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Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.01/A1

Topic: A.07. Developmental Disorders

Support: •NIH 5DP1MH110234-02 Pioneer Grant

Title: Neural mechaisms of chromatin remodeling: The role of cohesin complex proteins in memory and learning using *Drosophila melanogaster*

Authors: *K. A. EDWARDS, B. Z. KACSOH, G. BOSCO
Mol. and Systems Biol., Dartmouth Col., Hanover, NH

Abstract: Mutations in proteins that organize chromatin are implicated in neurodevelopmental disorders, such as Cornelia de Lange Syndrome (CdLS). CdLS patients present with microcephaly, intellectual disability, and autism spectrum disorder caused by heterozygous loss-of-function mutations in genes encoding the cohesin complex proteins and the cohesin-loading factor, Nipped-B (NipB). These proteins function together in regulating chromatin structure and gene transcription. Our objective is to determine how cohesin and cohesin-related proteins regulate memory and social learning in the fruit fly *Drosophila melanogaster*. In the presence of a larval endoparasitoid wasp, adult flies perform two behaviors: either decrease egg laying or lay their eggs on ethanol-laden food. Flies will remember this exposure following wasp removal. Our lab has shown that known long-term memory genes and a functional mushroom body (MB) are necessary for this memory. Adult flies (10 replicates 5 female and 1 male 3-5 days past eclosion (dpe)) are allowed to lay eggs on their choice of food made with water or 6% ethanol in the presence or absence of 3 female wasps. After 24 hours, wasps are removed and flies are moved to new enclosures each day with fresh food while the proprtion of eggs laid of ethanol-laden food is reported.
Flies that have been exposed to wasps will also communicate their exposure to naïve fruit flies which subsequently decrease egg laying. We use this behavior to assay social learning by placing wildtype *Canton-S* flies (12 replicates 10 female and 2 male 3-5 dpe) in one section of a two-part enclosure with or without 20 female wasps in the neighboring chamber for 24 hours. After measuring the egg depression of these “teachers”, each replicate is paired with naïve “student” flies (12 replicates 10 female and 2 male 3-5 dpe) in new enclosures with fresh food. The egg depression of students is recorded as a measure of learning. Using these assays, we have measured how flies with mutations in cohesin or NipB are able to remember and learn. We found that mutations in SMC1, a cohesin protein, impaired memory formation while flies with mutations in NipB have impaired memory retention. Additionally, flies with mutations in Rad21, another cohesin protein, are unable to learn while flies with mutations in SMC1 and NipB have

an impaired ability to learn. Because flies with NipB mutations have been observed to have abnormal MB structures while loss of SMC1 and Rad21 cause dendrite and pruning defects in the MB, we have evaluated MB structure of mutants across their development. We are investigating these phenotypes to evaluate influences of cohesin and cohesin-related proteins on memory and learning.

Disclosures: K.A. Edwards: None. **B.Z. Kacsoh:** None. **G. Bosco:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.02/A2

Topic: A.07. Developmental Disorders

Support: CHARGE Syndrome Foundation
FRQS Junior 1

Title: Chd7 regulates GABAergic network development in zebrafish

Authors: P. JAMADAGNI[1], E. SAMARUT[2], *K. PATTEN[1]
[1]Inst. Armand-Frappier, INRS, Laval, QC, Canada; [2]Neurosciences, CRCHUM, Montreal, QC, Canada

Abstract: Mutations in the ATP-dependent chromatin remodeller chromodomain, helicase, DNA binding (CHD) 7 are the primary cause of CHARGE syndrome and have been associated with autism spectrum disorders (ASD). However, the mechanisms by which mutations in CHD7 affect brain development and function are poorly understood. To address this question, we have developed a zebrafish *chd7* CRISPR/Cas9 knockout model owing to the suitability of this vertebrate model for the study of early neurodevelopment. *chd7* knockout (*chd7-/-*) zebrafish larvae exhibit a small head phenotype, defects in craniofacial cartilage development, heart defects and had no swim bladder. We also found that *chd7-/-* fish display aberrant axonal network development. Interestingly, the mutant fish displayed hyperactivity particularly during dark light cycle. It has been proposed that an aberrant inhibitory signaling in the brain is a mechanism underlying ASD; we thus next sought to perform a detailed analysis of the brain in our model. We observed a marked decrease in proliferation as well as significant decrease in GABAergic cells in *chd7* mutants. The decreased number of GABAergic cells in certain regions of the brain is due to a failure in the migration of these cells. Treatment with the GABA-A receptor antagonist pentylenetetrazol (PTZ) showed that *chd7* mutants exhibit an increased sensitivity to PTZ-induced seizure, providing further evidence for GABAergic deficits in *chd7* mutants. Using an unbiased whole transcriptomic approach, we identified many genes involved in cell proliferation, migration and cell adhesion that are dysregulated in *chd7* mutant. Together,

our findings indicate loss of chd7 results in a deficit of inhibitory neurons and suggest an essential role of chd7 in the brain neuronal network development.

Disclosures: P. Jamadagni: None. **E. Samarut:** None. **K. Patten:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.03/A3

Topic: A.07. Developmental Disorders

Support: Intramural NIH Grant Z01 NS003041-11

Title: Long distance plasticity of callosal connections: From men to mice

Authors: *D. SZCZUPAK[1,2], C. C. YEN[1], C. LIU[1], S.-H. CHOI[1], F. MEIRELES[3], C. VICTORINO[3], A. C. SILVA[1], R. LENT[3,2], F. F. TOVAR-MOLL[3,2]
[1]Natl. Inst. of Hlth., Bethesda, MD; [2]Federal Univ. of Rio de Janeiro, Rio de Janeiro, Brazil; [3]Inst. D'or for Res. and Educ., Rio de Janeiro, Brazil

Abstract: Brain plasticity is usually associated with microstructural changes, but it can also reflect a large macroscopic rewiring of the brain called long-distance plasticity (LDP). LDP was first described in humans with dysgenesis of the corpus callosum (dCC), a brain malformation in which some or all callosal fibers fail to find their natural tracts and end up forming completely new paths. So far, little is known about the detailed anatomical and temporal pattern of the development of those connections and, to date, no animal model could reproduce the full complexity of brain connectivity in this pathology. In the present study, we used ultra-high field diffusion-weighted MRI to map the underlying formation of white-matter fiber tracts in a long-known murine model of dCC, the Balb/c mouse. We observed that the Balb/c mouse has a large variability in size of the CC compared to wild type C57BL/6 mice (Figure 1A). Using the high-resolution DTI images, we also noticed that, compared to a normal C57BL/6 mouse, in which the inter-hemispheric fiber cross midline over a broad swath of the CC (Figure 1B), Balb/c mice have their interhemispheric connections cross midline in a very restricted point of the CC (Figure 1C). Balb/c mice have a whole brain reorganization (FIgure 2). These spontaneous abnormalities of the CC in Balb/c validate this strain as a suitable animal model to investigate the genetic origins of malformations of the CC, which may lead to a better understanding of how LDP occurs in humans.

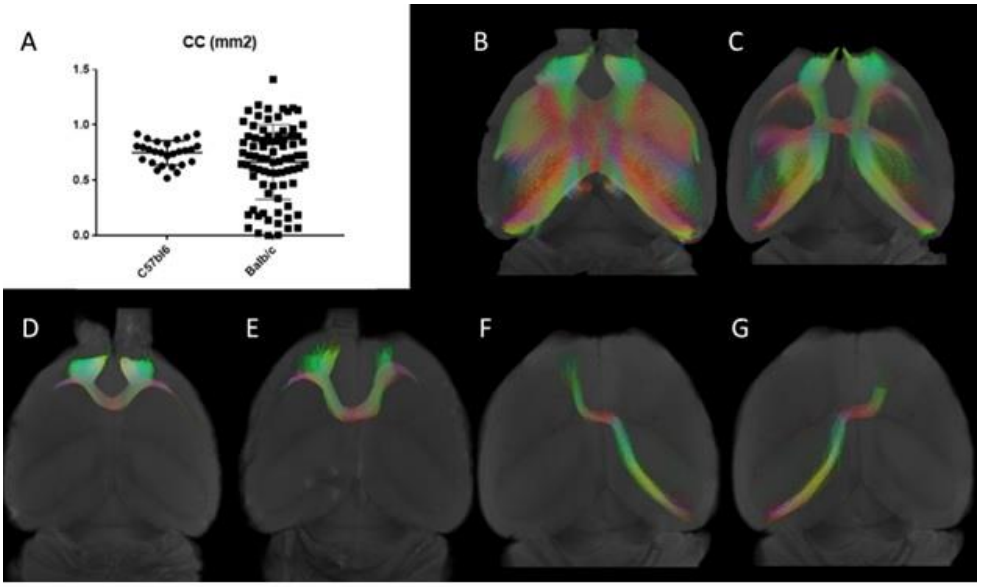

Figure 1. A. Graph showing the greater variability of the size of the CC in Balb/c as compared with controls, in axial view. B-G. Examples of the abnormal connectivity in Balb/c include the the global callosal network, where we can observe the whole interhemispheric network in (B) compared to a severely altered animal callosal network (C), connections of the prefrontal cortex, where in wild type mice occur through the genus of the CC (Figure 1D) but in Balb/c go through a CC remnant that is located more posteriorly than the genus of the CC (Figure 1E). Balb/c mice also show sigmoid bundles connecting contralateral posterior cortex to prefrontal cortex (Figure 1F, 1G). These sigmoids bundles do not exist in wild type mice, but were first discovered in human patients of dCC.

