embryonic Kidney (HEK293) cells, the patcherBot achieved a medium-high success rate of whole-cell recording (median: 80%; range: 60 to 100%; n=70 patch-clamp attempts) over a combined user-free operation of over 5 hours. In mouse VI cortical tissue slices, the patcherBot achieved lower success rate (median: 55%, range: 22 to 67%; n = 44 patch-clamp attempts), commensurate with the increased difficulty of patch-clamp in brain slices. Combining the patcherBot with existing single-cell activity imaging techniques could open the door to "circuit mappers", generalizable tools for profiling electrophysiology, morphology, and synaptic connectivity patterns in the brain on a single-cell level.

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Poster

345. Methods: Physiology and Circuitry I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 345.02/WW5

Topic: I.04. Physiological Methods

Support: NSF GRFP

NIMH U01-MH106027-01

Title: Capping patch clamp pipettes for improved gigaseal yield

Authors: *W. STOY¹, I. KOLB¹, G. L. HOLST², G. B. STANLEY¹, C. FOREST² ¹Wallace H. Coulter Dept. of Biomed. Engin., ²George W. Woodruff Sch. of Mechanical Engin., Georgia Inst. of Technol., Atlanta, GA

Abstract: Whole-cell patch clamping *in vivo* often requires a recording electrode, a pipette, to travel through several millimeters of heterogeneous brain tissue. During this process, called regional pipette localization, the tip of the pipette can encounter obstructions including the dura, pia, blood vessels, and ventricular membranes. When these obstructions are encountered, they often contaminate the pipette tip, preventing the formation of a gigaseal. Application of high positive pressure to the pipette to prevent contamination introduces intracellular solution to the extracellular space, causing high osmotic pressure on nearby cells as well as off-target staining if a contrast agent is present in the solution. To overcome these limitations we have developed several methods to maintain the cleanliness of pipette tips, including a method to avoid obstacles

encountered during regional pipette localization *in vivo*. Here, we present a reversible cap to protect the pipette tip during regional pipette localization. We demonstrate in vitro that this cap, a polymer microsphere, can be reversibly attached to the tip of a pipette with the application of suction, that under atmospheric pressure the microsphere remains attached to the pipette, and that the microsphere remains attached when moved through slices of brain tissue. We demonstrate that when a cell is penetrated with a freshly pulled pipette *in vitro*, it contaminates the pipette tip (visible as an increase in pipette tip resistance), which prevents this same pipette from gigasealing onto a second cell (0 gigaseals, 5 attempts). However, when a cell is penetrated with a capped pipette, there is no contamination following removal of the microsphere (no increase in pipette tip resistance) and gigaseals are reliably formed on subsequent cells at a rate similar to traditional *in vitro* slice patching (4 gigaseals, 5 attempts). These results indicate that a reversible cap may protect sensitive pipette tips from contamination during regional pipette localization.

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Poster

345. Methods: Physiology and Circuitry I

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Topic: I.04. Physiological Methods

Support: NIH grant 1-U01-MH106027-01

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NSF grant CCF-1409422

James S. McDonnell Foundation grant number 220020399

DSO National Laboratories of Singapore

Title: Cell membrane tracking in live brain tissue with differential interference contrast (DIC) microscopy

Authors: *J. LEE, I. KOLB, C. R. FOREST, C. J. ROZELL Georgia Inst. of Technol., Atlanta, GA

Abstract: Differential interference contrast (DIC) microscopy is a practical imaging modality for live-cell experiments. However, DIC microscopy of thick tissue samples (>100 μ m) is notoriously challenging for automatic cell segmentation due to the low signal-to-noise ratio (caused by increased probability of light scatter due to tissue thickness) and unclear cell

boundaries due to the presence of surrounding organic tissue. Real-time detection of cell boundaries is desirable for automating electrophysiology experiments, and current algorithms are unable to reliably detect DIC cell boundaries under such demanding conditions. We present the first DIC deconvolution and cell-segmentation algorithm to track membrane locations in DIC imagery of living brain tissue with high fidelity. The proposed algorithm is formulated as a regularized least-square optimization that incorporates (1) a filtering mechanism that effectively and efficiently suppresses boundary-corrupting cellular interference, and (2) a robust edgesparsity regularizer that exploits dynamical information with edge-tracking capabilities. Our algorithm was applied on the experimental setting of patch-clamp recording in brain tissue and it demonstrated state-of-the-art performance boundary-tracking errors of 1-2 μ m. In addition, the algorithm is designed to remove glass-pipette artifacts via image inpainting and this was tested on patch clamp video data from mouse brain tissue. Shown in the figure is (a) DIC Microscopy image with identified target cell and glass-pipette (b) Deconvolution and pipette-removal with inpainting using proposed algorithm (c) Segmentation output using global thresholding, with pipette mask.



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Poster

345. Methods: Physiology and Circuitry I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 345.04/WW7

Topic: D.04. Somatosensation: Touch

Support: DARPA HAPTIX N66001-15-C-4018

Title: Design and assessment of stimulation parameters for a novel peripheral nerve interface

Authors: A. KUNDU¹, E. PATRICK¹, A. FAHMY², R. MADLER², F. DELGADO³, S. W. CURRLIN⁴, K. J. OTTO², J. PRINCIPE¹, *A. GUNDUZ³, N. MAGHARI², M. OP DE BEECK⁵,

D. BRAEKEN⁵, R. BASHIRULLAH²

¹Electrical and Computer Engin., ³Biomed. Engin., ⁴BME, ²Univ. of Florida, Gainesville, FL; ⁵IMEC, Leuven, Belgium

Abstract: The implantable multimodal peripheral recording and stimulation system (IMPRESS) is a novel, bi-directional neural interface technology, consisting of a high-density (16 stimulation and 64 recording channel) interneural probe to be used in an advanced peripheral neuroprosthetic that decodes motor intent and encodes sensory feedback. The probe, which is a high-density (hd) version of a transverse intrafascicular multichannel electrode (TIME), consists of a CMOS chip that is embedded in a highly flexible and hermetic polyimide ribbon cable. The chip is designed to contain circuitry for multiplexing and on-site stimulation as well as the electrode sites. However, this work presents a first generation of the IMPRESS hd-TIME that does not include active CMOS circuitry on the silicon chip. Rather, a passive CMOS-compatible chip with matched form factor of the final design is fabricated with stimulation and recording electrodes, only. Also, a high precision, stimulating analog front-end (SAFE) controller integrated-circuit chip was designed to test efficacy of this 1st generation IMPRESS TIME for in vivo neural stimulation in a rat model. Establishing ideal stimulation protocol to elicit meaningful sensory feedback first requires understanding the effects of and optimizing a wide range of stimulation parameters. Thus, computational modeling was employed to fundamentally understand the optimum range of parameters (i.e. pulse amplitude, pulse width, and interpulse width) of a charge balanced, biphasic stimulus for an intrafascicular probe. Moreover, the model was used to assess whether pulse amplitude or pulse width modulation had an inherent advantage over the other for a sensory encoding scheme. We present the model results that allow us to narrow down the range of stimulus parameters as well as *in vivo* stimulation results of our 1st generation IMPRESS TIME. Pulse amplitude modulation to be used with frequency modulation was selected as an effective encoding scheme for IMPRESS and in vivo stimulation results show good selectivity between different fascicles in the sciatic nerve of the rat. This work is sponsored under the auspices of Dr. Doug Weber by the US Defense Advanced Research Projects Agency (DARPA) (contract No. N66001-15-C-4018 to the University of Florida).

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Poster

345. Methods: Physiology and Circuitry I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 345.05/WW8

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant 3F32NS095580-02S1

NIH Grant 4R01DA035913-04

Title: Novel approaches to optimize neuronal computational models

Authors: *R. BEN-SHALOM¹, K. KIM¹, K. J. BENDER²

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Abstract: Compartmental neuronal models are a powerful tool to both validate experimental observations and make quantifiable predictions. The quality of such predictions depends critically on model detail and accuracy. Model parameters, including the distribution and properties of ion channels and receptors, are often based on a combination of experimental observation and experimenter intuition. Unfortunately, while such "hand-tuned" models can capture some aspects of neuronal function, they are often not robust to perturbation, indicating that they have failed to identify optimal parameter conditions that accurately capture neuronal physiology. Optimization algorithms have been developed to address this issue. These algorithms search throughout the parameter space using a score function that compares model output to experimental data to identify simulations that reach a certain accuracy criterion. This process often generates multiple models, each with different underlying parameter sets that nevertheless all match the experimental data to similar extents. Identifying which of these models best reflects neuronal physiology remains a challenge.

Here, we demonstrate a new technique that identifies the optimal combination of score functions, which in turn shed light on how such scores can be improved with revised experimental stimuli. Using well-established models, we extensively sampled the parameter space at different distances from the original parameter set. By applying a battery of score functions and evaluating their performance, we could identify the most effective score function for parameter estimation. A similar analysis can identify the stimulation protocols that most effectively estimate correct parameter sets. Ultimately, we plan to apply these tools to guide which is the best experimental data set for neuronal parameter estimation, allowing for efficient optimization of physiologically realistic compartmental models.

Disclosures: R. Ben-Shalom: None. K. Kim: None. K.J. Bender: None.

Poster

345. Methods: Physiology and Circuitry I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 345.06/WW9

Topic: I.06. Computation, Modeling, and Simulation

Title: Topological classification of pyramidal cells in juvenile rat somatosensory cortex

Authors: *L. KANARI^{1,2}, Y. WANG³, Y. SHI⁴, S. RAMASWAMY², R. PERIN⁴, J. SHILLCOCK², K. HESS⁵, H. MARKRAM²

¹EPFL, Blue Brain Project, Geneve, Switzerland; ²EPFL, Blue Brain Project, Geneva, Switzerland; ³Tufts Univ., Needham, MA; ⁴Brain Mind Institute, EPFL, Lausanne, Switzerland; ⁵EPFL, Lausanne, Switzerland

Abstract: Pyramidal cells are the major excitatory neuron type in the cerebral cortex and represent 70-85% of all neurons in the mammalian cortex (Markram, Muller et al, 2015). The generic anatomy of PCs in the neocortex is characterized by a triangular soma, two distinct dendritic domains emanating from the base and apex of the soma (basal and apical dendrites, respectively), and a single axon. While it has been well established that PCs generally differ in their overall size of the apical dendrite in order to reach layer 1, the classification based on global morphological features obscures much of the diversification within each layer. On the other hand, a more detailed classification derived from the human expert assignment of classes is inconsistent and unsupported by feature classification techniques (DeFelipe et al, 2013). We use a topological method based on persistent homology methods to quantitatively capture the branching shapes of neurons, the Topological Morphology Descriptor (TMD, Kanari et al, 2016) to establish an objective classification scheme for cortical pyramidal cells. The TMD of neuronal trees allows different neuronal populations to be clearly distinguished, including those whose visual appearance is only subtly different, and therefore provides an objective classification of cells into discrete morphological classes.

The classification based on the TMD is sufficient to objectively classify cortical PCs and yields a quantitative measure of the uncertainty in the classification. Using this scheme, we found two major types of PC in layer 2, one in layer 3, three in layer 4, three in layer 5, and five types in layer 6. We conclude that a TMD classification scheme provides a reliable approach to standardizing the classification of neuronal morphologies, unlike previous methods.

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Poster

345. Methods: Physiology and Circuitry I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

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Topic: I.04. Physiological Methods

Support: NIH Grant U01MH105982

NIH R01 DC004551

NIH R01 DC009809
Title: ACQ4: An open-source software platform for developing patch-clamp experiments

Authors: *L. CAMPAGNOLA¹, M. B. KRATZ², S. SEEMAN¹, P. A. DAVOUDIAN¹, A. HOGGARTH¹, J. F. PERKINS¹, D. D. REID¹, T. JARSKY¹, P. B. MANIS² ¹Allen Inst. for Brain Sci., Seattle, WA; ²Dept Otolaryngol/Head & Neck Surgery, Univ. N Carolina-Chapel Hill, Chapel Hill, NC

Abstract: ACQ4 is a software development platform that provides a set of fully open-source tools for acquiring data in neurophysiology experiments, with emphasis on automation in patchclamp electrophysiology. Building from the modular components provided by ACQ4, we have prototyped and streamlined software for experiments ranging from simple patch-clamp recordings to photostimulation mapping, 2-photon calcium imaging, in vivo intrinsic imaging, and multipatch recordings. The core of ACQ4 consists of a set of device classes supporting patch-clamp amplifiers, cameras, laser scanning hardware, microscopes, and micromanipulators. These device classes are complemented by a set of user interface modules that each streamline different aspects of typical patch-clamp experiments, while a centralized management system facilitates communication and synchronization between devices and modules. All data acquisition is accompanied by comprehensive metadata collection, with particular care given to tracking physical and temporal relationships; synchronization between devices is handled and recorded automatically, and all spatial data such as images, pipette positions, and laser scanning patterns are recorded relative to the same coordinate system. ACQ4 is built on the free and open scientific Python stack, which helps us promote open, reproducible science while reducing the barriers to access.