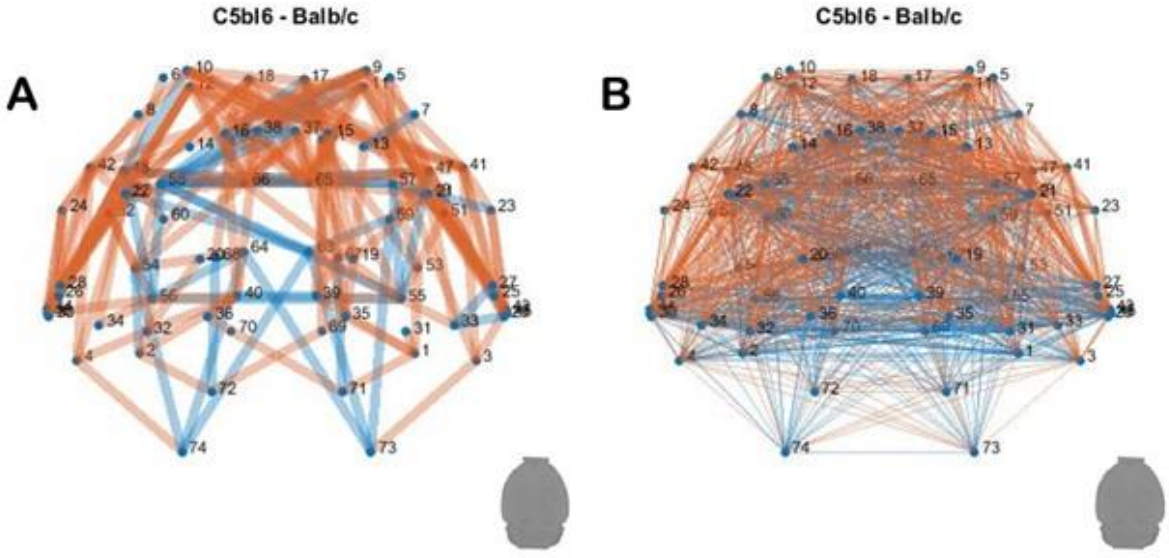

Figure 2. Binary connectome (A) and weighted connectome (B). Red indicates stronger connectivity in Balb/c compared to C57bl6 and blue represents stronger connectivity in C57bl6 compared to Balb/c. The narrowing of intrahemispheric connections in Balb/c mice leads to a completely different structural connectivity of cortical areas compared to that of C57BL/6 mice.

Disclosures: D. Szczupak: None. **C.C. Yen:** None. **C. Liu:** None. **S. Choi:** None. **F. Meireles:** None. **C. Victorino:** None. **A.C. Silva:** None. **R. Lent:** None. **F.F. Tovar-Moll:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.04/A4

Topic: A.01. Neurogenesis and Gliogenesis

Support: grants from National Key Basic Research Program of China
National Natural Science Foundation of China

Title: A3 regulates self-renewal maintenance of neural progenitors

Authors: *M. OU, Z.-G. LUO
Inst. of Neuroscience, Chinese Acad. of Scie, Shanghai city, China

Abstract: Gyrencephaly is a unique structure found in limited species including human beings. Since the expansion of neocortex providing the structure basis for much more neurons to form elaborate cortical network, it is of importance to investigate the underlying mechanism of cortex folding conformation. According to previous exploration, researchers have found that outer subventricular zone(OSVZ) is specially appears in species with complex neocortex. Further observations strengthen that outer radial glial cells may be a major source of neocortex expansion. Here, we suggest that A3 is involved in stemness maintenance of progenitor pool by adjusting mitotic process. At the level of mRNA expression, A3 is highly expressed in OSVZ human cortex detected by RNA sequencing. Consistently, in situ hybridization probe of A3 was enriched in VZ/SVZ during mouse early development. Applying in-utero electroporation, in vivo overexpression A3 yielded the potent increase of the number of proliferative progenitors. Meanwhile, A3 deficiency after RNAi causes neurogenesis defect both in vitro and in vivo assays. Furthermore, in several loss-of function experiments via CRISPR/Cas9-mediated genome editing, A3 knockdown in mouse perturb progenitors pool with mitotic delay and chromatin structure disrupted. A3 deletion mice showed deficient cortical thickness comparing with their wildtype littermate. Immunostaining displayed that projection neurons sitting in deeper layers of the cortex with Ctip2 (layer V) was diminished. Base on the potential function of A3 and related observation, our work propose that A3 plays a role in maintaining the neural progenitors and reveal the impact of mitosis delay on self-renew and development potency in corticogenesis.

Disclosures: M. Ou: None. **Z. Luo:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.05/A5

Topic: A.07. Developmental Disorders

Title: Constitutively active MEK1 signaling drives selective death of cortical parvalbumin-expressing GABAergic interneurons in mouse embryonic brain development

Authors: *M. HOLTER, G. R. BJORKLUND, S. SHAH, K. NISHIMURA, J. NEWBERN
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Abstract: Cortical GABAergic interneurons are a crucial population of inhibitory cells that comprise approximately 20% of the total cortical neuron population and can be identified by numerous distinct genetic profiles, morphological characteristics, firing properties, and patterns of connectivity. Cortical GABAergic interneuron deficits have been linked to several human neurological disorders including autism spectrum disorder, schizophrenia, epilepsy, and more recently, the RASopathies. The RASopathy family of neurodevelopmental disorders arise from perturbations of RAS/MAPK signaling and often result in a variety of neurological abnormalities. However, it is unclear how altered RAS/MAPK signaling affects the trajectory of cortical GABAergic interneuron development. To address this question, we generated mice expressing the GABAergic neuron-specific VGAT:Cre recombinase to selectively target a Cre-dependent caMEK1 (*Mek1*$^{S217/222Q}$) mutation to all GABAergic cells. Adult VGAT:Cre caMEK1 mutant mice displayed a noticeable reduction in total cortical GABAergic interneuron number in comparison to controls. Inspection of GABAergic interneuron subtypes revealed a selective reduction in parvalbumin-expressing GABAergic interneurons with no changes in somatostatin-expressing GABAergic interneuron number. We detected similar reductions in the proportion of parvalbumin interneurons in a separate MGE-derived mutant mouse line using Nkx2.1:Cre. Upon further investigation, we found that some nascent caMEK1-expressing GABAergic interneurons located in the subpallial mantle zone exhibit increased activated-caspase-3 labeling and apoptotic features. This suggests that early cell death is a key mechanism driving reduced cortical parvalbumin interneuron number in adult mutants. Previous work has shown that RASopathy mutations alter the expression and secretion of extracellular matrix components from astrocytes. Preliminary evidence shows that mutated GABAergic interneurons may also contribute to differences in perineuronal net formation as assessed by immunohistochemistry and GABAergic-specific RIPseq. Overall, these data implicate RAS/MAPK signaling in early parvalbumin interneuron cell death and the subsequent formation of cortical circuitry.

Disclosures: M. Holter: None. **G.R. Bjorklund:** None. **S. Shah:** None. **K. Nishimura:** None. **J. Newbern:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.06/A6

Topic: A.01. Neurogenesis and Gliogenesis

Support: Dart Neuroscience, LLC
EY011261
EY027437
EY019005

Title: Repeated exposure to brief periods of enhanced visual experience rehabilitates the injured brain in xenopus tadpoles

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

Abstract: Traumatic brain injuries introduce functional and structural circuit deficits that must be repaired for an organism to regain function. In *Xenopus laevis* tadpoles, as in other systems, injury induces neurogenesis and the newly-generated neurons then integrate into the existing circuit, however, the mechanisms governing this integration are poorly understood. We developed an injury model in which tadpoles are given a penetrating stab wound which damages the optic tectal circuit and impairs visuomotor behavior. Development of visuomotor circuit function in *Xenopus* is driven by sensory activity. We tested whether providing enhanced visual experience affects circuit recovery from injury. We found that providing animals with brief periods of enhanced visual stimulation starting 24 hours after injury increased synaptic inputs and circuit integration of newly generated neurons, and sped behavioral recovery. To investigate mechanisms of activity-mediated recovery from injury, we interfered with NMDA receptor function. Ifenprodil, which blocks GluN2B subunit containing NMDA receptors impaired dendritic arbor elaboration. GluN2B knockdown blocked functional integration of neurons generated in response to injury and prevented behavioral recovery. We conclude sensory activity mediated by GluN2B-containing NMDARs mediates structural and functional recovery of the tectal circuit following injury in *Xenopus* tadpoles.

Disclosures: A.C. Gambrill: None. **R.L. Faulkner:** None. **C.R. McKeown:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.07/A7

Topic: A.07. Developmental Disorders

Support: NIH Grant R01DE022830

Title: Pak1ip1 gene mutation results in neural crest-dependent developmental defects

Authors: *A. DE CRESCENZO, A. A. PANOUTSOPOULOS, A. LEE, K. S. ZARBALIS
Pathology and Lab. Med., Univ. of California Davis, Davis, CA

Abstract: Neural crest cells (NCCs) appear early in development during neurulation and are required for proper histogenesis of a variety of tissues and organs that importantly, include the peripheral nervous system and structures of the craniofacial skeleton. NCCs have also recently been the center of attention in stem cell-based research due to their regenerative multipotent abilities. Important developmental aspects of NCCs generation, migration, and differentiation and their subsceptibilty to gene mutation remain unresolved debilitating deficits that arise from abnormalities of these processes, Here, we report on a mutant mouse with a missense mutation in the Pak1ip1 gene, which encodes a protein necessary for 60S ribosomal subunit formation. The homozygous mutant of this line showed severe developmental defects, including orofacial cleft affecting palate and maxillae, cranial nerve maldevelopment, generalized hypoplasia, and embryonic lethality. Analyzing the rate of proliferation/cell death in the wild-type vs mutant, we found that the apparent craniofacial phenotypes were generated by a loss of NCC. The TUNEL assay, assessing cell death, confirmed that the rate of cell death in mutants is higher than that of the wild-type, while a pHH3 immunofluorescent analysis, targeting currently dividing cells, showed a deep decrease in proliferative activity in the mutant when compared to the normal condition. Based on these findings, we propose an explanation for the craniofacial defects seen in the mutants involving the loss of NCCs during early development. According to our hypothesis, the low numbers of NCCs would prevent them from reaching the farthest region from their origin, which are incidentally the frontonasal prominences. As Pak1ip1 is pivotal for proper ribosome activity, its functional loss would most likely translate in nuclear stress and subsequent Tp53 up-regulation. Therefore, we analyzed the expression levels of Tp53 and registered, in the mutant, an increased Tp53 activity and G1 cell cycle arrest in neuroepithelial cells, which give rise to the neural crest. Our findings illustrate that the developmental abnormalities observed in the Pak1ip1 mutants are predominantly based on the specific loss of NCCs during development and point towards pharmacological or genetic Tp53 interference as a potential rescue strategy for Pak1ip1 loss-of-function.

Disclosures: A. De Crescenzo: None. **A.A. Panoutsopoulos:** None. **A. Lee:** None. **K.S. Zarbalis:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.08/A8

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH EY011261
Dart NeuroScience, LLC
Hahn Family Foundation

Title: Nutrient restriction causes reversible G2 arrest in xenopus neuronal progenitors

Authors: *C. R. MCKEOWN, H. T. CLINE
The Scripps Res. Inst., La Jolla, CA

Abstract: Nutrient status affects the developing brain, yet the effect of nutrient restriction and food availability on a cellular level *in vivo* is poorly understood. In the absence of external nutrients, *Xenopus laevis* tadpoles enter a period of developmental stasis during which neural progenitor cell proliferation is drastically reduced, with proliferation synchronously resuming when food becomes available. Here we investigate the mechanisms by which neural progenitors halt cell division in response to nutrient restriction and then re-enter the cell cycle upon feeding. We demonstrate that nutrient restriction causes tectal progenitors to stop progression through the cell cycle after S phase, and that the reintroduction of nutrients triggers progenitors to synchronously re-enter the cell cycle at M-phase, suggesting cells in stasis are paused at G2. Consistent with a model for G2 arrest, we find that levels of phosphorylated cdc2 are decreased upon stasis entry and return upon the resumption of feeding. We demonstrate that progenitors along the tectal midline have increased DNA content in response to nutrient restriction, further supporting a G2 arrest model. We also show that initiation of the nutrient-restriction-induced G2 pausing is rapamycin-insensitive, but cell cycle re-entry requires mTOR signaling. This capacity of neural progenitors to pause cell cycle progression in G2 provides a mechanism to control proliferation in response to nutrient availability and yet allows cells to be poised to divide quickly when nutrients become available. This may be a general cellular mechanism that allows for developmental flexibility during times of limited resources.

Disclosures: C.R. McKeown: None. **H.T. Cline:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.09/A9

Topic: A.01. Neurogenesis and Gliogenesis

Support: A grant from the Ministry of Education, Culture, Science, Sports and Technology of Japan (16H06829)

Title: Developmental changes in Gpnmb expression in the prenatal rat brain

Authors: *S. YOKOYAMA[1,2], H. ZHU[2]
[1]Kanazawa Univ., Kanazawa, Japan; [2]Res. Ctr. for Child Mental Develop., Kanazawa, Japan

Abstract: The glycoprotein non-metastatic melanoma B (Gpnmb), a type-I transmembrane protein, is produced by various types of normal cells including melanocytes, osteoclasts, osteoblasts, and dendritic cells in peripheral blood, as well as by various tumor cells. An increasing number of studies have described that Gpnmb is abundantly expressed in invasive glioblastomas, suggesting its involvement in tumor progression and metastasis. Previously we reported that Gpnmb is produced by macrophages and microglia in the normal central nervous system of postnatal and adult rats (Huang, J.-J. *et al.*, Brain and Behavior 2, 85-96, 2012; Yokoyama, S. Soc. Neurosci. Abstr., 674.14, 2016) and by cells in the choroid plexus epithelium, ventricular-subventricular zone and neocortex in the embryonic rat brain (Yokoyama, S. and Zhu, H. Soc. Neurosci. Abstr., 197.14, 2017). The purpose of this study was to define more in detail these Gpnmb-immunoreactive (IR) cells in the embryonic brain. At E10, Gpnmb-IR was only faintly detected in the ventricular wall. At E13, Gpnmb-IR cells were present in the lateral ventricle wall, extending radial fibers from ventricular zone to pial surface. These Gpnmb-IR cells were positive for nestin and vimentin, markers for radial glial cells. At E16 and E19, regional difference in Gpnmb-IR became prominent. Gpnmb-IR was predominantly distributed in the choroid plexus of the lateral and third ventricle; the soma of the Gpnmb-IR cells migrated to the subventricular zone. These Gpnmb-IR cells were frequently co-stained with specific markers including Sox2 for neural stem cells, doublecortin for neuroblasts, and bromodeoxyuridine for cell proliferation, as well as radial glia markers. These data suggest that Gpnmb is involved in the neural development in the embryonic brain.

Disclosures: S. Yokoyama: None. **H. Zhu:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.10/A10

Topic: A.07. Developmental Disorders

Support: Sharon Stewart Aniridia Research Trust
ARCS Foundation

Title: Consequences of heterozygous loss-of-function mutations to PAX6 in the adult mammalian brain

Authors: *M. K. GRANT[1], A. M. BOBILEV[3], A. M. RASYS[1], A. E. BRANCH[4], J. B. BYERS[1], H. SCHRIEVER[1], K. HEKMATYAR[2], J. D. LAUDERDALE[1]
[1]Cell. Biol., [2]Bio-Imaging Res. Ctr., Univ. of Georgia, Athens, GA; [3]Psychiatry, Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [4]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Aniridia is a congenital and progressive disorder affecting approximately 1 in 83,000 live births. Although the disorder is most well known for its ocular phenotypes, the condition has a several other abnormalities, which are only recently emerging as prominent features of the disorder. These include neural, sensory, cognitive, and auditory processing deficits. Development of aniridia in humans is predominately caused by heterozygous loss-of-function mutations in the *PAX6* gene, a highly conserved transcription factor critical for normal eye and brain development. PAX6 has been implicated in aspects of central nervous system development such as patterning, regionalization, and the formation of neural circuits; however, PAX6's role in the adult brain have yet to be described. Our lab has utilized 3T MRI to show structural changes in the brains of aniridia patients as compared to their *PAX6*-normal comparisons. Consistent with other reports, we found reductions to major fiber tracts such as the anterior commissure, posterior commissure, and optic chiasm in addition to lack of or reduction to the pineal gland. The cellular basis for these changes are not well understood, so we have turned to the rodent model of aniridia, *Small eye*, where we can utilize a variety of tools to assess *Pax6* expression and the neural consequences of mutations in the brain. The current study employed MRI using a 7T Agilent system to acquire structural brain images using 3D T2 weighted fast spin echo sequences, volumetric analysis, histological examination, *Pax6* transgenic mouse lines, and tissue clearing of the adult brain to examine the consequences of loss of one functional copy of the *Pax6* gene. Results indicate that while our rodent model recapitulates certain structural changes seen in our human population such as the optic chiasm, it does not capture all of the structural changes seen in our aniridia patients. Our results suggest that within our human population there are potentially modifier effects contributing to the structural brain changes we

see. We are currently using the whole tissue clearing method, iDISCO, to help us better understand the role PAX6 plays in the adult brain and the consequences of heterozygous loss-of-function of this gene. Collectively, these data allow us to visualize the overlap between adult *Pax6* expression and structural brain variants, and provide new hypotheses regarding the effects of early versus adult *PAX6* haploinsufficiency in the mammalian brain. Implementation of this approach also provides a novel platform for investigating the link between gene expression and neural structure and connectivity, with broad applications for neurogenetic research.