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Poster

345. Methods: Physiology and Circuitry I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 345.08/WW11

Topic: H.01. Animal Cognition and Behavior

Support: Kaken-hi (15H05569)

Kaken-hi(15H01417)

Title: A method for cardiac pacing together with local field potential recordings from the brain in a freely moving rat

Authors: *Y. SHIKANO, T. SASAKI, Y. IKEGAYA

Pharmaceut. Sci., Univ. of Tokyo, Tokyo, Japan

Abstract: The brain and the heart are interconnected to each other. However, it remains largely unknown whether cardiac oscillatory activity could impact brain activity. To address this issue, we developed a novel technique that reversibly controls cardiac rhythm by electrically stimulating peripheral tissues in a freely moving rat. The method can be combined with electrophysiological recordings of systemic bioelectrical signals, including cortical local field potential signals, electrocardiogram, electromyogram, and respiratory rhythm. For heartbeat acceleration, electric stimulation was directly applied to the heart through two wire electrodes attached in the vicinity of the sinus node and the apex, allowing us to set a heart rate with a range between 400 bpm and 750 bpm. For heartbeat deceleration, electric stimulation was applied to the efferent vagus nerve, leading to a decrease in a heart rate as low as 240 bpm. Electrical artifacts included in brain recording channels could be removed by subtraction of averaged artifacts from the original electrical signals. We expect that this technique serves as a tool to examine cardiac activity-triggered modulation of brain functions and behaviors.



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Poster

345. Methods: Physiology and Circuitry I

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Topic: D.06. Audition

Support: NIH NIDCD R01DC015527

PA Lions Hearing Research Foundation

Burroughs Wellcome Fund Career Award

Human Frontiers in Science Foundation

Title: Meso-scale structure and quotidian variation of neuronal networks estimated from twophoton imaging of mouse auditory cortex

Authors: *R. BETZEL¹, K. C. WOOD², C. F. ANGELONI², M. N. GEFFEN², D. S. BASSETT¹ ¹Dept. of Bioengineering, ²Univ. of Pennsylvania, Philadelphia, PA

Abstract: Neural systems can be modeled as complex networks in which neural elements (cells, populations, or brain areas) are represented as nodes linked to one another through structural or functional connections (synapses, projections, and fiber bundles or statistically interdependent activity). The resulting "network" (or "graph") can be analyzed using mathematical tools from network science and graph theory to quantify the system's topological organization and better understand its function [1].

While the network-based approach has become common in the analysis of large-scale neural systems (i.e. MRI, EEG, MEG), few studies have used network science to study the organization of biological neural networks reconstructed at the cellular level. Even the most fundamental questions, such as how best to estimate the presence/absence and weights of connections between cells, remain largely unanswered [2].

Here, we record spontaneous and stimulus-evoked activity from the same set of cells in mouse auditory cortex over four days using two-photon calcium imaging. We reconstruct functional networks in which cells are linked to one another by edges weighted according to the maximum lagged correlation of their filtered fluorescence traces [3]. We show that the global structure of the network is largely preserved across days, as measured by the correlation of edge weights. We further show that network meso-scale architecture, i.e. partitions of cells into topologically-related sub-networks called "communities", are also largely stable, revealing consistently anticorrelated clusters of cells [4]. Finally, we show that despite quotidian stability, over shorter timescales (~minutes), network meso-scale structure is variable, revealing a stable core and fluctuating periphery [5].

The methods presented here are flexible and easily extended to additional datasets, opening the possibility of studying cellular level network organization of neural systems and how that organization is modulated by stimuli and disease.

DS Bassett & O Sporns (2017). "Network neuroscience." Nat. Neuro. 20(3), 353-364.
MD Humphries (2017). Dynamical networks: finding, measuring, and tracking neural

population activity using network theory. bioRxiv (http://dx.doi.org/10.1101/115485).

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networks. J. Comp. Net., 3(2), 221-248. [5] MP Rombach, MA Porter, JH Fowler, PJ Mucha (2014). Core-periphery structure in networks. SIAM J. App. Math., 74(1), 167-190.

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Poster

345. Methods: Physiology and Circuitry I

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Topic: I.04. Physiological Methods

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Title: Microcoil-driven responses induced by magnetic stimulation spatially restricted to the local surface of the mouse auditory cortex *In vivo*

Authors: *H. OSANAI, S. MINUSA, T. TATENO Hokkaido Univ., Sapporo, Hokkaido, Japan

Abstract: Transcranial magnetic stimulation has been utilized as a non-invasive method to treat neurological disorders (e.g. tinnitus) and to investigate nervous system functions. However, the large coil size of the stimulation and the less focal stimulation prevent the chronic application. Recently, micro-magnetic stimulation (μ MS), which utilizes a micro coil with implantable size (< 0.5 mm diameter), was proven to be capable of activating the neural circuitry in vitro and in vivo. Although most μ MS studies examined its effects on single neurons, little has been known about the effect on brain tissues. Here, we demonstrate μ MS can non-invasively induce the local neural activity in the mice auditory cortex in vivo using the multi-channel and ECoG electrode recordings. By stimulating from the cortical surface, we found that the local field potentials and the firing activities were evoked locally in cortical layer 2/3, and they were propagated into the deeper layers. We also examined characteristics of the horizontal locality in the μ MS-driven neuronal activity. The result indicates that field potential responses at the sites close to the micro

coil were profoundly (three times) larger than those at the sites horizontally apart (> 1200 μ m) from the coil. Additionally, we found that μ MS-driven neuronal responses depended on the coil orientation to the surface, implying that the direction of the electric field was critical for the extent of neuronal activation. Our results can provide the basis for a future improvement of non-invasive μ MS devices for the chronic neurotherapeutic applications at the local-circuitry level.

Disclosures: H. Osanai: None. S. Minusa: None. T. Tateno: None.

Poster

345. Methods: Physiology and Circuitry I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 345.11/WW14

Topic: F.08. Biological Rhythms and Sleep

Title: Implication of synchronous spiking to the auditory steady-state response interpretation: An EEG study

Authors: *I. GRISKOVA-BULANOVA¹, A. VOICIKAS², C. PACORET³ ²Inst. of Biosci., ¹Vilnius Univ., Vilnius, Lithuania; ³Dept. of Basic Neurosciences, Univ. of Geneva, Geneva, Switzerland

Abstract: Introduction: Auditory steady-state response (ASSR) is widely used in neuroscience and is regarded as a periodic response to a periodic stimulation, for example click trains. However, the nature of ASSRs is still an open question, giving some evidence to both the superposition of evoked potentials (Capilla et al. 2011; Bohórquez and Özdamar 2008) and to the entrainment of the network (Ross et al. 2000; Picton et al. 2003) theories. At the neural level, the phenomena of synchronous spiking in response to clicks is acknowledged (Eggermont 1994), appearing on the time-frequency representation as a sharp broad-band gamma response (Pavlov et al. 2012). As this phenomenon is detectable on EEG recordings, this study proposes to revisit ASSR nature in the light of spiking-network interactions.

Methods: 64 channel EEG was recorded in a sample of healthy volunteers (n=40, 19-46 years). Click (1.5ms white noise bursts) trains of 500 ms were presented binaurally at 60 dB with ISI at 700-1000 ms through earphones in the following frequency ranges with 1 Hz steps: 12-30Hz and 35-55Hz with 80 trials per frequency. EEG data were conventionally cleaned. The inter-trial phase coherence (ITPC) of the cycle for each stimulation frequency was extracted at the subject level for analysis of spiking profiles. Waveform grand averages of the responses to click stimulation were created and re-averaged by cycle. The waveforms and the ITPCs were evaluated 1) aligned to the click stimulus and 2) to the synchronous spiking event, i.e. sharp – broadband gamma burst, allowing observation of time-locked and phase-locked components of the response.

Results: A grand average of waveform aligned on the click stimulus revealed the time-locked

peaks (No, Na, Pb, Nb, P1) for all stimulation frequencies, suggesting an event-based response. Inter-trial phase coherence revealed one broad band gamma (30-100Hz) bursts at ~21ms delay from the stimulus for all stimulation frequencies. The alignment of cycles on this event resulted in observation of phase-locking for frequencies above 30Hz.

Conclusion: The study shows the superposition of evoked potentials below 30Hz (Miyazaki et al. 2013) and the entrainment from the constructive interference of the electric field created by overlapping responses to local synchronous spiking at higher rates. The entrainment is phase-locked to the cortical synchronous spiking and not to the auditory stimuli. Entrainment in this case, is a local consequence of the local synchronous spiking and its involvement in the process of information transmission of clicks should be addressed further.

Disclosures: I. Griskova-Bulanova: None. A. Voicikas: None. C. Pacoret: None.

Poster

345. Methods: Physiology and Circuitry I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 345.12/WW15

Topic: D.06. Audition

Support: DFG Grant SPP1665

Leibniz Institute for Neurobiology Special Project

Title: Chronic recording of single units in rodent auditory cortex using carbon nanotube coated tetrodes

Authors: *K. TAKAGAKI^{1,2,3}, Z. XIA^{1,2}, G. ARIAS GIL^{1,2}, M. T. LIPPERT^{1,3}, F. W. OHL^{1,2,3} ¹Systems Physiol. of Learning (SPL), Leibniz Inst. for Neurobio., Magdeburg, Germany; ²Inst. of Biol. (IBIO), Otto-von-Guericke Univ., Magdeburg, Germany; ³Ctr. for Behavioral Brain Sci. (CBBS), Magdeburg, Germany

Abstract: We use tetrodes coated with carbon nanotubes to study single-unit activity in the rodent auditory cortex during learning. Carbon nanotubes allow us to lower the impedance of our tetrodes from approx. 600 k Ohm down to approx. 10 k Ohm. This low impedance is stable over months, both *ex vivo* and *in vivo*. The low impedance and resulting high signal-to-noise ratio of our recordings allow us to record stable single units in behaving rodents over weeks. In this poster, we present evidence for the stability of our recordings, and characterize physiological changes in this unit-activity as animals learn a cortically-dependent GO/NOGO auditory discrimination task.

Disclosures: K. Takagaki: None. Z. Xia: None. G. Arias Gil: None. M.T. Lippert: None. F.W. Ohl: None.

Poster

345. Methods: Physiology and Circuitry I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 345.13/WW16

Topic: I.04. Physiological Methods

Title: A real-time processing technique for 40 Hz auditory steady-state response: The parameters and effect of surgical interventions

Authors: *S.-I. HIRANO, Y. NISHIKAWA

Osaka Dent. Univ., Osaka, Japan

Abstract: Brain surgery sometimes involves a risk of causing nervous system dysfunction due to surgical manipulation. Many neurophysiological monitoring methods have been introduced to solve this problem. Each method has advantages and disadvantages, but none meet the definite need for an intraoperative monitor. To realize an intraoperative acoustic monitor that will provide a rapid functional alert, a real-time technique for processing the 40 Hz auditory steady-state response (40Hz-ASR) was investigated in cerebellopontine angle surgery.

Twelve rabbits underwent surgical interventions. The 40Hz-ASR during the interventions was recorded. The response was recorded between a silver ball electrode that was implanted at the bregma and needle electrodes that were inserted at the roots of the bilateral pinnae. Auditory clicks were successively presented at 40 Hz. In order to fulfil the demand, the signals of the 40Hz-ASR were immediately digitized and analyzed by the Fast Fourier Transformation (FFT) method. The spectral power (SP) and the phase coherence measure (PC) were calculated simultaneously. A real-time technique for processing the 40Hz-ASR was studied under a variety of experimental conditions.

This analytical method proved the 40 Hz-specificity of this response. The PC increased as the number of sweeps that were averaged increased. In contrast, the SP declined. In the verification experiment that was conducted to seek the optimum parameters of this series, both the PC and SP reached a plateau at an average of approximately 30 times. At a sample size of 15 or 20, the dispersion of PC values was minimal. The calculation of PC at an average of 30 times and a sample size of 15 to 20 permits evaluation of the 40Hz-ASR with sufficient reliability and sensitivity. Under general anesthesia, the PC showed no change, where the SP showed considerable attenuation. Surgical lesioning on the acoustic nerve or the brainstem caused a worsening of both the PC and the SP. The magnitude of deterioration was in proportion to the extent of lesioning.

Properties of the 40Hz-ASR that have been identified previously were reconfirmed in this realtime analysis, and the optimum values for each parameter, which were determined in early experiments in this sequence, permitted real-time monitoring with sufficient accuracy and rapidity. Our results indicate that the on-line measurement of the PC and SP enables rapid evaluation of acoustic and brainstem functions, which may be applicable in intraoperative monitoring. The author has obtained approval from the Institutional Animal Research Committee prior to launching this study, and has no COI to declare.

Disclosures: S. Hirano: None. Y. Nishikawa: None.