Disclosures: M.K. Grant: None. **A.M. Bobilev:** None. **A.M. Rasys:** None. **A.E. Branch:** None. **J.B. Byers:** None. **H. Schriever:** None. **K. Hekmatyar:** None. **J.D. Lauderdale:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.11/A11

Topic: A.01. Neurogenesis and Gliogenesis

Support: University of Iowa OVPRED
Iowa Neuroscience Institute

Title: Cortical and cerebellar neurodegeneration in the absence of the nuclear protein Akirin2

Authors: *S. L. PEEK[1], J. A. WEINER[2]
[2]Iowa Neurosci. Inst., [1]Univ. of Iowa, Iowa City, IA

Abstract: The role of chromatin remodeling machinery in neurodevelopment is becoming increasingly more apparent. Akirin2 (Aki2) is a highly conserved nuclear protein believed to act as an intermediary between transcription factors (e.g., NFκB, Twist) and BAF (SWI/SNF) chromatin remodelers with roles in immunity and myogenesis. Although Aki2 is expressed prominently in neuronal progenitors, postmitotic neurons, and astrocytes, its function in the brain was, until recently, entirely unexplored. Using conditional *Aki2* knockout mice, our laboratory has shown that restricted loss of Aki2 in early cortical progenitors results in disrupted proliferation, aberrant neuronal differentiation, and massive apoptosis (Bosch et al., *Neural Development,* 2016). The role of Aki2 in *postmitotic* neurons in the postnatal brain, and the molecular mechanisms through which it regulates neuronal development and maturation, remain unknown. To test the hypothesis that Aki2 regulates patterns of gene expression critical for the maturation, maintenance, and survival of postmitotic neurons, we utilized *CaMKII-Cre* and *Pcp2-Cre* mouse lines to delete the *Aki2* gene from excitatory neurons of the forebrain or postmitotic Purkinje cells of the cerebellum, respectively. In *CamKII-Cre;Aki2*$^{fl/fl}$ mice, Aki2 expression is lost from excitatory cortical neurons by ~P18. By P50, the cortex is significantly thinner in knockout mice, with fewer neurons and reduced dendrite arborization. Expression of

GFAP is significantly increased, likely indicative of reactive gliosis. By P150, *CamKII-Cre;Aki2*$^{fl/fl}$ mice are significantly smaller than control littermates and cortical layers are severely thinned, with evidence of neurodegeneration. In *Pcp2-Cre;Aki2*$^{fl/fl}$ mice, Aki2 expression is lost from Purkinje cells at ~P6. Purkinje cell axon degeneration is already apparent 4 days after *Aki2* loss. At P35, there are fewer Purkinje cell somata, the molecular layer, containing Purkinje cell dendrites, is thinner, and Bergmann Glia also upregulate GFAP. *Pcp2-Cre;Aki2*$^{fl/fl}$ mice develop a tremor by P30 that progresses with age. Together, these data indicate crucial roles for Aki2 in the maturation and survival of postmitotic neurons. Given Aki2's role in regulating specific patterns of gene expression, current efforts are focused on generating transcriptomic data from knockout cortical and cerebellar neurons in order to identify downstream molecular mechanisms.

Disclosures: S.L. Peek: None. **J.A. Weiner:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.12/A12

Topic: A.03. Stem Cells and Reprogramming

Support: DARPA Contract # HR0011-17-C-0026

Title: Nervous system plasticity and regeneration in Hydra

Authors: *A. S. PRIMACK, S. SIEBERT, C. JULIANO
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Abstract: In the small freshwater cnidarian *Hydra,* all differentiated cells in the homeostatic adult animal are replaced every 12-20 days, including the entire nervous system. *Hydra* is also able to regenerate its nervous system following catastrophic injury. Our ultimate goal is to understand the general principles of *Hydra* neural plasticity in both homeostatic and regenerative conditions. As a first step towards this goal, we are using single cell RNA-sequencing (scRNA-seq) to build a complete molecular map of the *Hydra* nervous system. We have thus far identified eight neuron subtypes with unique molecular signatures and are mapping the location of these subtypes in the *Hydra* nervous system using in situ hybridization. Based on our preliminary scRNA-seq data and published literature, we hypothesize that continual renewal of the *Hydra* nervous system under homeostatic conditions is accomplished by a combination of two mechanisms: 1) specification of new neurons from stem cells (neurogenesis) and 2) transdifferentiation between neuron subtypes. In our future work, we aim to use our scRNA-seq data to identify and test transcription factors unique to neurogenesis and transdifferentiation, thus gaining insight into the regulatory control of nervous system plasticity. Additionally, we plan to build transgenic reporter lines to quantify the number of neurogenesis and transdifferentiation

events that occur during both homeostatic maintenance and regeneration of the nervous system. Through these exploratory studies, we hope to elucidate the molecular mechanisms that underlie neuronal plasticity and regeneration.

Disclosures: A.S. Primack: None. **S. Siebert:** None. **C. Juliano:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.13/A13

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH
The Zvi and Ofra Meitar Family Fund

Title: Early migratory neurons of the olfactory placode, the oral ectoderm, and the cephalic epithelium adjacent to the forebrain

Authors: *I. BYSTRON
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Abstract: A variety of studies have already highlighted cellular and molecular differences between human brain development and that of other mammals. At present, it is universally accepted that the early cephalic mesenchyme contain migratory neurons produced from progenitor cells in the neurogenic placodes and the neural crest, in all mammalian species. We demonstrate that the human cephalic epithelium adjacent to the forebrain contain hitherto unrecognized stem cell niches generating precocious migratory neurons, which are distinct from the pioneer neurons of the olfactory placode, and the neurons of the neural crest origin. Human embryos from Carnegie stages (CS) 10-17 (29-41 days post-conception) were obtained from the Human Developmental Biology Resource UK. We used a number of cell-specific and proliferative markers to reveal the phenotypic characteristics and migratory pathways of the first neurons in the cephalic ectoderm and mesenchyme. We developed a new approach to reconstruct cells in sections of the human ectoderm, diencephalon, cortical wall, and retina by rapid, high-resolution volume rendering of multichannel 3D confocal data sets from a Zeiss LSM 710 confocal microscope. The majority of precocious TU-20-positive ectodermal neurons appear to migrate tangentially within the ectoderm, and some delaminate from the epithelium to coalesce within the mecenhyme surrounding the rostral telencephalon. Intriguingly these neuronal populations form the first connections between the several regions of the embryonic telencephalon and the early cephalic ectoderm. Some neurons migrate into periocular mesenchyme and extend non-axonal processes through the prospective pigment epithelium into the neural retina. Others invade the presumptive cortical wall perhaps providing additional

signalling information to the local stem cell niche. The onset of local neurogenesis in ventral diencephalon presides the generation of neurons within the ectoderm at the roof of the future oral cavity. The fibers of the neurons located in the oral ectoderm form a dense network along the basement membrane adjacent to the ventral hypothalamus by CS13. Pioneer olfactory neurons constitute a distinct migratory population at CS 13-14. Their processes penetrate the rostro-ventral cerebral wall by CS17.
Thus the human cephalic epithelium adjacent to the forebrain contain hitherto unrecognized stem cell niches generating precocious neurons with distinct migratory routes.
Supported by The Zvi and Ofra Meitar Family Fund. The human embryonic and fetal material was provided by the Joint MRC / Wellcome Trust (grant # 099175/Z/12/Z) Human Developmental Biology Resource.

Disclosures: I. Bystron: None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.14/A14

Topic: A.01. Neurogenesis and Gliogenesis

Support: R01MH080434
R01MH078972
R21NS095632
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UW Vilas Trust
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Title: FMRP regulates adult neural stem cell maintenance

Authors: *X. ZHAO[1], Y. LI[2], M. E. STOCKTON[2], Y. ZHAO[2], J. L. MILLER[2], I. BHUIYAN[2]
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Abstract: Fragile X syndrome (FXS) is the most prevalent inherited intellectual disability, resulting from a loss of fragile X mental retardation protein (FMRP). FXS patients suffer lifelong cognitive disabilities, but the function of FMRP in the adult brain and the mechanism underlying age-related cognitive decline in FXS remain unclear. We have previously shown that FMRP deficiency leads to aberrant activation of neural stem cells residing in the hippocampus of young adult mice leading to impaired cognitive deficits. Here we investigated whether over-activation of neural stem cells lead to stem cell depletion in older mice. We found that that in mature adult (6 month old) FMRP- deficient mice, there is a significant reduction in the numbers of adult

neuronal stem cells leading to reduced new neuron production and cognitive deficits. Our work reveals an important role for FMRP in adult neural stem cell maintenance and present a potential novel therapeutic strategy for treating mature adult FXS patients.

Disclosures: X. Zhao: None. **Y. Li:** None. **M.E. Stockton:** None. **Y. Zhao:** None. **J.L. Miller:** None. **I. Bhuiyan:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NRF grant 2017R1A2B4004289
the Korea Brain Research Institute basic research program (18-BR-04)
the DGIST Convergence Science Center Program (18-BD-04) of the Ministry of Science, ICT and Future Planning of Korea

Title: Autophagy mediates astrogenesis of adult hippocampal neural stem cells

Authors: *S.-H. JEONG, S. HA, K. YI, J. J. CHU, S.-W. YU
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Abstract: Neural stem cells (NSCs) have the ability to self-renew and differentiate into neurons, oligodendrocytes and astrocytes. Highly dynamic nature of NSC differentiation requires the intimate involvement of catabolic processes, such as autophagy. Autophagy is a major intracellular degradation pathway for cellular homeostasis and remodeling. Autophagy is important for mammalian development and its role in neurogenesis has recently drawn much attention. However, little is known how autophagy is associated with differentiation of NSCs into other neural lineages. Here, we report that autophagy plays a critical role for adult astrogenesis. Autophagy flux increased at the early time points, but then returned to the normal level during differentiation of adult hippocampal neural stem (HCN) cells into astrocytes. Genetic suppression of autophagy by stable knockdown of Atg7 or CRISPR-cas9-mediated knockout (KO) of p62 impaired astrogenesis, while reintroduction of p62 recovered astrogenesis in p62 KO HCN cells. Taken together, our findings demonstrate that autophagy plays a key role in astrogenesis of adult NSCs.

Disclosures: S. Jeong: None. **S. Ha:** None. **K. Yi:** None. **J.J. Chu:** None. **S. Yu:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.16/A16

Topic: A.01. Neurogenesis and Gliogenesis

Support: MSU Alliaance Funds

Title: Functional *in vivo* screen of TSC1&2 missense mutants associated with ASD in cortical GABAergic interneurons

Authors: D. WUNDRACH, S. M. BILINOVICH, A. M. STAFFORD, D. B. CAMPBELL, J. W. PROKOP, *D. VOGT
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Abstract: The genes underlying symptoms of Tuberous sclerosis (TS), TSC1 and TSC2, have been assessed for mutations over several years. Notably, many of these mutations are missense and of unknown impact. We wanted to understand the functional impact of these mutations in the human TSC1 and 2 genes and their effect on their encoded proteins, Hamartin and Tuberin, respectively. Hamartin and Tuberin form an obligatory complex that indirectly inhibits the activity of the mammalian target of rapamycin complex 1 (mTORC1). Moreover, mTORC1 activity has been implicated as a biological hub for many autism spectrum disorder (ASD) risk genes, and in turn, a TS diagnosis has a high comorbid rate of ASD. Herein, we will test the impact that missense mutations discovered in TSC1 and 2, with a co-diagnosis of ASD, have on protein function. We are currently assessing these mutations through cellular and biochemical assays. In addition, we are performing these experiments in cortical GABAergic interneurons, a cell type altered by deletion of Tsc1, and a likely cell type involved in TS pathogenesis. We have also discovered novel phenotypes regulated by Tsc1 in mouse cortical GABAergic interneurons and will explore the role of these mutations in the respective phenotypes. In addition to the cellular and biochemical assessments, we will ascertain how human TSC1 and 2 mutations impact the cellular morphology and molecular identity of this important cell group. Overall, these data have the potential to uncover novel signaling mechanisms that lead to symptoms in TS and broaden our understanding of how human missense mutations impact the function of Hamartin and Tuberin.

Disclosures: D. Wundrach: None. **S.M. Bilinovich:** None. **A.M. Stafford:** None. **D.B. Campbell:** None. **J.W. Prokop:** None. **D. Vogt:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.17/A17

Topic: A.01. Neurogenesis and Gliogenesis

Support: DAAD

Title: Efficient generation of dopaminergic neurons by transdifferentiation of cortical GABAergic neurons

Authors: *A. RAINA, S. U. MAHAJANI, M. BAEHR, S. KUEGLER
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Abstract: Objective: The generation of functional dopaminergic neurons from fibroblasts and induced pluripotent stem cells (iPSCs) is tedious and time-consuming, therefore the objective was to efficiently generate dopaminergic neurons by transdifferentiation of readily available primary post-mitotic cortical neurons using transcription factors ASCL1, NURR1, LMX1A, and PITX3. **Methods:** Adeno-Associated Viral vectors were used to deliver each transcription factor alone and in different combinations in primary cortical neurons. 150,000 cells were seeded in each well of 24 well plates, transduced at day in vitro 0, and examined by immunocytochemistry for tyrosine hydroxylase (TH), a mature dopaminergic neuronal marker, 7 days after transduction. **Results:** We demonstrated that dopaminergic neurons can be efficiently generated in 7 days *in vitro* (div) by transdifferentiation of post-mitotic neurons. Overexpression of ASCL1, NURR1, and LMX1A together yielded 5-10% (12-18% in 14 div) of TH positive neurons, whereas ASCL1 alone, LMX1A alone and both of them together did not yield any TH positive neurons. NURR1 alone yielded 2% of TH positive neurons. A combination of LMX1A and NURR1, and ASCL1 and NURR1 yielded 2-5% of TH positive neurons. While only a sub-set of cortical neurons were transdifferentiated, it was found that 53-63% of GABAergic neurons were TH positive when NURR1 alone was overexpressed. LMX1A, NURR1, and PITX3 were functional as each of them when expressed in human iPSCs were able to pattern and differentiate human iPSCs into TH positive neurons, acting as a control system. In summary, results suggested that NURR1 was found to be a critical transcription factor in combination with ASCL1 and LMX1A for transdifferentiation of post-mitotic GABAergic neurons into dopaminergic neurons. **Conclusion:** Efficient generation of dopaminergic neurons from post-mitotic GABAergic neurons is the first ever evidence of transdifferentiation of terminally differentiated neuronal cells.

Disclosures: A. Raina: None. **S.U. Mahajani:** None. **M. Baehr:** None. **S. Kuegler:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.18/A18

Topic: A.01. Neurogenesis and Gliogenesis

Title: The role of ECE2 in the human brain developmental disorder periventricular heterotopia

Authors: *I. Y. BUCHSBAUM[1,2], G. GIORGIO[3], C. KYROUSI[1], A. O'NEILL[4,5], S. R. ROBERTSON[5], S. CAPPELLO[1,2]
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Abstract: Malformations of the human neocortex, such as periventricular heterotopia (PH), result from disturbances in the tightly regulated processes of brain development. In PH, new-born neurons fail to migrate to their destined place, leading to malpositioned grey matter along the lateral ventricles. Only mutations in a few genes (FLNA, ARFGEF2, FAT4, DCHS1) are known to cause this neuronal migration disorder in humans. To identify new molecular pathways involved in brain development and to find novel genes involved in PH, trio-based Whole Exome Sequencing was performed. Here, we concentrate on the identified candidate gene endothelin converting enzyme-2 (ECE2).
Mouse models carrying mutations in genes identified in patients with cortical malformation only partially recapitulate expected cortical phenotypes. Thus, in addition to manipulating the expression of the candidate gene in the developing mouse brain, alternative model systems are needed that are more similar to the developing human brain. Induced pluripotent stem cells derived from human somatic cells (hiPSCs) were used to generate neural precursor cells and neurons (2D, [Boyer *et al.*, 2012]), as well as cerebral organoids (COs, 3D, [Lancaster *et al.*, 2013]) as model systems for the developing human brain.
In our *in vitro* human model systems, we identified ECE2 to be expressed in vesicles of both neural progenitor cells and, at a higher level, in neurons. Global pharmacological inhibition of ECE2 activity in 2D caused a change in the dynamics of young migrating neurons, whereas its acute knockdown (KD) or overexpression had only mild effects. This hints at the involvement of mostly non-cell-autonomous mechanisms. Upon inhibition of ECE2 in COs, the production of neurons was reduced and radial glia (RG) polarity was disturbed. Acute KD of ECE2 by electroporation into ventricles of COs led to an increased amount of heterotopic neurons in the vicinity of electroporated radial glia cells, partially recapitulating the patient morphological phenotype. Thus, ECE2 expression may be necessary for correct morphology of radial glia, which are used as scaffold for neuronal migration to the cortical plate.