Poster

345. Methods: Physiology and Circuitry I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 345.14/WW17

Topic: I.04. Physiological Methods

Support: NRF-CRP10-2012-01

Title: Wireless flexible neural clip achieves deep visceral nerve targeting for micturition in rats

Authors: *N. V. THAKOR^{1,2,3}, S. LEE^{2,3}, W. PEH^{2,3}, S.-C. YEN^{2,3}, C. LEE^{2,3} ¹Biomed. Engin. Dept., Johns Hopkins Univ., Baltimore, MD; ²Singapore Inst. for Neurotechnology (SiNAPSE), Singapore, Singapore; ³Electrical and Computer Engin., Natl. Univ. of Singapore, Singapore

Abstract: Background: Bladder dysfunction is a widely studied area in the field of neuromodulation that remains a major healthcare challenge in the daily lives of patients. The pelvic nerve is a promising stimulation target for the control of bladder function as it provides autonomic efferent outputs to contract the bladder detrusor muscle. However, the pelvic nerves are small visceral nerves located deep inside the body, which leads to difficulties using current electrodes in implantation, as well as maintaining contact for reliable stimulation. Here, we demonstrate a flexible neural clip interface (NCI) that is suitable for implantation on small pelvic nerves for bladder neuromodulation.

Methods: Bladder pressure and urine output were measured and detected during pelvic nerve stimulation to assess functionality. Stimulation parameters were biphasic rectangular waveforms with a frequency of 10 Hz, 150 μ s phase width, duration of 5 seconds, and amplitudes that ranged from 100 to 1000 μ A. All acquired data were then analyzed using custom MATLAB code. Pressure data were low-pass filtered at 30 Hz, and a 5 second window prior to each electrical stimulation was taken as the baseline to calculate changes in pressure.

Results: The clip design enabled easy and reliable implantation on the pelvic nerve in a manner analogous to clipping a paper clip. We performed repetitive stimulation using supra-threshold amplitudes to show the reproducibility of bladder voiding through pelvic nerve stimulation using the NCI (n = 8 trials). Repeatable voiding responses were obtained in all 8 trials during a single recording session, with pressure changes. To examine how reliable our NCI worked across different animals, we reused the same NCI for experiments carried out in another rat on a

different day. We found that in both rats, applying higher stimulation amplitude caused greater peak changes in intra-bladder pressure, and decreased the time taken to reach the peak pressure, indicating that the NCI can be reliably implanted without much loss in electrode performance, and is mechanically robust for handling across different experiments.

Conclusions: The present findings indicate that the pelvic nerve can be stimulated in a graded manner to control bladder contractions leading to reproducible voiding events comparable with other published techniques. Notably, we showed that our novel flexible clip electrode can provide effective electrical contact with the pelvic nerve for reliable and repeatable stimulation in acute *in vivo* anesthetized animals.

Disclosures: N.V. Thakor: None. S. Lee: None. W. Peh: None. S. Yen: None. C. Lee: None.

Poster

345. Methods: Physiology and Circuitry I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 345.15/WW18

Topic: I.06. Computation, Modeling, and Simulation

Support: F32 DA041282

R01 NS086570-01

85110-PHI-14

DA013429-16

Title: Recapitulation of the neurovascular unit (NVU) in a microfluidic platform using primary human cells

Authors: *A. M. ANDREWS¹, S. H. RAMIREZ²

¹Pathology, Temple Univ., Philadelphia, PA; ²Dept. of Pathology and Lab. Med., Temple Univ. Sch. of Med., Philadelphia, PA

Abstract: Endothelial cells (ECs) form the basis of the blood-brain barrier (BBB), a physical barrier that selectively restricts transport into the brain. *In vitro* models can provide significant insight into BBB physiology, mechanisms of human disease pathology, toxicology and drug delivery. In terms of BBB models, co-culture microfluidic chips are an advanced model allowing for the recreation of 3D architecture and mechanical forces (fluid flow). Here, we detail the use of human fetal tissue as a source to build syngenic tri-cultures from the same host in a microfluidic chip that recapitulates the *in vivo* neurovascular unit. Aside from the syngenic aspect to this model, the microfluidic chips allow for inclusion of cell-cell interactions through the ability of CNS cells to extend into the "column-matrix" to make connections with the ECs.

We show that our brain ECs formed a fully functional barrier as assessed by the restriction of fluorescently labeled tracers (sodium fluorescein, NaFluo, 3 kDa Cascade Blue-Dextran, 40 kDa Tetramethyrhodamine-Dextran) in the EC flow compartment. Morphological findings include, an EC appearance that is consistent with cells exposed to fluidic flow and an astrocytic configuration that resembles the physiologic cytoarchitecture. Additionally, assessment of known triggers of BBB dysfunction, produced increases in the apparent permeability in the reconstituted NVU. Overall, we show the potential to develop human syngenic BBB models in microfluidic chips, which provide important functional aspects of the *in vivo* NVU environment.

Disclosures: A.M. Andrews: None. S.H. Ramirez: None.

Poster

345. Methods: Physiology and Circuitry I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 345.16/WW19

Topic: B.09. Physiological Properties of Neurons

Support: GlaxoSmithKline

Defense Advance Research Projects Agency (DARPA)

Title: Thin-film softening multi-contact cuff electrodes: current steering and sub-chronic neuromodulation

Authors: *M. A. GONZÁLEZ GONZÁLEZ, A. G. HERNANDEZ REYNOSO, G. S. BENDALE, L. PATIL, A. KANNEGANTI, R. MODI, A. JOSHI IMRE, W. VOIT, M. I. ROMERO-ORTEGA

Bioengineering Dept., Univ. of Texas At Dallas, Dallas, TX

Abstract: Silicone cuff electrodes are used clinically for the stimulation of the tibial nerve in patients with drop foot, the vagus nerve (VN) for multiple conditions including epilepsy, depression and tinnitus, and the pudendal nerve for lower urinary tract disorders. While these electrodes are effective, the material, cuff size and stiffness of these devices, often cause fibrosis, increasing electrode impedance, and eventually leading to failure. In addition, most cuff electrodes stimulate the entire nerve sometimes recruiting undesired fascicles causing deleterious side-effects. In VN stimulation (VNs), unwanted fiber activation cause arrhythmias and laryngopharyngeal dysfunction. In this study, we photo-lithographically fabricated thin-film cuff electrodes using a shape memory polymer (thiol-ene/acrylate; SMP) which softness at 37°C from GPa to 500 MPa elastic modulus. A 16-electrode array with both titanium nitride (TiN) and gold sites raging from 3300-18500 um² in size, and from 0.8 K Ω to 1 M Ω in impedance, were fabricated and bonded to Omnetics connectors. The electrodes were tested in acute and sub-

chronic implantations onto the sciatic nerve (ScN) and the stimulation tested by the evoked EMG activity. Successful recording were confirmed during bladder filling in cuffs placed onto the pelvic nerve (PN). We confirmed the selective stimulation of the tibial and peroneal fascicles from cuff wrapped around the ScN by the evoked selective plantar- and dorsi-flexion movements, and respective EMG activity from the gastrocnemius and tibialis anterior muscles. Thirty days after implantation, the animals showed a reduced fibrotic tissue, (50%) compared to commercial silicon cuffs. In summary, we present a flexible, thin and sensitive multi-contact cuff electrode that can selectively stimulate specific nerve fascicles while inducing minimal fibrotic response. Together, the data supports the use of softening multi-contact cuff electrodes as a viable alternative for peripheral nerve neuromoduation.

Disclosures: M.A. González González: None. **A.G. Hernandez Reynoso:** None. **G.S. Bendale:** None. **L. Patil:** None. **A. Kanneganti:** None. **R. Modi:** Other; GlaxoSmithKline (GSK), Defense Advanced Research Projects Agency (DARPA). **A. Joshi Imre:** None. **W. Voit:** Other; GlaxoSmithKline (GSK), Defense Advanced Research Projects Agency (DARPA). **M.I. Romero-Ortega:** None.

Poster

345. Methods: Physiology and Circuitry I

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 345.17/WW20

Topic: B.10. Network Interactions

Support: Department of Veterans Affairs Rehabilitation R&D Career Development Award (CDA2) IK2-RXX002013 to HIC

Title: Characterization of cerebral organoid activity using two-photon calcium imaging and acute electrophysiology techniques

Authors: ***A. NEMES**¹, D. JGAMADZE², J. T. LIM², M. SCHAFF³, C. ADAM², J. A. WOLF², H.-C. I. CHEN⁴

¹The Univ. of Pennsylvania, Philadelphia, PA; ²Neurosurg., ³Univ. of Pennsylvania, Philadelphia, PA; ⁴Dept. of Neurosurg., Univ. of Pennsylvania Dept. of Neurosurg., Philadelphia, PA

Abstract: <u>Introduction:</u> The emergence of cerebral organoids provides a new approach for modeling human neurological disease and potentially repairing brain circuitry. These neural tissues, derived from the self-organization of pluripotent stem cells, recapitulate many brain-specific structures, including the layered architecture of cerebral cortex. While technical refinements in organoid generation protocols continue to improve the degree to which organoids mimic the brain, less is known about the neuronal and network activity of these entities. We

sought to study the early development of organoid activity.

<u>Methods</u>: We grew cerebral organoids from a federally improved embryonic stem cell line (H9) and a healthy volunteer induced pluripotent stem cell line using two different differentiation protocols (Pasca et al., 2015 and Qian et al., 2016). At different time points, we measured the calcium activity of Fluo-4 loaded organoids using two-photon microscopy. Videos of this calcium activity were analyzed using a semi-automated process. We also performed acute electrophysiology using laminar multi-electrode probes.

<u>Results:</u> In the two-photon experiments, we observed spontaneous calcium activity starting on differentiation day (dd) 50. There were a higher frequency of calcium events per cell at dd80 compared to dd50. Additionally, dd80 organoids had groups of cells with synchronous calcium activity. Although single units could not be resolved at this time point, electrophysiological recordings demonstrated a significant increase in multi-unit voltage amplitude at later time points compared to earlier time points. This activity was effectively eliminated with a depolarization block induced by the addition of potassium chloride.

<u>Conclusion</u>: The recently published paper by Quadrato et al. showed that cerebral organoids do not generate spontaneous action potentials until 8 months of growth. Our results demonstrate that cerebral organoids develop spontaneous calcium and electrical activity as early as dd50. The clustering of calcium activity at dd80 is suggestive of the development of some level of network structure. Further studies are required to better characterize this early activity and correlate it with the development of spontaneous single-unit activity.

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Poster

346. Data Analysis and Statistics: Neuronal Networks

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 346.01/WW21

Topic: I.07. Data Analysis and Statistics

Title: Pyloric rhythm extraction using signal processing tools

Authors: ***F. DOS SANTOS**, K. LAM, P. ANDRAS Keele Univ., Newcastle, United Kingdom

Abstract: Voltage sensitive dyes (VSDs) are used to record membrane voltage changes by measuring the corresponding changes in absorbance or fluorescence.

Voltage-sensitive dye imaging (VSDi) has been used in the stomatogastric ganglion (STG) of the *Cancer pagurus* the past decade, however identification of individual neurons is difficult due to the noise in the data.

The Pyloric Rhythm (PR) in the STG is characterised by a tri-phasic rhythm with phases

corresponding to the activity of the lateral pyloric (LP), pyloric (PY) and pyloric dilator (PD) neurons. The PR activity can be recorded in the lateral ventricular nerve (lvn).

We present our work on a signal decomposition method, using signal-processing tools to identify the neurons of the pyloric rhythm (PR).

In a first stage we identify the slow wave activity of neurons showing the PR, matching them with the signal recorded from the *lvn* with a blind sample. The match between the VSD recorded activity of pyloric neurons and the *lvn* recording of the PR is almost perfect.

To test our method we used three PY neurons with injected dye (these were previously identified by intracellular recording) as ground truth. Our results show that the proposed method works accurately. We were able to identify the PY bursts in each cycle allowing the reconstruction of the PY phase of the pyloric cycle recorded in the lvn. We were able to calculate accurately the duty cycle for the PY cells.

Future work will involve finding accurately the duty cycle of all PR cells and possibly some gastric mill rhythm cells without requiring the intracellular recording of the neurons. This will make possible the study of the complete STG network with a simple bath imaging settings. Figure 1a) shows the pyloric rhythm extracted from the image recording data, figure 1b) shows the extracted rhythm of the three pyloric cells overlaid with the lvn recoding.



Disclosures: F. Dos Santos: None. K. Lam: None. P. Andras: None.