Accordingly, acute KD of Ece2 in the developing mouse brain, lead to partial delamination of progenitor cells, giving rise to ectopic neuronal cluster formation.
Altogether, new candidate genes for neurodevelopmental disorders can be identified by whole exome sequencing and studied by combining *in vitro* human models and *in vivo* animal models. COs can be used to recapitulate patient phenotypes and to decipher pathogenic mechanisms based on both disturbed RG scaffold and malfunctional neuronal migration.

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Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

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Program #/Poster #: 550.19/A19

Topic: A.01. Neurogenesis and Gliogenesis

Support: PRIN 2015-2015W729WH_005 to ATP and GDC

Title: Herpes simplex type-1 (HSV-1) infection inhibits adult hippocampal neurogenesis in mice via amyloid-β protein (Aβ) accumulation

Authors: *D. D. LI PUMA[1], R. PIACENTINI[1], L. LEONE[1], K. GIRONI[1], M. E. MARCOCCI[2], G. DE CHIARA[3], A. T. PALAMARA[4,2], C. GRASSI[1]
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Abstract: In previous studies we reported that HSV-1 infection of cultured neurons induced the proteolytic processing of Amyloid Precursor Protein (APP) and intracellular accumulation of amyloid-β protein (Aβ) thus impairing synaptic transmission (Piacentini&Li Puma et al., 2015). Here we investigated the effects of HSV-1 on proliferation and differentiation of hippocampal neural stem cells (NSCs) isolated from newborn mice and the role played by Aβ in these effects. We found that HSV-1 infection (0.5 MOI) induced Aβ accumulation in cultured NSCs and reduced their proliferation (-30% *vs*. mock-infected NSCs, 24 h post-infection, assessed by Ki67 expression and BrdU incorporation; $P<0.05$). Infected NSCs also exhibited decreased neuronal differentiation, evaluated by MAP2 immunoreactivity. Indeed, HSV-1 infection reduced the percentage of $MAP2^+$ NSCs by 35%, 42% and 38% ($P<0.05$ *vs*. mock-infected cultures) after 3, 6 and 9 days of differentiation, respectively. Accordingly, the percentage of NSCs expressing the glial marker GFAP increased ($P<0.05$ *vs*. mock-infected cultures). The HSV-1-induced inhibition of NSC proliferation and differentiation depended on Aβ accumulation as

demonstrated by the reversion of these effects after treatment of infected NSCs with 4G8 antibody or β- and γ- secretase inhibitors. We then extended our studies to an *in vivo* model of recurrent virus infections. HSV-1 was inoculated in 1-month old C57/bl6 mice by snout abrasion in order to induce latency and virus was subsequently reactivated twice by thermal stress at 1-month intervals. One week before sacrifice mice were injected with BrdU and hippocampal neurogenesis was studied by immunohistochemistry. The number of $BrdU^+$ cells and newly generated neurons (i.e., cells positive for both BrdU and the neuronal marker doublecortin, DCX) was significantly reduced in the dentate gyrus (DG) of HSV-1-infected mice (-28% and -48%, respectively *vs*. mock; $P<0.05$). Instead, hippocampal neurogenesis was not altered in the DG of HSV-1-infected APP KO mice thus confirming the involvement of Aβ in HSV-1-driven effects on NSCs. Western blot (WB) experiments investigating DCX and NeuroD1 protein expression confirmed immunohistochemical data. The reduction of proliferation and differentiation of virus-infected NSCs observed both *in vitro* and *in vivo* did not depend on cell death, as assessed by Vybrant apoptosis assay and WB analysis of BAX/Bcl2 expression. Collectively, our results demonstrate that HSV-1 infection reduces hippocampal NSC proliferation and their neuronal differentiation via intracellular accumulation of Aβ.

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Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.20/A20

Topic: A.01. Neurogenesis and Gliogenesis

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Title: Physiological maturation and network integration of non-proliferative neuronal precursors in the adult murine piriform cortex

Authors: *B. BENEDETTI[1,2], R. KÖNIG[1,3,2], D. DANNEHL[5,1,2], C. KREUTZER[1,2], M. BELLES[6], M. RITTER[4], T. M. WEIGER[7], J. NACHER[6], M. ENGELHARDT[5], L. AIGNER[3,2], S. COUILLARD-DESPRES[1,2]
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Abstract: In the adult murine piriform cortex, non-proliferative neuronal precursors express the protein doublecortin (DCX) and eventually become mature neurons. The physiology of these cells and their relevance for brain functions are unknown. Here we investigated the neuronal precursors' structural and functional maturation questioning whether they eventually become equivalent to other principal neurons of this brain region. Patch clamp experiments and morphometric analysis of axon initial segment were carried out in acute brain slices and histological sections respectively. Accordingly, the fate of precursors was traced in transgenic mice (DCX/dsRED and DCX-CreRT::CAG-fl/eGFP) where these cells can be fluorescently labeled, studied throughout maturation and compared to other age-matched principal neurons. Young precursors (tangled cells) and immature neurons (young complex cells) were analyzed in 2 - 4 months old mice, and more mature (old) complex cells were analyzed in 4 - 8 months old mice. Tangled cells were small, virtually received no synaptic input and produced scarce action potential. Young complex cells received sparse synaptic input, produced low action potential frequencies, had small capacitance, small inward and outward currents and slow action potential kinetics. Young complex cells were in this respect similar to early postnatal (P03 - 04) immature principal neurons. Old complex cells displayed increased amount of synaptic input, larger capacitance, larger inward and outward current, and sharper action potential kinetics, but retained low action potential firing frequencies and developed a remarkably high rheobase, implying limited excitability. Furthermore, the axon initial segment of old complex cells was shorter than that of other aged-matched principal neurons. Strikingly, while principal neurons typically received a mixed glutamatergic and GABAergic synaptic input, gabazine completely blocked postsynaptic currents in complex cells of any age spontaneous suggesting exclusive GABAergic input. On one hand, the odd functional features of complex cells challenge their relevance for the adult brain; on the other, their unique features suggest that these cells are new coding elements in the piriform cortex rather than the simple replacement or addition of homologous coding units to the preexisting network components.

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Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

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Program #/Poster #: 550.21/A21

Topic: A.01. Neurogenesis and Gliogenesis

Support: Danish Council for Independent Research Grant DFF-7017-00071
Innovation Fund Denmark Grant BrainStem 4108-00008B

Title: Anatomical and molecular characterization of the developing entorhinal cortex neuronal circuit in the pig

Authors: *Y. LIU[1], T. B. BERGMANN[1], J. M. P. VIDAL[1], Y. MORI[2,3], M. PIHL[1], P. D. THOMSEN[1], P. HYTTEL[1], K. MØLLGÅRD[4], M. P. WITTER[5], V. J. HALL[1]
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Abstract: The entorhinal cortex (EC) acts as a gateway for information traveling in and out of the hippocampal formation and is important for spatial memory. The stellate cell (SC) resides in the medial EC, projects to the dentate gyrus and contributes to both the grid and border cell phenotypes. However, very little is known about the molecular identity of SCs. SCs express reelin (RELN+) and calbindin negative (CALB1-) and this differentiates them from the other main principle neurons. The aim of our project is to develop novel differentiation protocols to produce SCs from induced pluripotent stem cells (iPSCs). We therefore decided to probe the developing EC to identify key growth factors and cytokines that are critical for the formation of SCs. We selected the pig as a model, since it may better reflect human EC development compared to the rodent, due to its gyrencephalic anatomy. Embryonic brain tissue was obtained from a local slaughterhouse. To identify the period of neurogenesis and EC development, we performed Nissl staining and postmortem structural MRI on brains collected at embryonic day (E) 40, 48, 60, 70, 80, 100 and postnatal day (P) 75 (gestation length 114 days, n=3). We identified cells which have entorhinal cortex cytoarchitecture properties at the Layer II border as early as E49 and a more mature EC cytoarchitecture was observed at E60 in the ventral telencephalon, within the piriform lobe. Furthermore, using histological parameters, we could delineate the lateral (LEC) from the medial EC (MEC). Furthermore, immunolabelling revealed that the posterior EC, containing the MEC, presented neurogenesis already by E40. Radial glia cells were found in the ventricular zone (VZ) and expressed GFAP/BLBP, SOX2 and PAX6. Further, a marker of intermediate progenitors, TBR2, was detected in the sub-VZ. Interestingly, we found GFAP+/BLBP+ cells, even in the ependymal layer at P75 (23.31%). We identified a population of RELN+/CALB1-/MAP2+ neurons at the superficial border of Layer II appearing at E60, which we presume to be the SCs. The percentage of these cells in Layer II increased from 1.87% of the population in the cortical plate at E60 to 51.77% within Layer II by E100. We compared RELN expression in the pig to that in 21 week old human fetal EC, and found no RELN+ neurons in the Layer II in the human. This study has led to the characterization of the developing EC in a new species and documented when SCs arise in a large mammal. We are currently analysing single-cell sequencing data from the embryonic brains, allowing us to identify growth factors and cytokines important for SC development. This may eventually lead to the creation of novel SC differentiation protocols from human iPSCs.