Poster

346. Data Analysis and Statistics: Neuronal Networks

Location: Halls A-C

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Program#/Poster#: 346.02/WW22

Topic: I.07. Data Analysis and Statistics

Support: Australian Research Council grant DP140101968

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International Neuroinformatics Coordinating Facility, Seed Grant

Title: Mesoscopic inter-areal connectivity of marmoset cortex: Comparison with mouse and macaque monkey

Authors: P. MAJKA^{1,2}, *P. THEODONI^{3,4}, D. H. RESER⁵, M. G. ROSA^{6,2}, X.-J. WANG^{4,3,7} ¹Lab. of Neuroinformatics, Nencki Inst. of Exptl. Biol. PAS, Warsaw, Poland; ²Australian Res. Council Ctr. of Excellence for Integrative Brain Function, Canberra, Australia; ³New York Univ. Shanghai, Shanghai, China; ⁴NYU-ECNU Inst. of Brain and Cognitive Sci. at NYU Shanghai, Shanghai, China; ⁵Monash Rural Hlth., Monash Univ., Churchill, Australia; ⁶Biomedicine Discovery Inst. and Dept. of Physiol., Monash Univ., Melbourne, Australia; ⁷Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: The human brain is >3,000 larger than the mouse brain. Yet, key elements of their basic neural architecture are conserved. Understanding how variations in brain morphology relate to measures of structural connectivity is essential to allow an understanding of how the brain processes information, and gives rise to cognitive functions, in different species.Recent tract tracing studies in macaque and mouse [1-3] have revealed conserved cortical network properties. Here we provide an intermediate point of comparison between these two mammals by analyzing the first collective database of the weighted and directed cortico-cortical connectivity obtained from >75 retrograde tracer injections in the cerebral cortex of the common marmoset (Callithrix jacchus), a small (~350g) monkey. In addition, a consensus of cortical areas between macaque and marmoset is proposed, which allows direct comparisons of the connectivity profiles between homologous cortical areas (or groups of areas) in these two primate species. We found that the obtained inter-areal connectivity matrix is dense, similar to results in macaque and mouse. The connection weights are heterogeneous, as they span over five orders of magnitude and are log-normally distributed. Additionally, similar to macaque and mouse, the inter-areal distances are normally distributed and the inter-areal connection probability decays with wiring distance as well as with the functional similarity distance between two cortical areas. Moreover, all three species show similar three-motif distribution, clique distribution, and have a dense core structure. A key network property found in macaque and mouse is that the probability of axons of a given length decays exponentially, the so-called exponential distance rule, which can predict many structural connectivity properties [4,5]. We found that this rule stands also for the marmoset and that the decay rate scales with brain size with a power law, from which the decay factor for the human brain can be predicted.

Our consistently collected tract-tracing data provide the cornerstone for future studies of the network characteristics of the brain in marmosets, as well as for comparative studies involving other mammalian species. Our results suggest conserved properties of the connectivity matrix across mammals, while allowing estimates of quantitative parameters that may result in different information processing and cognitive characteristics.

[1] Markov et al. Cereb Cortex 24:17-36 (2014)[2] Oh et al. Nature 508:207-214 (2014)[3]

Zingg et al. Cell 156:1096-1111 (2014)[4] Ercsey-Ravasz et al. Neuron 80:184-197 (2013)[5] Horvát et al. PLoS Biol 14(7):e1002512 (2016)

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Poster

346. Data Analysis and Statistics: Neuronal Networks

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 346.03/WW23

Topic: I.07. Data Analysis and Statistics

Title: Distinct structure-function dependencies across different connectivity scales in the rat brain

Authors: *M. STRAATHOF¹, M. R. T. SINKE¹, T. J. M. ROELOFS^{1,2}, E. L. A. BLEZER¹, O. SCHMITT⁴, W. M. OTTE^{1,3}, R. M. DIJKHUIZEN¹

¹Biomed. MR Imaging and Spectroscopy Group, Ctr. for Image Sci., ²Dept. of Translational Neuroscience, Brain Ctr. Rudolf Magnus, ³Dept. of Pediatric Neurology, Brain Ctr. Rudolf Magnus, Univ. Med. Ctr. Utrecht, Utrecht, Netherlands; ⁴Inst. für Anatomie Rostock, Universitätsmedizin Rostock, Rostock, Germany

Abstract: Resting-state fMRI and diffusion MRI have contributed to the elucidation of neural network connectivity in healthy and diseased brains. Partial correspondence between functional connectivity and diffusion-weighted tract reconstructions suggests a structure-function dependency in the brain, but the exact relationship remains unclear. Diffusion-weighted tract reconstructions only map the macroscale connections and may contain spurious tracts. In contrast, neuronal tracers allow detailed histological visualization of monosynaptic directed axonal connections at the cellular level. We aimed to further characterize the structure-function relationship by performing specific dependency mapping at the macro- and mesolevel, for which we combined high-resolution datasets of functional and structural MRI and neuronal tracing in rat brains from independent datasets.

Resting-state fMRI time-series were acquired under 1.5% isoflurane anesthesia at 9.4T in 19 young adult male Sprague Dawley rats and 14 adult male Wistar rats. Time-series were motion-corrected and band pass filtered between 0.1-0.01 Hz. Sixty diffusion-weighted images were acquired in ten post-mortem brains from adult male Wistar rats at 150-µm isotropic resolution (b=3842 s/mm²). Tracts were reconstructed with state-of-the-art constrained spherical deconvolution tractography. Images were matched with a Paxinos and Watson brain atlas. Macroscale functional connectivity and tract-based connectivity were determined between 84 bilateral cortical and 8 subcortical regions. Mesoscale neurotracer-based connectivity was obtained from the NeuroVIISAS database (~7800 tract-tracing publications). Structure-function

relationships were mapped for homotopic and non-homotopic regions, cortical-cortical and cortical-subcortical pairs, within and between hemispheres.

We found a positive relation between functional and macroscale structural connectivity in both datasets for homotopic and non-homotopic connections (r=0.17-0.52). This differed from the relationship between functional connectivity and mesoscale structural connectivity. There we found a – dataset consistent –negative correlation between homotopic cortical regions (r=-0.25 & r=-0.34) and no clear relationship for non-homotopic connections.

Our study suggests a distinct dependency between functional connectivity and underlying axonal wiring. While functional and structural connectivity corresponded positively at macroscopic level, indefinite correspondence at mesoscale level implies the importance of hierarchical characterization for thorough assessment of whole-brain connectivity.

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Poster

346. Data Analysis and Statistics: Neuronal Networks

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DARPA Young Faculty Award

Boston University Biomedical Engineering Department

Title: Mild blast injury acutely changes intracellular calcium in hippocampal neurons and reduces the coding ability of the neuronal network

Authors: *K. R. HANSEN, G. DEWALT, A. I. MOHAMMED, H.-A. TSENG, V. SALIGRAMA, B. NAZER, W. D. ELDRED, X. HAN Boston Univ., Boston, MA

Abstract: Traumatic brain injury (TBI) represents an important public health concern for both veterans and the general public. Mild brain injury can result from head trauma and is often associated with changes in mental state or consciousness. Much of what is understood about brain injury comes from long term studies in cell signaling and anatomical pathology, but very little is understood about the transient functional changes that occur in neurons in response to brain injury. Using a wide field Ca^{2+} imaging technique our lab recently developed, we characterized the intracellular Ca²⁺ dynamics of thousands of individual hippocampal neurons in awake mice upon mild blast directed towards a freely moving cranium. We found that immediately following blast, the intracellular Ca^{2+} levels decreased over a time scale of minutes. In addition to slow changes in the baseline intracellular Ca²⁺ levels, we also found a wide spread reduction in neuronal spiking activity measured as Ca^{2+} transients at the time scale of seconds. At a population level, the reductions in individual neuronal activity resulted in reduced network information coding ability as estimated with Shannon entropy. Blast induced changes in both intracellular Ca²⁺ levels and neuronal activity patterns gradually recovered in approximately one hour. Together, these results demonstrate that mild blast leads to profound changes in both the function of individual neurons and the computational ability of the population of cells in the neuronal network, and highlight the potential of applying such imaging techniques to study blast induced pathologies.

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Poster

346. Data Analysis and Statistics: Neuronal Networks

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 346.05/WW25

Topic: I.07. Data Analysis and Statistics

Support: NIH 1DP2NS082126

NIH 1R01NS087950-01

DARPA Young Faculty Award

Title: Selective modulation of neural population In vivo by ultrasound stimulation

Authors: *H.-A. TSENG, A. MOHAMMED, S. BENSUSSEN, K. R. HANSEN, X. HAN Boston Univ., Boston, MA

Abstract: Non-invasive neural stimulation techniques are critically needed for medical application. Ultrasound, due to its non-invasive nature, has been of interest over the years as a potential non-invasive neural stimulation technique that can be focused into intact brains. For

example, stimulations over mouse motor cortex induced muscle contraction, and stimulation over human sensory cortex hindered sensory discrimination ability. However, it is unclear how neurons or neural networks respond to ultrasound stimulation in intact brain.

To examine the effect of ultrasound stimulation on individual neurons and neural networks in vivo, we applied ultrasound stimulations at various intensities to the brains of awake and head-fixed mice, and recorded neural activity in two brain areas: the hippocampus and the motor cortex. Calcium indicator (GCaMP6f) was expressed in neurons and observed with wide-field microscope. We found that, ultrasound selectively modulate different neuron populations at different intensity levels. The modulation effects were either activation or suppression, and often sustained several seconds after stimulation. These results highlight the potential of ultrasound stimulation as a non-invasive neural stimulation technique, and the stimulation effects can be tuned by altering ultrasound strength.

Disclosures: H. Tseng: None. A. Mohammed: None. S. Bensussen: None. K.R. Hansen: None. X. Han: None.

Poster

346. Data Analysis and Statistics: Neuronal Networks

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Topic: I.07. Data Analysis and Statistics

Support: NIH 1DP2NS082126

Title: Cholinergic and parvalbumin interneurons coordinate distinct aspects of dorsal striatal network dynamics during voluntary movement

Authors: *M. ROMANO¹, W. HOWE³, H. GRITTON², D. ZEMEL², M. BUCKLIN², X. HAN²

¹Grad. Program in Neurosci., ²Dept. of Biomed. Engin., Boston Univ., Boston, MA; ³Dept. of Neurosci., Icahn Sch. of Med. at Mt Sinai, New York City, NY

Abstract: Medium spiny projection neurons (MSNs) are the principal outputs of the striatum. Local interneuron populations are thought to organize the activity of MSNs to support the multiple behaviors mediated through striatal function. However, the sparse distribution of these interneurons has made it difficult to assess their capacity to modulate MSN network activity *in vivo*. Here, we build upon recent improvements in wide-field optical imaging to monitor the activity of two major interneuron classes, parvalbumin (PV) and cholinergic (ChI) cell types, in tandem with large networks of MSNs, in mice engaged in voluntary motor output. We reveal evidence that the activity of PV interneurons strongly gates the activity of nearby, but not far away, MSNs to modulate the gain of on-going motor output. Surprisingly, the activity of these neurons was largely unsynchronized across the dorsal striatum, arguing against broad and nonselective inhibition facilitating movement selection. Cholinergic interneurons (ChIs) in contrast, poorly tracked overall movement, but engaged and synchronized MSN activity over hundreds of microns during discrete changes in ongoing motor plans. Our combined evidence demonstrates the unique capacity of striatal interneurons to organize networks of MSNs in support of distinct aspects of motor control.

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Poster

346. Data Analysis and Statistics: Neuronal Networks

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Topic: I.07. Data Analysis and Statistics

Support: NIH 1DP2NS082126

Title: Discrete networks of neurons differentially track conditioned and unconditioned stimuli in the hippocampus through the process of learning and extinction

Authors: *M. ABDULKERIM, K. R. HANSEN, A. I. MOHAMMED, H.-A. TSENG, H. GRITTON, X. HAN Biomed. Engin., Boston Univ., Boston, MA

Abstract: The hippocampus is a region of the brain important for new memory formation and recall and is essential for trace-learning. We previously applied wide-field microscopy and calcium imaging to monitor CA1 neural activity in a well characterized trace eyeblink task. We found that through the process of learning approximately ~25% of neurons in the hippocampus exhibit task relevant activity. As a follow-up, we recently examined how extinction training, within this task, alters the response properties of these neurons. In addition, we asked if extinction training recruits new neurons within the hippocampus as a function of new learning. We applied trial-by trial neural network analysis to determine how stability of the network changes across each state of training and whether the state of the network is a reliable indicator of trial performance. We discovered that individual neurons were poor predictors of trial outcome on a trial-by trial basis but that networks of neurons were strong predictors of behavior. We also found that the responsivity of discrete and separable populations of neurons became largely dissociable as new learning occurred and that each particular population of neurons was more or less influential in network computations depending on the training stage of the animal.

Disclosures: M. Abdulkerim: None. K.R. Hansen: None. A.I. Mohammed: None. H. Tseng: None. H. Gritton: None. X. Han: None.

Poster

346. Data Analysis and Statistics: Neuronal Networks

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Topic: I.07. Data Analysis and Statistics

Support: NIH 1DP2NS082126

NIH 1R01NS087950-01

Title: Automatic fluorescence intensity based adaptive thresholding (FIBAT) for rapid image segmentation of large scale calcium imaging datasets

Authors: H.-A. TSENG¹, S. SHEN³, K. R. HANSEN², R. WU³, J. SI³, *X. HAN² ²Biomed. Engin., ¹Boston Univ., Boston, MA; ³Sch. of Electrical, Computer and Energy Engin., Arizona State Univ., Tempe, AZ

Abstract: Recent development in high performance calcium indicators enabled researchers to observe neural activity from a large number of neurons over days or weeks, generating increasingly large datasets. Such large datasets pose great challenges to neuroscientists. One critical step in processing imaging datasets is to identify regions of interest (ROIs), where each ROI corresponds to an individual neuron, so that the fluorescence of each ROI can be used to understand neural activity patterns. Although manual selection of ROIs has been a common approach for small imaging datasets, it is not suitable for large datasets due to its labor-intensive and time-consuming nature. Thus, an algorithm that automatically identifies ROIs is critically needed to analyze large imaging datasets. We developed an algorithm for rapid automatic image segmentation using adaptive thresholding of fluorescence intensity, an algorithm we named automatic fluorescence intensity based adaptive thresholding (FIBAT). We first generated a static image representing the mean intensity of each pixel over time. We then applied an intensity threshold globally to the static image to isolate potential ROIs. The threshold was adaptively adjusted so that the largest number of ROIs was obtained. We then applied adaptive threshold locally around the immediately surrounding area of each ROI identified, segmenting each ROI into smaller ROIs if possible. This process was iterated multiple times to capture as many ROIs possibly. We tested the algorithm on two calcium imaging datasets, one from the hippocampus area and anther from the striatum area, where neurons are different in sizes. Compared to manual selection of ROIs, FIBAT successfully identified about 80% of the manually generated ROIs. Upon further visual examination of false positive ROIs identified by FIBAT, we found that many are neurons missed by manual selection. Overall, the performance of our algorithm is

comparable with manual selection, and can outperform manual selection by identifying ROIs with low intensity that were often missed by manual selection.

Disclosures: H. Tseng: None. S. Shen: None. K.R. Hansen: None. R. Wu: None. J. Si: None. X. Han: None.

Poster

346. Data Analysis and Statistics: Neuronal Networks

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James S. McDonnell

DARPA

NSF GRF

Title: Low-dimensional representations of learning in multi-trial datasets

Authors: ***A. H. WILLIAMS**¹, B. POOLE¹, N. MAHESWARANATHAN¹, T. H. KIM¹, F. WANG¹, S. VYAS¹, K. V. SHENOY¹, M. J. SCHNITZER², T. G. KOLDA³, S. GANGULI¹ ¹Stanford Univ., Stanford, CA; ²Stanford Univ. Dept. of Biol., Stanford, CA; ³Sandia Natl. Labs., Livermore, CA

Abstract: Dimensionality reduction methods, such as Principal Components Analysis (PCA), provide compact statistical summaries of large population recordings and thus have become a cornerstone tool for modern neural data analysis [1]. Existing methods reduce the dimensionality of fast, within-trial neural dynamics, but ignore how dynamics change over repeated behavioral trials. These across-trial trends are of broad interest as neural correlates of attention, memory consolidation, learning, and other internal cognitive states.

We present two approaches - canonical polyadic (CP) tensor decomposition and time-warped principal components analysis (twPCA) - that reduce the dimensionality of within-trial firing rates and find low-dimensional representations for across-trial dynamics. CP tensor decomposition, an existing and well-studied statistical technique [2], formally generalizes

existing network models of low-dimensional gain modulation (e.g. [3]), while twPCA models temporal shifts and scalings of neural dynamics, similar to dynamic time warping (DTW). These complementary methods can be jointly applied to neural data to accommodate low-dimensional changes in both amplitude and timing.

In experimental datasets collected from different species, brain regions, and behavioral tasks, these unsupervised methods recover sub-populations of neurons with interpretable within-trial as well as across-trial dynamics, reflecting experimental conditions, rewards, behavioral strategies, and motivational states. CP decomposition identified functional types of excitatory neurons in prelimbic cortex while mice switched navigational strategies in a four-armed maze. In separate experiments, CP decomposition and twPCA characterized neural dynamics while a Rhesus macaque learned to compensate for a visuomotor rotation in a brain-machine interface task. We have developed open-source computational tools for fitting these models to neural data [4,5]. [1] Cunningham JP, Yu BM (2014). Nat Neurosci. 17:11, 1500-1509.

[2] Kolda TG, Bader BW (2009). SIAM Review. 51:3, 455-500.

[3] Gorris RLT, Movshon JA, Simoncelli EP (2014). Nat Neurosci 17:6, pp. 858-865.

[4] https://github.com/ganguli-lab/twpca

[5] https://github.com/ahwillia/tensortools

Disclosures: A.H. Williams: None. B. Poole: None. N. Maheswaranathan: None. T.H. Kim: None. F. Wang: None. S. Vyas: None. K.V. Shenoy: None. M.J. Schnitzer: None. T.G. Kolda: None. S. Ganguli: None.

Poster

346. Data Analysis and Statistics: Neuronal Networks

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Topic: I.07. Data Analysis and Statistics

Support: KAKENHI (No. 15K01845)

Title: Detection of rodent social behavior using machine learning based method with convolutional neural network

Authors: *H. MUKAI, I. YABE, Y. OKADA, T. INOUE Meiji Univ., Kawasaki, Japan

Abstract: Determination of rodent behavior from video images obtained in experimental record is important in numerous branches of neuroscience. Freezing is most frequently studied phenomenon related to wide range of intrinsic behaviors such as contextual fear conditioning which is thought to mainly depend on the hippocampus as well as auditory fear conditioning, where amygdala plays a major role. Not restricted to the behaviors in individual animals,

freezing behavior has been studied in researches of social interaction, namely observational fear, which is associated with neuronal activities in the cingulate cortex. Existing methods of automated analysis, partly rerouting time-consuming and labor-intensive manual image identification in large data sets, are mostly depending on simple image feature analysis, not incorporating recent huge progress of machine learning, including deep learning. Introducing the results of machine learning methods has potential power allowing more efficient analysis for more complex phenomena in rodent behavior. To achieve the goal, we employed convolutional neural network (CNN) for the current study. CNNs are widely used neural network which are making tremendous success in recent computer visual object recognition contests. Among number of CNN implementation for various objectives, we employed C/C++ based, relatively light weight CNN for our purpose because the network could work with small computer resources. Training neural network has been carried out on PC with GPGPU (General purpose graphic processing unit) with labelled data. We are currently applying our machine learning based method to data obtained in recording social interactions of mice during/after observational fear. We are obtaining parallel results with conventional image processing method for simple freezing behavior, and further pursuing to identify a novel classification index of rodent behavior by the network. Our method is expected to be a better alternative to analyze vast amounts of recorded behavior data in many fields of neuroscience. Acknowledgement: This work is partly supported by KAKENHI (No. 15K01845) to H.M.

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Poster

346. Data Analysis and Statistics: Neuronal Networks

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Title: A novel probabilistic framework for estimating of neural connections from partially observed neural spikes

Authors: *T. IWASAKI¹, H. HINO², M. TATSUNO³, S. AKAHO⁴, N. MURATA¹ ¹Dept. of Electrical Engin. and Biosci., Waseda Univ., Tokyo, Japan; ²Dept. of Computer Sci., Univ. of Tsukuba, Tsukuba, Japan; ³Dept. of Neurosci., Univ. Lethbridge, Lethbridge, AB, Canada; ⁴Mathematical Neuroinformatics Group,, Natl. Inst. of Advanced Industrial Sci. and Technol., Tsukuba, Japan

Abstract: Plasticity is one of the most important features of the nervous systems that enables animals to adjust their behavior to an ever-changing external world. A major mechanism of plasticity is the changes in synaptic efficacy between neurons, and therefore estimation of neural connections is crucial for investigating information processing in the brain. Although many analysis methods have been proposed to this end, most of them suffer from a mathematical difficulty that stems from the fact that activity of only a limited number of neurons are available. That is, many existing methods ignore the effects from unobserved neurons. To overcome this difficulty, a novel probabilistic framework is proposed for estimating neural connections from partially observed spikes. We extract connections between observed neurons by explicitly quantifying influence from unobserved neurons to observed neurons. By modeling all possible propagations of influence over multiple neurons, the proposed method is able to eliminate pseudo-correlations; the pseudo-correlation is the problem that an indirect connection is detected as a direct connection. The method provides a macroscopic measure of connection within a temporal window of interest and it can be used for selecting the specific connections for further analyses. The proposed method is validated by artificial data and its applicability to real electrophysiological data is demonstrated as well.

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Poster

346. Data Analysis and Statistics: Neuronal Networks

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Support: NIH R01 HD086088

Contract 70155, Epic Medical C&I

Title: An efficient modeling approach for brain connectivity analysis of saltatory pneumotactile velocity stimulus

Authors: *K. JUNG¹, H. OH², J. LEE¹, S. M. BARLOW^{3,4}

¹Educational Psychology and Leadership/IMMAP, Texas Tech. Univ., Lubbock, TX; ²Neurosci., Baylor Col. of Med., Houston, TX; ³Special Educ. and Communication Disorders, ⁴Ctr. for Brain-Biology-Behavior, Univ. of Nebraska, Lincoln, NE

Abstract: Previous fMRI studies of the sensorimotor system have demonstrated the location, velocity, and direction of tactile stimuli on the skin's surface are discriminable features of sensorimotor processing. To better understand the representation and processing of dynamic saltatory tactile stimuli in the sensorimotor cortex, brain connectivity is utilized to delineate how sensorimotor areas are connected and how they influence each other. Here, we employed a pathanalytic structural equation modeling (SEM) approach for such a brain connectivity analysis of moving tactile stimuli on the glabrous skin of the digits, called path-analytic generalized structured component analysis (GSCA; Hwang & Takane, 2014). Path-analytic GSCA enables specification and estimation of the relationships among ROIs. For parameter estimation, pathanalytic GSCA applies a least squares estimation method, and the standard errors of parameter estimates are estimated by the bootstrap method. As such, path-analytic GSCA is computationally efficient in dealing with more elaborate and complicated brain connectivity models without recourse to distributional assumptions. We applied path-analytic GSCA to a bidirectionally connected structural model of the BOLD response time courses data extracted from four ROIs chosen a priori to investigate primary sensorimotor regions contralaterally to the two different velocity stimuli (5cm/s and 25cm/s) on the right hand (BA3b_Left(L), BA1_L, BA3a_L, and BA44_L) in neurotypical adults (n=11). The result of our analysis revealed that there were statistically significant differences between the 5cm/s and 25cm/s stimuli on the structural paths in the hypothesized model. Future work will apply Dynamic GSCA to timeseries data to further delineate connections not only within the sensorimotor control network but also within somatosensory related areas such as cerebellum and posterior parietal cortex (PPC) and the influence of reorganization of these connections on fine motor control in individuals with stroke.

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Poster

346. Data Analysis and Statistics: Neuronal Networks

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Support: ONR-YIP 26-1302-8750

Simons Collaboration on the Global Brain

Howard Hughes Medical Institute

Title: Unsupervised latent variable extraction from neural data to characterize processing across states

Authors: *R. CHAUDHURI¹, B. GERCEK¹, B. PANDEY¹, I. R. FIETE² ¹Ctr. for Learning and Memory, Univ. of Texas at Austin, Austin, TX; ²Ctr. for Learning and Memory, The Univ. of Texas at Austin, Austin, TX

Abstract: In circuits at the sensory and motor periphery and in rare instances in cognitive areas, the identity of the primary independent variable represented by the neural responses is known. This is the exception: In many brain regions or in different states (e.g. sleep), the independent encoded variable is unknown. To what extent is it possible to discover the identity of the independent variable and decode its value from neural data, without direct knowledge of what it is? We present efforts to discover structure in multiple single-unit data, extract the unknown independent variable, and perform fully unsupervised decoding of it. Our method involves parameterizing low-dimensional manifold structure in high-dimensional data. We apply it to recordings from the mouse thalamus and postsubiculum (Peyrache et al., 2015) to examine neural circuit dynamics during awake exploration, REM, and slow-wave sleep (SWS). The method discovers a ring structure, which it parameterizes to extract a circular variable, consistent with head direction coding in these circuits. The accuracy of unsupervised decoding of the latent variable on many sessions is on par with decoders constructed using head direction. Per-neuron tuning curves for the latent variable match (up to rotation) the actual head direction tuning curves. REM states and a subset of SWS states fall on the waking manifold, showingwithout the assumptions involved in applying awake-state encoding models to sleep decodingthat sleep and awake states share the same structure. Unlike waking, REM states perform a random walk on the circular variable, while SWS states sample a subset of angle values and make large jumps between them.

Thus, we show it is possible to discover the structure of a variable that drives neural responses and decode it without knowing its identity, simply from time-series neural data. Such approaches can help understand encoding and dynamics in neural circuits, across behavioral states.