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Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.22/A22

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH NINDS Grant F31NS100360-01

Title: Inhibition of an autocrine activity-dependent insulin signal is required for sensory neuron differentiation in *C. elegans*

Authors: *L. BAYER HOROWITZ, J. BRANDT, N. RINGSTAD
NYU Sch. of Medicine, Skirball Inst., New York, NY

Abstract: The nervous system comprises diverse and highly specialized neuron-types, each expressing a unique set of genes that defines its functional properties. What molecular mechanisms generate diverse neuron types remains unknown. Study of the *C. elegans* chemosensory BAG neurons showed that a p38 Mitogen Activated Protein kinase (MAPK), PMK-3, is required for their proper differentiation. How p38 MAPKs function in neurodifferentiation is poorly understood. Through analysis of mutations that restore gene expression to *pmk-3* mutant BAG cells, we found that genes that promote neural activity and secretion antagonize *pmk-3* dependent gene expression in developing BAG neurons. Silencing BAG neural activity also sufficed to restore gene expression to *pmk-3* mutant BAG cells, suggesting that an autocrine activity-dependent signal antagonizes PMK-3-dependent neurodifferentiation. To determine the identity of the secreted signal, we used RNA-Seq to transcriptionally profile wild-type and *pmk-3* mutant BAG neurons. We found that *pmk-3* mutant BAG neurons overexpressed multiple insulin like peptides (ILPs) as compared to wild-type neurons, indicating that *pmk-3* inhibits expression of ILPs. To test whether ILPs are the signal that antagonizes PMK-3 dependent neurodifferentiation, we overexpressed the dominant negative ILP *daf-28(sa191)* in *pmk-3* mutant BAG cells to disrupt insulin production and release from BAG. This manipulation was sufficient to restore gene expression and function to *pmk-3* mutant BAG neurons. Furthermore, BAG cell-specific knock-down of the insulin receptor homolog, DAF-2, also restored gene expression and function to *pmk-3* mutant BAG cells. Together our data delineate a mechanism through which p38 MAPKs promote proper sensory neuron differentiation by inhibiting an autocrine and activity-dependent insulin signal that represses expression of a BAG neuron fate. These findings reveal an unexpected role for insulin signaling in nervous system development and suggest that insulin-like factors are at the nexus of

intrinsic genetic programs and extrinsic signaling mechanisms that regulate neuronal differentiation.

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Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.23/A23

Topic: A.03. Stem Cells and Reprogramming

Title: Genome-wide definition of regulatory regions and transcripts during the transition from pluripotent to neural-restricted stem cells

Authors: *V. MENEGHINI[1], M. LUCIANI[2], L. PETITI[3], I. CIFOLA[3], C. PEANO[4], A. GRITTI[2], A. MICCIO[1]
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Abstract: Human fetal-derived neural stem/progenitor cells (hfNSCs) are under clinical evaluation for several neurodegenerative diseases. These cells display a favorable safety profile but require immunosuppression upon allogeneic transplantation in patients. In this scenario, obtaining *bona-fide* neural stem/progenitor cell (NSC) populations from human induced pluripotent stem cells (hiPSCs) is relevant for the development of autologous approaches to treat neurological disorders. We have recently generated hiPSC-derived NSCs (hiPS-NSCs) sharing phenotypic and functional identity with hfNSCs, providing proof-of-principle of their potential application in *ex vivo* gene therapy protocols for metachromatic leukodystrophy, a demyelinating genetic disease. The transcriptional and epigenetic mechanisms underlying hiPSC commitment towards the neural lineage need to be investigated to optimize the production and to define the safety profile of hiPS-NSCs in the perspective of their potential clinical application. In this study, genome-wide transcriptomic analysis revealed a strong downregulation of transcription factors regulating pluripotency, cell cycle and cancer-related pathways and the concomitant appearance of a distinct “neural signature” in hiPS-NSCs (without donor- or clone-related bias) highlighting the role of known and unknown master regulators of the transition from pluripotent to neural-restricted stem cells. Computational integration of RNA-seq and ChIP-seq data showed a dramatic change in the usage of cell-specific enhancers and super-enhancers during hiPSC neural differentiation suggesting their major role in the generation and maintenance of hiPS-NSC population. Differences in the transcriptomic and epigenetic profiles of hiPS-NSCs and hfNSCs

can be ascribed to culture conditions, regionalization and differentiation potential, with no major signs of activation/misregulation of potential cancer-related pathways directly attributable to a pluripotent "memory" or abnormal differentiation. We envisage that combining genetic and epigenetic analyses will clarify the dynamic changes occurring during hiPSC neural fate, helping to define a consistent "NSC signature" that might aid strategies for increasing safety and efficiency of hiPS-NSC populations to be used for cell therapy approaches.

Disclosures: V. Meneghini: None. **M. Luciani:** None. **L. Petiti:** None. **I. Cifola:** None. **C. Peano:** None. **A. Gritti:** None. **A. Miccio:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.24/A24

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant U01-MH106882
CIRM Grant TG2-01158

Title: Hippocampal neurons from human pluripotent stem cells enables modeling of connectivity *in vitro*

Authors: *A. SARKAR[1], A. MEI[1], S. STERN[2], A. C. M. PAQUOLA[2], M. SOKHIREV[2], H. KIM[2], F. H. GAGE[1]
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Abstract: Despite widespread interest in using human induced pluripotent stem cells (hiPSCs) in neurological disease modeling, a suitable model system to study human neuronal connectivity is lacking. Here, we report a comprehensive and efficient differentiation paradigm for hiPSCs that generate multiple CA3 pyramidal neuron subtypes as detected by single-cell RNA sequencing (RNA-seq). This differentiation paradigm exhibits characteristics of neuronal network maturation, and rabies virus tracing revealed synaptic connections between stem cell-derived dentate gyrus (DG) and CA3 neurons in vitro recapitulating the neuronal connectivity within the hippocampus. Because hippocampal dysfunction has been implicated in schizophrenia, we applied DG and CA3 differentiation paradigms to schizophrenia-patient-derived hiPSCs. We detected reduced activity in DG-CA3 co-culture and deficits in spontaneous and evoked activity in CA3 neurons from schizophrenia-patient-derived hiPSCs. Our approach offers critical insights into the network activity aspects of schizophrenia and may serve as a promising tool for modeling diseases with hippocampal vulnerability.

Disclosures: A. Sarkar: None. **A. Mei:** None. **S. Stern:** None. **A.C.M. paquola:** None. **M. Sokhirev:** None. **H. Kim:** None. **F.H. Gage:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.25/A25

Topic: A.01. Neurogenesis and Gliogenesis

Support: Department of Science and Technology grant no NG67993

Title: Co-morbid HIV-Tat and morphine attenuate human neural stem cell neurogenesis and alter proteins regulating neural functions

Authors: *S. MALIK, P. SETH
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Abstract: Prevalence of neuro-developmental disorders in perinatally HIV-infected children or in infants born to opioid abusing mothers suggest perturbations in neural stem/progenitor cell (NPC) functions further leading to neurobehavioral abnormalities. Since co-morbid HIV and opioids have been shown to affect the proliferative potential of NPCs, we investigated if multipotency of these cells is compromised. Human fetal NPCs were exposed to co-morbid HIV-protein, Tat and morphine and differentiation into neuronal lineage was assessed. Comprehensive gene analysis revealed reduced expression of genes involved in maintenance of NPC pool and initiation of differentiation, and simultaneous increase in certain basic helix-loop-helix (bHLH) transcriptional repressors such as *Hey* and *Hes.* Further programming of NPCs into neuronal lineage in presence of co-morbid HIV-Tat and morphine exposure revealed compromised neurogenesis which may serve as a confounding factor for HIV Associated Neurocognitive Disorders (HANDs). Following neurogenesis for up to two weeks in culture with simultaneous HIV-1 Tat and morphine exposure revealed down-regulation of several genes involved in cell adhesion, establishment and maintenance of neuronal connections and synapse assembly. Ours is the first study which has looked into neuronal differentiation of human NPCs with co-morbid HIV-1 Tat and morphine exposure and provides a new facet to HIV-drug abuse co-morbidity that may have far reaching clinical consequences both in paediatric as well as adult neuroAIDS.