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Poster

346. Data Analysis and Statistics: Neuronal Networks

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Support: NIH Grant NS030549

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NIH Grant 050474

Title: Determination of awake and sleep stages using objective detection criteria and analyses of long-term continuous intrahippocampal LFP recordings and video activity measures in mice

Authors: L. MOLNÁR¹, I. FERANDO², *I. MODY³

¹Electrical Engin., Sapientia Hungarian Univ. of Transylvania, Targu Mures, Romania; ²Neurol., UCLA, David Geffen Sch. of Med., Los Angeles, CA; ³Dept Neurol., UCLA Sch. Med., Los Angeles, CA

Abstract: Numerous algorithms exist to determine separate sleep stages and awake periods in mice. However, most of the methods for identifying such stages rely on subjective thresholds for their detection. We wanted to devise a method that does not rely on such thresholds, but separates the behavioral stages based on the intrinsic properties of the various oscillatory events in the LFP. We recorded CA1 or dentate gyrus δ , θ and γ oscillations (2048 Hz sampling) in adult mice freely moving in their home cages. The mice were kept with ad libitum access to water and food, on a 12:12 hours dark(D):light(L) cycle. The animals were also continuously monitored using an infrared sensitive video camera that recorded digital video files at 10-11 frames/s through the iSpy software. This software also calculated the movement and activity (A) of the animals by a digital frame-to-frame subtraction procedure. Each 12 hrs, for the L and D periods, recordings were down-sampled for the detection of δ , θ and γ oscillations. Using Igor Pro, the traces were band-pass filtered with an FIR filter between 1-4 Hz for δ , 5-12 Hz for θ , and 30-120 Hz for γ oscillations. The RMS values of 8 s epochs (0.5 or 1 s shifts) were obtained from the filtered recordings in each bandwidth and all-point histograms of δRMS , θRMS and γ RMS values were plotted for 12 hr L and D periods. In addition, the "peakiness" (P) of the θ oscillations was calculated and given values corresponding to the separation of its power from neighboring frequencies. We then calculated the ratio of $\gamma RMS/\delta RMS$ (G/D) for the duration of the recordings. The histograms of G/D showed a bimodal distribution well fitted by two Gaussians. The Gaussian with a lower mean and a very low variance could be easily separated. This distribution was then used to define the non-REM sleep periods (NREM) using a Gaussian probability-weighted function. Once the NREM episodes were defined, we separated the epochs outside the NREM into A and REM periods using an Euclidean vector space normalization and weighting of four vectors: A, θ RMS, P and γ RMS. Judging by the similarities between the durations of the various behavioral stages obtained by our method and those previously published, our approach accurately determined the behavioral stages of the animals without the requirement of recording EMG activity and subjective thresholding.

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Poster

346. Data Analysis and Statistics: Neuronal Networks

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Support: IARPA Contract No. 2012-12050800010

Title: Toward a common, extensible cloud architecture for images to graphs

Authors: *N. DRENKOW¹, P. ADAIR², D. HILL², J. LI², R. NORMAN-TENAZAS², R. RAIS², K. TORGAS², B. A. WESTER³, W. R. GRAY RONCAL⁴ ¹JHU/APL, Laurel, MD; ²Johns Hopkins Univ., Baltimore, MD; ³Johns Hopkins Univ. Applied Physics Lab., Laurel, MD; ⁴Johns Hopkins Univ. Applied Physics Lab., Baltimore, MD

Abstract: Electron microscopy (EM) datasets used for building and analyzing connectomes will soon approach petabyte-scale. It is well understood that trained human annotation of EM datasets is accurate, yet time-consuming, costly, and not scalable. While automated computer vision and machine learning approaches are poised to tackle many challenges of building connectomes in a fraction of the time and cost, many state-of-the-art algorithms have been focused on task performance but not necessarily scalability. In a similar vein, these algorithms are often evaluated on small datasets, but not packaged in a reusable or reproducible way, thus making it difficult for other researchers to employ them on a much larger scale.

Efforts at JHU/APL are focused on improvements and adaptations of these state-of-the-art algorithms in combination with novel, efficient uses of cloud-based computing and Docker to operate at the scale of current and anticipated EM datasets. This operation is critically important to support our generation of ground truth data, proofreading efforts, and performer evaluation under the MICrONS program.

In building connectomes via automated approaches, we've addressed scalability at critical levels of the processing hierarchy. At the algorithmic level, APL has developed and adapted state-of-the-art algorithms for processing large volumetric data. In particular, APL has successfully implemented, modified, and trained deep learning U-Net models to perform both dense membrane and synapse segmentation. To-date, most existing deep learning techniques produce voxel-wise or supervoxel-wise labelings. While the latter method enables some degree of scalability, our approach provides dense segmentation at a rate that is orders of magnitude faster than naive approaches without sacrificing accuracy.

At the workflow level, APL has leveraged Docker and cloud-based solutions to enable efficient and repeatable processing of large volumetric datasets stored in the Block and Object Storage Service (BOSS). Through the use of Docker and Amazon Web Services (including their Elastic Container Service, Simple Queueing Service, and Batch), we're able to massively parallelize volume processing while maintaining high throughput. Combining the algorithmic and workflow accelerations, we're able to produce membrane and synapse segmentations for the entire Kasthuri 2015 (100x80x55 um3) dataset in approximately six hours using only eight K80 GPUs. These segmentations then enable faster, targeted ground-truth generation and proofreading under MICrONS.

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Poster

346. Data Analysis and Statistics: Neuronal Networks

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Topic: I.07. Data Analysis and Statistics

Title: Single-subject source analysis from continuous resting state EEG

Authors: *A. C. TANG, W. R. FUNG, Y. HUA, L. QIN The Univ. of Hong Kong, Pokfulam, Hong Kong

Abstract: Resting state functional magnetic resonance imaging (rfMRI) studies have revealed organized patterns of brain activity, reflecting local and global synchronized activity within and across distinct functional brain regions. As fMRI measurement reflects changes in blood oxygen level, synchronized brain activity captured by rfMRI is of a different time scale from patterns of synchronization resulting from rapid changes in electroencephalograhy (EEG) associated with synaptic transmission. The relatively low cost and wide availability of EEG makes it a potential candidate for frequent and routine monitoring of human brain function. Here taking advantage of the millisecond temporal resolution of EEG, we used Second Order Blind Identification (SOBI) a blind source separation (BSS) method to uncover both local and global synchronized neuronal activity from continuous high-density EEG data (128 channels) collected during 5 min task-less resting period without using a shielded room. Across 9 participants (19 to 72 years of age), we were able to identify SOBI components with highly regular sensor-space projection maps reflecting (1) local synchronized neuronal activity along both dorsal and ventral visual pathways and in temporal and frontal cortices; (2) global synchronized neuronal activities spanning posterior-anterior parts of the brain. A subset of these components can be reliably observed across all participants while others show more individual differences. The most notable is the identification of the following component types: (1) components whose topography resemble those previously reported global network showing face-stimuli elicited P300 response; (2) components whose topography suggest a spatial origin of V4; (3) components whose topography suggest multiple spatial origins from the frontal lobes, including regions associated with

language processing. These maps raise the possibility of localizing synchronized ongoing neuronal activities to specific functional brain regions filling the gap from the rfMRI studies. The fact that such functionally distinctive maps can be obtained from 5 min EEG data suggests the feasibility of using highly portable EEG systems in frequent monitoring of brain activity from specific functional brain regions.

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Poster

346. Data Analysis and Statistics: Neuronal Networks

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Topic: I.07. Data Analysis and Statistics

Title: Novelty reduces trial-to-trial variability in the latency of cortical neuronal responses

Authors: *G. OUYANG¹, Y. HUA², W. R. FUNG², C. ZHOU³, A. C. TANG² ¹Psychology, Ernst-moritz-arndt-Universität Greifswald, Greifswald, Germany; ²Lab. of Neurosci. for Educ., The Univ. of Hong Kong, Pokfulam, Hong Kong; ³Dept. of Physics, Ctr. for Nonlinear Studies, Inst. of Computat. and Theoretical Studies, Hong Kong Baptist Univ., Kowloon Tong, Hong Kong

Abstract: Under resting or task free conditions, the human brain maintains a highly variable ongoing activity, shown as oscillatory EEG signals recorded from the scalp. Such on-going activity contributes to trial-to-trial variation in the latency of the event-related potentials (ERPs) response to identical stimulation. In typical cognitive studies, such trial-to-trial variations are dealt with by averaging across trials of stimulus presentation and across different subjects to obtain the socalled grand average. Here by applying Second Order Blind Identification (SOBI) to singlesubject hd-EEG data, we first extracted neuronal sources, including both early sensory areas and higher order processing areas down the cortical processing stream. We then compared the latency variability in single-trial ERPs of these SOBI recovered cortical neuronal sources during a visual color oddball task, where the participants pressed a button in response to the low probability color stimulus (20% of 250 trials). For different time windows selected to capture the early and late temporal components of an ERP, we estimated single trial response latency by calculating the cross-correlation between a single trial and the averaged ERP waveforms. Variance tests revealed a significant difference between the high versus low frequency color stimuli conditions (ps < .01). Most surprising is the direction of this difference---the variability in LATE response to low frequency stimuli was significantly smaller than that of the high frequency stimuli despite the former having a smaller number of trials. In contrast, no significant variance differences were found for the EARLY ERP components. This result suggests that a major source of trial-to-trial variations may arise from top-down evaluation of the incoming

feed-forward signals and that novelty may recruit stronger attention to enhance learning, which manifests itself as increased response reliability in the top-down efferent signals broadcasted to multiple brain regions. This data set also offers additional opportunities to further test the hypothesis that top-down modulation of neuronal activity of the lower functional brain regions by higher functional regions underlies the greater variation in the late, as opposed to the early, ERP component of the early stage brain regions.

Disclosures: G. Ouyang: None. Y. Hua: None. W.R. Fung: None. C. Zhou: None. A.C. Tang: None.

Poster

346. Data Analysis and Statistics: Neuronal Networks

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 346.18/WW38

Topic: I.07. Data Analysis and Statistics

Title: Online unsupervised spike sorting using an artificial STDP neural network

Authors: M. BERNERT^{1,2}, *B. YVERT¹

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Abstract: Extracellular neural recordings typically consist of the superposed activity of multiple neurons. To study ensemble dynamics, it is important to isolate the activity of single neurons. Automatic unsupervised spike-sorting is still a challenging problem, especially when largescaled neural recordings are considered. Moreover, applications such as brain-computer interfaces require real-time implementation. Most spike-sorting methods use four main steps: data filtering, action potential detection, feature extraction and clustering. However these methods often require supervision or offline processing. To overcome these limitations, we propose a fully unsupervised method based on a spike-timing dependent plasticity (STDP) artificial neural network, a type of network able to perform unsupervised learning. We considered a network constituted of three layers of neurons and one attention neuron, connected in a feedforward manner. The input signal, band-pass filtered, is continuously fed into the input layer. The spike trains of the output layer directly reflect the detected and sorted action potentials from the input signal. Each layer of the network has a specific role. The input layer fires at regular time steps, and the emitted spike train encodes the shape of the input signal within a sliding time window, thanks to input neurons encoding for different signal amplitudes at different delays. The attention neuron, which receives the input spike train, detects action potentials in the signal thanks to a short-term plasticity rule, and modulates the intermediate and output layer. The intermediate layer learns to recognize short signal shapes encoded by the input layer thanks to an STDP rule. Finally the output layer learns intermediate layer patterns thanks to an STDP rule, with the ability to adapt to the pattern size thanks to an intrinsic plasticity rule. We evaluated this method on several sets of artificially generated data with known truth and also on real extracellular recordings associated with intracellular recordings. On average we obtained comparable or better performances than the open-source spike-sorting software Osort and Wave_clus, especially in case of low SNR. Overall the proposed approach thus provides a robust online automatic and fully unsupervised spike sorting method.

Disclosures: M. Bernert: None. B. Yvert: None.

Poster

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Title: Dynamic neural stitching: Learning consistent neural population dynamics from separately recorded neural populations across months using LFADS

Authors: *D. J. O'SHEA^{1,2}, C. PANDARINATH⁶, J. COLLINS⁷, R. JOZEFOWICZ⁷, E. TRAUTMANN³, S. D. STAVISKY⁴, J. C. KAO², M. M. CHURCHLAND⁸, M. T. KAUFMAN¹⁰, J. M. HENDERSON¹¹, K. V. SHENOY^{12,5}, L. ABBOTT⁹, D. SUSSILLO^{2,5,7} ²Electrical Engin., ³Neurosciences, ⁴Neurosurg., ⁵Stanford Neurosciences Inst., ¹Stanford Univ., Stanford, CA; ⁶Biomed. Engin., Emory Univ. / Georgia Tech., Atlanta, GA; ⁷Google Brain, Mountain View, CA; ⁸Neurosci., ⁹Columbia Univ., New York, NY; ¹⁰Neurosci., Cold Spring Harbor Lab., Cold Spring Harbor, NY; ¹¹Dept Neurosurgy, R 227, Stanford Univ. Dept. of Neurosurg., Stanford, CA; ¹²EE, BioE & Neurobio., Howard Hughes Med. Inst. - Stanford Univers, Stanford, CA

Abstract: Increasing evidence suggests that the activity of large populations of neurons is often well-described by low-dimensional dynamics. Measurements of these neural populations are often limited to recordings from separate populations of neurons observed in many separate experimental sessions. With this approach, it is typical for the recorded neurons to be completely non-overlapping across sessions. This makes understanding the dynamics of the aggregated neural population difficult, especially at the level of single trials. Here we introduce "dynamic neural stitching", an adaptation of LFADS (Latent Factor Analysis via Dynamical Systems, see

Pandarinath et al. SfN 2017) which infers common latent dynamics from separately recorded, single-trial neural population activity. LFADS learns one consistent dynamical system to describe all the data, and session-specific transformations that map from these (latent) dynamics to the neural activity observed in each individual session.

This approach is motivated by the assumption that in an experimental design where the subject is engaged in a similar behavior across recording sessions, groups of neurons recorded from the same brain region should share the same underlying dynamics. We tested this approach using 45 sequentially recorded spiking datasets (24 ch. Plexon V-probes) from macaque M1 and PMd during a center-out instructed-delay reaching task. We trained one multi-session LFADS model as well as 45 individual, single-session models for comparison. The multi-session model's latent states were considerably more informative about single-trial behavior, including cross-validated predictions of hand kinematics ($p < 10^{-6}$) and reaction times ($p < 10^{-6}$). The latent neural population state trajectories of the stitched model varied by reach direction but were highly consistent across recording sessions, implying that LFADS found a common low-dimensional latent state governing the population activity across recording sessions.

New multi-electrode and imaging technologies enable recording of ever-larger populations of neurons, but it is often practical to collect more cells over many sessions. Dynamic neural stitching enables neuroscientists to move beyond trial-averaged analyses of sequentially recorded datasets, and instead to infer common latent dynamics from the individual trials of each dataset. Understanding these dynamics may help to unravel the computations performed by neural circuits and to clarify the relationship between single-trial neural population state and behavior.

Disclosures: D.J. O'Shea: None. **C. Pandarinath:** None. **J. Collins:** None. **R. Jozefowicz:** None. **E. Trautmann:** None. **S.D. Stavisky:** None. **J.C. Kao:** None. **M.M. Churchland:** None. **M.T. Kaufman:** None. **J.M. Henderson:** None. **K.V. Shenoy:** F. Consulting Fees (e.g., advisory boards); Neuralink Inc., consultant, Cognescent, Scientific Advisory Board, Heal, Scientific Advisory Board. **L. Abbott:** None. **D. Sussillo:** None.

Poster

346. Data Analysis and Statistics: Neuronal Networks

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Title: NETCAL: An interactive platform for large-scale, NETwork and population dynamics analysis of CALcium imaging recordings

Authors: *J. G. ORLANDI^{1,4,5}, S. FERNÁNDEZ-GARCÍA^{5,7,8}, A. COMELLA-BOLLA^{5,7,8}, M. MASANA^{5,7,8}, G. GARCÍA-DÍAZ BARRIGA^{5,7,8}, M. YAGHOUBI¹, J.-M. CANALS^{5,7,8}, M. A. COLICOS^{2,3}, J. DAVIDSEN¹, J. ALBERCH^{5,7,8}, J. SORIANO^{4,6} ¹Dept. of Physics and Astronomy, ²Dept. of Physiol. & Pharmacology, Fac. of Med., ³The Hotchkiss Brain Inst., Univ. of Calgary, Calgary, AB, Canada; ⁴Departament de Física de la Matèria Condensada, Facultat de Física, ⁵Departament de Biomedicina, Facultat de Medicina, ⁶Inst. of Complex Systems (UBICS), Univ. de Barcelona, Barcelona, Spain; ⁷Inst. d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; ⁸Ctr. de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain

Abstract: Calcium imaging has become the preferred technique in neuroscience to simultaneously record the activity of thousands of cells. Yet most tools to analyze the recordings are rather rudimentary or require extensive knowledge of other disciplines, from machine learning to big data and network theory. Moreover, there is often a disconnection between the experiments and their analysis and outcome, usually taking place days apart and/or being done by different people.

We present NETCAL, a MATLAB-built, dedicated software platform to record, manage and analyze high-speed high-resolution calcium imaging experiments. Its ease of use, interactive graphical interface and exhaustive documentation is aimed to wet-lab researchers, but it will also meet the needs of any experienced data scientist through its plugin and scripting system. We have developed a large set of tools and incorporated state-of-the-art algorithms and toolboxes for large-scale analysis of network and population dynamics. Analyses include: automated cell detection (both static and dynamic); trace and population sorting through machine learning, clustering and pattern recognition; bursting dynamics; spike detection; network inference (from functional networks to causal relations); and many more. Several of these tools are also available in real-time, e.g. cells and spikes can be monitored during the actual recording, giving the researcher extensive feedback on the progress of the experiment.

We have tested and used the software in several different experimental preparations and laboratory equipment. For instance, NETCAL has been used to test the viability and performance of differentiation protocols from human induced pluripotent stem cells (hISPCs); to characterize the individual and collective behavior of dissociated cortical and striatal cultures from Huntington's disease (HD) mouse models; to reveal the communication between neurons and astrocytes in rat hippocampal cultures; and to detect propagating activity patterns in cortical cultures. Although NETCAL has been developed for calcium recordings in cultures, we have successfully tested and used it in other preparations, e.g., in-vivo calcium imaging and multi-

electrode arrays.

Our platform has been developed by scientists for scientists, to promote and foster the development of tools for the replication and validation of experimental results. The software is highly modular, and its implementation provides easy extendability to adapt it to the specific requirements of any research group.

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Poster

346. Data Analysis and Statistics: Neuronal Networks

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Title: Detection of irregularly firing inspiratory neurons in the pre-Bötzinger complex based on spatio-temporal optical imaging data analysis

Authors: *F. MIWAKEICHI

The Inst. of Statistical Mathematics, Tachikawa-Shi, Japan

Abstract: Spontaneous synchronous neuronal activity is fundamental phenomena to maintain homeostasis. One of the essential brain function to sustain the life is respiration, and it has been known that spontaneous neural synchronization in the pre-Bötzinger complex (preBötC) generates inspiratory bursts which can be observed as local filed potential (LFP) signal. Recent development of imaging techniques, such as Ca^{2+} imaging system, enables us to simultaneously record multicellular activity to investigate neuronal circuitry. Cross-correlation analysis with a reference function defined from LFP signal for whole temporal domain has been used to detect neurons that involve inspiratory burst. Though this approach is simple and can be straight forwardly applied to the data, only the regularly firing (clock-like) neurons, which involves most of inspiratory burst, can be detected. The ratio of regularly firing neurons among the neuron in

the imaging data is about 10% and the other neurons are irregularly activated. Then question arises; what is the role of other neurons to generate respiratory rhythm? In order to elucidate the behavior of irregularly firing (non-clock-like) neurons, we applied cross-correlation analysis for each inspiratory burst with a sliding temporal window. Through this analysis we detected as many as seven times the number of the irregularly firing neurons than regularly firing neurons with discriminating the types of neuron, such as excitatory, glycinergic and GABAergic inhibitory neurons. We found that activation timing of regularly firing neurons were synchronized and kept the same time delay relative to the onset of the burst in LFP signal. On the other hand, the activation timing of irregularly firing neurons were also irregular. We also found that the number of firing neurons affected the amplitude of LFP signal for each inspiratory burst. We will further discuss the applicability of the causality analysis among the neurons in this poster.

Disclosures: F. Miwakeichi: A. Employment/Salary (full or part-time):; full, Department of Statistical Modeling, The Institute of Statistical Mathematics, Tokyo, Japan, Department of Statistical Science, School of Multidisciplinary Sciences, The Graduate University for Advanced Studies, Tokyo, Japan.

Poster

346. Data Analysis and Statistics: Neuronal Networks

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Title: A fast image registration framework for light-sheet fluorescence microcopy of neural activities

Authors: *H. HUI¹, H. MENG¹, C. HU¹, X. YANG¹, J. TIAN² ¹Key Lab. of Mol. Imaging, CAS, Inst. of Automation, Chinese Acad. of Scienc, Beijing, China; ²Key Lab. of Mol. Imaging, CAS, Beijing City, China

Abstract: The ability of fast and single-neuron resolution imaging of neural activities enables light-sheet fluorescence microscopy (LSFM) as a powerful imaging technique in functional
neural connection applications. The state-of-art LSFM imaging system can record the neuronal activities of entire brain for small animal, such as zebrafish or C. elegans at single-neuron resolution. However, the stimulated and spontaneous movements in animal brain result in inconsistent neuron positions during recording process. In this work, we address the problem of real-time registration of neural positions in stacks of LSFM images. This is necessary to register brain structures and activities. To achieve real-time registration of neural activities, we present a fast rigid registration architecture by implementation of Graphics Processing Unit (GPU). In this approach, the image stack was preprocessed on GPU by mean stretching to reduce the computation effort. The present image was registered to the previous stack that considered as reference. A fast Fourier transform (FFT) algorithm was used for calculating the shift of the image stack. The calculations for image registration were performed in different threads while the preparation functionality was refactored and called only once by the master thread. We implemented our registration algorithm on NVIDIA Quadro K4200 GPU under Compute Unified Device Architecture (CUDA) programming environment. The experimental results showed that the registration computation can speed-up to 550ms for a full high-resolution brain image. The registration can be accelerated using more GPUs in the architecture. Our approach also has potential to be used for other dynamic image registrations in biomedical applications.

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Poster

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Title: LFADS: A deep learning technique to precisely estimate neural population dynamics on single trials

Authors: *C. PANDARINATH¹, D. J. O'SHEA², J. COLLINS⁵, R. JOZEFOWICZ⁵, S. D. STAVISKY³, J. C. KAO⁴, E. TRAUTMANN², M. M. CHURCHLAND⁶, M. T. KAUFMAN⁸, S. RYU⁹, J. M. HENDERSON¹⁰, K. V. SHENOY¹¹, L. ABBOTT⁷, D. SUSSILLO⁵ ¹Biomed. Engin., Emory Univ. / Georgia Tech., Atlanta, GA; ³Neurosurg., ⁴Electrical Engin., ²Stanford Univ., Stanford, CA; ⁵Google Brain, Mountain View, CA; ⁶Neurosci., ⁷Columbia Univ., New York, NY; ⁸Neurosci., Cold Spring Harbor Lab., Cold Spring Harbor, NY; ⁹Dept Neurosurg, Palo Alto Med. Fndn., Palo Alto, CA; ¹⁰Dept Neurosurgy, R 227, Stanford Univ. Dept. of Neurosurg., Stanford, CA; ¹¹EE, BioE & Neurobio., Howard Hughes Med. Inst. -Stanford Univers, Stanford, CA

Abstract: Neuroscience is experiencing a data revolution in which simultaneous recording of many hundreds or thousands of neurons is revealing structure in population activity that is not apparent from single-neuron responses. This structure is typically extracted from trial-averaged data. Single-trial analyses are challenging due to incomplete sampling of the neural population, trial-to-trial behavioral variability, and fluctuations in action potential timing. We introduce Latent Factor Analysis via Dynamical Systems (LFADS; Sussillo et al. arXiv 2016), a deep learning method to infer latent dynamics from single-trial spiking data. LFADS uses a nonlinear dynamical system to model dynamics underlying observed population activity and to infer "denoised" firing rates for each neuron on a single-trial basis.

We tested the ability of LFADS to denoise single-trial spiking activity in motor cortical data with simultaneously recorded neural populations. In related work (O'Shea et al. SfN 2017) we extend LFADS to combine data from separately recorded populations to improve inference of underlying dynamics.

By denoising the observed spiking activity, LFADS enabled prediction of behavior with new levels of accuracy. We decoded reach kinematics from LFADS-denoised activity, and compared against alternative techniques on a dataset in which monkeys made straight and curved reaches. Denoising using LFADS outperformed smoothing spikes or Gaussian Process Factor Analysis (avg R2=0.91 for LFADS, v. 0.64/0.61 for smoothing/GPFA). We also compared performance as a function of population size and found that LFADS using ~35 neurons outperformed the alternatives applied to the full population of 202 neurons.

Further, LFADS uncovered known dynamic features of motor cortical activity on multiple timescales, e.g., 15-40 Hz LFP (Donoghue et al. J Neurophys 1998), and slow oscillations previously been characterized at the trial average level (Churchland et al. Nature 2012). LFADS revealed consistent dynamics on the single-trial level (~2300 individual trials, 108 reach conditions), and the inferred latent states were predictive of neurons that were held-out from model training (measured by likelihood of held-out neurons' spikes).

Finally, with its dynamical model, LFADS can infer when the observed data deviates from expected dynamics. When applied to M1/PMd data from a task in which unexpected perturbations occur mid-trial, LFADS inferred the presence, type and timing of the perturbations. In sum, LFADS accurately models neural population dynamics on single trials, opening the door to a detailed understanding their role in computation, and ultimately in driving behavior.

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Poster

346. Data Analysis and Statistics: Neuronal Networks

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Title: Automated 3D EM and functional brain image co-registration using deep learning

Authors: *F. LONG¹, A. BLECKERT¹, J. REIMER², E. FROUDARAKIS², A. TOLIAS^{2,3}, R. YOUNG¹, D. NUNO¹, C. REID¹, L. NG¹ ¹Allen Inst. For Brain Sci., Seattle, WA; ²rtment of Neurosci., Baylor Col. of Med., Houston,

TX; ³Dept. of Electrical and Computer Engin., Rice Univ., Houston, TX

Abstract: To gain insight into the relationship between the activity of neurons and their connectivity in neocortical circuits, we are using imaging and computational approaches to coregister neurophysiological and neuroanatomical datasets in the primary visual cortex of the mouse. We use transmission electron microscopy camera array to acquire 3D high-resolution images of mouse V1 to reconstruct brain anatomy at the cellular level. We use two-photon calcium imaging to observe functional activities of neurons in the same area. To correlate brain structures and neuron activities in large scale, we are developing deep learning based approach to co-register EM and functional data. Our method uses a small chunk of manually co-registered EM and functional image as the training set. In the training phase, it takes small pair-wise patches of EM and functional data as input to a deep convolutional neural network (CNN), which outputs 1 or 0, indicating the small patch of EM and functional image match or not. In the testing period, we generated a pool of candidate spatial transformations between EM and functional data. Each transformation brings the two sets of data into the same canonical space, making them comparable. We then generate pair-wise patch samples for each transformation and use the trained CNN to predict whether they belong to class 1 (match) or 0 (non-match). We then selected the transformation that produces the maximum percentage of matched samples as the best registration. Our preliminary result on a small dataset has proved the effectiveness of the method. Like other Allen Institute products, data and tools developed will be made publicly available for the research community.

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Poster

346. Data Analysis and Statistics: Neuronal Networks

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Title: Deep convolution neural networks and multi-scale inputs better classify suspicious breast masses

Authors: *A. J. LONSBERRY, S. PICKARD, R. QUINN

Mechanical Engin., Case Western Reserve Univ., Cleveland, OH

Abstract: We demonstrate a computational system comprised of deep neural networks that is able to classify suspicious masses in images with 100% accuracy. Breast cancer screening, with mammography being one of the most employed early screening methods, affects millions of women. Although mammography as currently practiced is invaluable, improvements in its accuracy would be beneficial. According to Smith-Bindman et al., America has twice as many callbacks for questionable mammograms when compared to the UK, with 11% of screening mammograms assessed as abnormal. These callbacks result in women receiving further testing, such as invasive biopsies, and result in 3% of reassessed cases being confirmed as cancer (0.3% of total number of mammograms). This more aggressive callbacks was attributed by Smith-Bindman et al. in large part to fear of lawsuits and has resulted in an increase in sensitivity of mammography at the cost of specificity. The work here is poised to 1) increase the specificity of the mammography screening modality while not sacrificing sensitivity and 2) mitigate unnecessary callbacks and biopsies.

Deep learning neural networks have previously been applied to medical imaging diagnostics with good results. Jiao et al. developed a system capable of classifying suspicious masses as malign or benign with 97% accuracy. Our technique is able to perform the same task with 100% accuracy. This is achieved by using a residual network (Res-Net) (He et al.), a type of deep convolution neural network with a specialized structure. Res-Nets use shortcut connections across constitutional layers to mitigate problems with gradients shrinking to near zero or tending to infinity. In our work, we diverge from the common convolution neural network input structure, and rather than input one image at a time, input three images centered on the same location, but with different scales. The multi-scale input provides fine detail of the suspected tumor as well as surrounding tissue.

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Poster

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Topic: I.07. Data Analysis and Statistics

Title: Bayesian inference about the brain's effective connectivity using intracranial EEG data

Authors: *T. ZHANG, *T. ZHANG Statistics, Univ. of Virginia, Charlottesville, VA

Abstract: We use ordinary differential equations (ODEs) to model the human brain as a continuous time dynamic system consisting of biophysically interacting components, i.e., brain regions. In contrast to existing ODE models for fMRI and EEG data that focus on directional connectivity among only a few brain regions, we propose a high-dimensional ODE model motivated by statistical considerations to explore connectivity among many small brain regions recorded by intracranial EEG (ECoG). The new model is widely applicableto characterize various brain regions' oscillatory activity and their network of connectivity in a cluster structure. We develop a unified Bayesian framework to quantify the inadequacy in the proposed ODE model for the complex brain system, identify clusters and strongly connected brain regions, and map the brain's directional network where each network edge denotes a significant directional effect exerted by one brain region over another. We apply the proposed ODE model and Bayesian method to EGoG data sets, studying the brain network changes over time.

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Poster

346. Data Analysis and Statistics: Neuronal Networks

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Title: Effects of global signal regression and head movement on connectivity analysis using resting state functional magnetic resonance imaging

Authors: *T. IIDAKA¹, T. KOGATA¹, E. BAGARINAO²

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Abstract: The functional network within the brain has been investigated with resting state (rs-) connectivity analysis in human participants. Changes in network connectivity in the brain have been reported in patients with neuropsychiatric disorders such as dementia, schizophrenia, and autism. Analysis of rs-connectivity using functional magnetic resonance imaging (fMRI) has been recognized as a powerful tool for developing disease-specific biomarkers. However, data obtained with rs-fMRI are confounded by signals originating from physiological and motionrelated artifacts, which may obscure result significance. To counter these confounding factors, global signal regression (GSR) has been used for data and the motion parameter threshold (MPT) for participants. However, how these methods affect the results of functional connectivity (FC) has never been clarified. In the present study, we systematically investigated the effects of GSR and MPT on FC analysis. Data were extracted from a multicenter database (Autism Brain Image Data Exchange) from five sites (all boys, including patients with autism (ASD) and controls (CTL), mean age: 10.8 years). Participants were selected and divided into three groups as per the manufacturer of the MRI scanner: group-G (General Electric, sites EMC and SDSU), group-P (Philips, site KKI), and group-S (Siemens, sites OHSU and UCLA). The number of participants was 89 in group-G (ASD/CTL, 44/45), 76 in group-P (38/38), and 79 in group-S (45/34). The rsfMRI data were preprocessed using SPM12 and DPARSFA, and signal changes in 90 regions, defined by the standard template, were extracted. The signal time courses were transformed into a 90 \times 90 FC matrix for each participant. The effects of GSR on mean and skewness of FC were tested collectively in ASD and CTL, and within each group. Excessive correlation (greater than 2 SD of the mean), which caused skewness of FC, was compared before and after GSR. Finally, the effects of head motion on the skewness of FC were investigated by excluding participants according to the MPT. The results showed that GSR significantly (p < 0.01) decreased mean FC to zero in both ASD and CTL, and in all groups. The significant (p < 0.05) difference observed in mean FC between ASD and CTL diminished after GSR. GSR significantly (p < 0.05) increased the number of regions with excessive correlation. Finally, the MPT significantly (p < p0.05) decreased the skewness of FC only after GSR application. These results indicate that GSR diminishes differences in FC between ASD and CTL, but does not eliminate excessive correlations. The effect of the MPT is relatively small and there is a tradeoff between data quality and sample size.

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Poster

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Capes

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Title: Longitudinal graph analysis of functional connectivity of mild cognitive impairment and AD patients

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Abstract: As a contribution to the longitudinal research of Alzheimer's disease (AD), this study united the capabilities of graph theory and the resting state functional connectivity (RS-fMRI) technique. The main objective was to track changes in the topological configuration of functional connectivity networks of patients with AD and mild cognitive impairment (MCI). The images analyzed were of subjects from the neuropsychology and dementia outpatient clinic of a school-hospital in Brazil, who had two examinations. MR acquisitions were done on a 3T Philips Achieva scanner, while subjects avoided attention demanding activity. The interval between examinations was on average 52 ± 30 weeks. In the first examination there were 15 healthy controls (C) (12 women, 69 ± 6 years old), 22 MCI patients (14 women, 70 ± 6 years old), and 14 AD (9 women, 73 ± 8 years old). For the second examination, three participants changed diagnosis, so there were 14 C (11 women, 70 ± 6 years old), 21 MCI (14 women, 71 ± 6 years old), and 16 AD (10 women, 74 ± 8 years old). Image preprocessing included scrubbing of flagged volumes (Power et al., NeuroImage 105, 2015) besides the usual steps. For the graphs, 264 regions of interest represented the nodes (Power et al., Neuron 72, 2011); while the pairwise Pearson correlation coefficient of the extracted time series represented edges. Graphs were binarized with correlation thresholds. Selected graph metrics and their variance were calculated: degree, characteristic path length, clustering coefficient, and betweenness centrality. Also calculated were the small world coefficient and the local efficiency of 10 selected nodes. Mixed effects linear models were applied with fixed effects for diagnostic group and random intercepts for each individual. Later, education, time between acquisitions and confound variables age and a nuisance movement parameter were also tested. Contrary to similar studies, there were no significant results, only hints for the variance of some

metrics reflecting effects of the progression of the disease. The lack of results may be associated to the methodology for removing artifacts, a lack of statistical power, and the adoption of global metrics.

In conclusion, data from more observations is necessary, as well as improvements during acquisitions, quality control of the images, and the construction of graphs. The preferred metrics and tests should be local and nonparametric.

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Poster

346. Data Analysis and Statistics: Neuronal Networks

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 346.29/WW49

Topic: I.07. Data Analysis and Statistics

Title: The local functional connectivity of the seizure onset and peri-seizure onset areas

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Abstract: Objective. To determine the band-related local functional connectivity (BRLFC) of the seizure onset area (SOA) and the peri-SOA.

Methods. This study was conducted on 18 unselected adult patients with intractable epilepsy undergoing icEEG monitoring for surgery. Intracranial EEG electrode contacts were located from post-implantation CT and MR images and registered to the MRI of a standard brain to allow interpretation of results from all patients in the same space. A 1 hr icEEG epoch, recorded during wake and removed in time from seizure occurrence, was studied. Coherence was estimated for all pairs of electrode contacts ipsilateral to the SOA in delta, theta, alpha, beta, gamma and a high frequency band. The BRLFC of each electrode contact was estimated as the average band-related coherence between it and all electrode contacts within a specified spatial window.

Results. The average BRLFC was significantly non-zero, and there was an inverse relationship between average BRLFC and distance from the SOA primarily in the beta, gamma and high frequency bands. The contacts ipsilateral to the SOA were separated into four sets based on radial distance to the SOA: (1) S: SOA contacts, (2) P: all contacts within 3 cm of the SOA but not including SOA contacts, (3) Q: all contacts from 4 to 7 cm of the SOA, and (4) R: all contacts from 8 to 12 cm of the SOA. The mean BRLFC for all contacts in each of these four

sets was calculated as well as the ratios of the mean values of BRLFC for S, P, Q, and R. The most pronounced significant (p < 0.05) differences in these values were observed between P and R in gamma BRLFC evaluated for a mid-radial distance window.

Conclusions. A graded relationship was observed between BRLFC and distance to the SOA, for specific frequency bands, such that electrode contacts with the greatest connectivity were in the SOA or in the immediate peri-SOA and those with the lowest connectivity were at a distance of several cm from the SOA.

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Poster

346. Data Analysis and Statistics: Neuronal Networks

Location: Halls A-C

Time: Monday, November 13, 2017, 8:00 AM - 12:00 PM

Program#/Poster#: 346.30/WW50

Topic: I.07. Data Analysis and Statistics

Title: Graph Theory network analysis of functional connectivity in Mild Cognitive Impairment

Authors: *A. V. MEDVEDEV¹, R. S. TURNER²

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Abstract: Early detection of Alzheimer's disease (AD), which is critical for the effective treatment, calls for new functional biomarkers of the disease. Resting state functional connectivity (rsFC) is promising for the development of such biomarkers because it has been suggested to be able to detect pathological process earlier than cognitive tests. This premise is supported by the existing evidence that rsFC patterns experience significant changes in several neurological and psychiatric disorders (Seeley et al 2009; Cabral et al 2014). Resting state FC has been successfully studied using fMRI but there are serious limitations in its use in the elderly population. Electroencephalography (EEG) has shown promise as a possible new biomarker for AD and Mild Cognitive Impairment (MCI) (Moretti 2015). With the development of more accurate methods for source reconstruction and new software tools, it becomes possible to use scalp EEG to produce interpretable 3D images of the brain (Dubovik et al 2012). We recorded high density EEG signals in seven MCI subjects and seven age-matched healthy participants during the resting state (5 min). Data preprocessing included artifact removal with a special effort to remove myogenic activity using Independent Component Analysis (Olbrich et al 2011). rsFC was measured by imaginary coherence which is insensitive to volume conductance effects (Nolte et al 2004). The time courses of imaginary Fourier coefficients (FieldTrip toolbox, Oostenveld et al 2011) averaged within physiological frequency bands were subjected to source reconstruction using swLORETA (BESA, GmbH, Germany). The time courses of frequencyspecific 3D images were analyzed to derive rsFC maps and the global parameters of rsFC for controls and MCI subjects were analyzed with Graph Theory using CONN software (Whitfield-Gabrieli and Nieto-Castanon 2012).

Second-level analysis revealed significant group differences in Global Efficiency of brain networks within specific frequency bands. Thus, Global Efficiency within the alpha band (10-12 Hz) was significantly lower in MCI subjects especially in the parietal cortex. The network analysis in the beta band (14-28 Hz) revealed significantly lower Global Efficiency in the MCI group especially in the left hemisphere.

These data suggest that high density EEG reinforced by source reconstruction techniques is sensitive to detect the aberrant patterns of connectivity in MCI, a precursor of Alzheimer's disease. Thus, the global parameters of functional connectivity can be used for the development of new biomarkers for early diagnosis of MCI and AD as well as for quantitative assessment of treatments and rehabilitation strategies.

Disclosures: A.V. Medvedev: None. R.S. Turner: None.