Disclosures: S. Malik: None. **P. Seth:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.26/A26

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant U01 MH103365-03
NARSAD Grant R13912

Title: Integrative multi-omics analyses of iPSC-derived brain organoids identify early determinants of human cortical development

Authors: *A. AMIRI[1], G. COPPOLA[1], S. SCUDERI[1], F. WU[1], T. ROYCHOWDHURY[2], F. LIU[1], S. POCHAREDDY[1], Y. SHIN[1], M. GERSTEIN[1], N. SESTAN[1], A. ABYZOV[2], F. M. VACCARINO[1]
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Abstract: Gene regulatory regions of the human genome active in the prenatal human cerebral cortex are thought to drive human brain evolution, and contain loci that confer risk for neuropsychiatric disorders. These stages are impossible to model in a longitudinal, dynamic fashion using postmortem brain tissue. Here, by comparing human forebrain organoids derived from induced pluripotent stem cells (iPSCs) and isogenic fetal cerebral cortex, we demonstrate that, on the transcriptome and epigenome level, organoids model embryonic and early fetal cerebral cortical development before 16 week post conception. By combined analyses of histone marks, transcriptome and chromatin conformation in organoids and fetal cortex, we reveal the longitudinal dynamics of transcripts and enhancer elements at stages that bridge neural stem cell proliferation with neurogenesis. We found that the transition from neural stem cells to cortical progenitors is characterized by the largest number of differentially expressed genes (71%; 3,436 out of 4,835) and differentially active enhancers (76%; 15,485 out of 20,356), the majority of which were unique to this transition. A large fraction (34%) of the enhancer acted as gene repressors. Based on expression/activity profile across differentiation days of organoids we constructed networks of transcript/enhancer modules. These modules revealed only six and four global patterns of expression and activity changes, respectively (i.e., supermodules). Furthermore, we observed convergence of expression and enhancer modules, suggesting co-regulation by common upstream mechanisms. Specific transcriptome and enhancer modules were enriched with autism/developmental disorders associated genes or enhancers that gained activity during human brain evolution, while enhancers active at different stages of neurodevelopment were differentially enriched for personal variants in subjects with autism and developmental disorders. Lastly the identified enhancers were enriched around GWAS loci of

psychiatric disorders. Combined, the evidencesuggests the likely very early onset of these diseases and point to genes and regulatory elements related to the disease onset.

Disclosures: A. Amiri: None. **G. Coppola:** None. **S. Scuderi:** None. **F. Wu:** None. **T. Roychowdhury:** None. **F. Liu:** None. **S. Pochareddy:** None. **Y. Shin:** None. **M. Gerstein:** None. **N. Sestan:** None. **A. Abyzov:** None. **F.M. Vaccarino:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.27/A27

Topic: A.03. Stem Cells and Reprogramming

Support: R56-AG058063
U54-HL127365

Title: A dynamic view of the proteomic landscape during differentiation of human ReNcell-VM neural progenitor cells

Authors: *Y. SONG[1,2], M. J. BERBERICH[2], K. SUBRAMANIAN[1], S. RODRIGUEZ[2], R. EVERLEY[2], T. J. MITCHISON[1], P. K. SORGER[2]
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Abstract: Neural differentiation requires finely-tuned temporal and spatial alterations in gene expression, protein modification, and signalling. To study the molecular and phenotypic changes during differentiation of neural progenitors, we used a highly reproducible and robust human neuroprogenitor model: the immortalized ReNcell VM cell. To advance our understanding of the complex regulation critical for brain development, we applied state-of-the-art multiplexed mass spectrometry and high-content-live-imaging, correlating quantitative changes in ~8,900 proteins and ~3,500 phosphoproteins (comprising ~11,000 phosphorylation sites) with phenotypical changes at 10 stages of neural differentiation in ReNcell VM system over 15 days. Total proteomes and immunofluorescence confirmed that ReNcell VM gave rise to neurons, astrocytes, and oligodendrocytes. Proteomics and phosphoproteomics results suggested consistent changes in pathways underlying cytoskeletal rearrangement, cell phase transition, neuronal migration, axon guidance, glial differentiation, neurotrophic signalling, extracellular matrix regulation, etc. Furthermore, the poly-selective CDK and GSK3 inhibitor kenpaullone and the HMG-CoA reductase inhibitor mevastatin have previously been reported to promote neural differentiation; proteomic and imaging data were therefore also collected from cells treated with these drugs. These studies provide a systematic set of data on progenitor cell differentiation and

drug perturbation to better understand the underlying regulatory networks and future applications of neural stem cells in health and disease.

Disclosures: Y. Song: None. **M.J. Berberich:** None. **K. Subramanian:** None. **S. Rodriguez:** None. **R. Everley:** None. **T.J. Mitchison:** None. **P.K. Sorger:** None.

Poster

550. Neurogenesis and Gliogenesis: Neuronal Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 550.28/A28

Topic: A.01. Neurogenesis and Gliogenesis

Support: MOE Tier 1 R-181-000-179-114
NUS Start-up R-181-000-155-133

Title: Role of piRNA and interacting exosome components in neuronal differentiation from human embryonal carcinoma cells

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Abstract: piRNAs have been reported to exist in neurons and to play some interesting somatic functions, including neurite outgrowth and maintenance of memory. However, whether piRNAs are induced to express and play any prominent role during neuronal differentiation remain unknown. Hence, we first profiled the piRNA expression in NT2 cells, a human embryonal carcinoma line, treated with *all-trans* retinoic acid (ATRA) for six days and fifteen days. It was noted that ATRA induced a dramatic change in the expression profile of piRNAs as compared to control treatment. Among the piRNAs that show a consistently increasing expression (Fold change > 2.0) over the course of ATRA treatment, we manually validated one sequence, piR-6, by Northern blot. To answer the question whether this piRNA is proactively involved in regulating neuronal differentiation, we overexpressed piR-6 in ATRA-treated NT2 cells and observed that some neuronal markers such as MAP2 and TUBB3 were further elevated as compared to regular ATRA-treated cells. Reciprocally, when this piRNA sequence was quenched by inhibitory oligonucleotides, the ATRA-induced expression of neuronal markers was significantly suppressed. To elucidate how the piRNA could modulate neuronal differentiation, we performed RNA pulldown in NT2 cells using the piR-6 mimics and analyzed the precipitates by mass spectrometry. Remarkably, piR-6 was found to precipitate exoribonucleases DIS3 and DIS3L2, both containing a single-stranded oligonucleotide binding domain, the cold shock domain (CSD). RNA immunoprecipitation confirmed that the two enzymes associate with this piRNA sequence. While DIS3 is mainly localized in the nucleus and DIS3L2 mostly

cytoplasmic, RNA FISH revealed the presence of piR-6 in both the cytoplasm and nucleus of ATRA-treated NT2 cells. Interestingly, when DIS3 or DIS3L2 was knocked down, the expression of neuronal markers was significantly reduced in ATRA-treated NT2 cells, which could not be restored even with piR-6 overexpression, suggesting that the piR-6/DIS3/DIS3L2 interaction could be essential for the normal neuronal differentiation. Given that DIS3 and DIS3L2 are directly involved in exosome-related degradation of RNAs, it is conceivable that some piRNA sequence could guide these exoribonucleases to degrade stemness transcripts and early neurogenic transcripts, hence facilitating the neuronal differentiation process. This study has suggested a novel somatic function of piRNAs, potentially related to the exosome-mediated RNA degradation, in the context of neurogenesis.

Disclosures: Q. Hu: None. **C.S. Subhramanyam:** None.

Poster

551. Neurogenesis and Gliogenesis: Glial Development and Interaction With Neurons

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 551.01/A29

Topic: A.01. Neurogenesis and Gliogenesis

Support: UABC 19va Convocatoria Interna to T.N.O.B
Conacyt-México 255087 to A.O.

Title: GLAST expression in different developmental stages of marine echinoderms

Authors: A. B. BENÍTEZ-MATA[1], R. A. ZÚÑIGA-ASTORGA[1], F. CORREA-SANDOVAL[1], A. ORTEGA[2], *T. N. OLIVARES-BAÑUELOS[1]
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Abstract: Radial Glial cells (RGC) have preserved characteristics in the *Deuterostomy* and *Protostomy* taxa, and their presence has been confirmed in adults of both monophyletic groups. Within the *Deuterostomy* taxon is the phylum *Echinodermata*, molecularly related to the chordates. Echinoderms are invertebrate organisms with a relatively simple Central Nervous System (CNS) in which RGC are the only and largest type of glial cells. Adult echinoderms are able to regenerate their CNS, which makes them suitable models in biomedical research for the neurodegeneration of fully developed mammals, including humans. Nevertheless, in echinoderms, the existence of RGC in their early development stages (represented by 4-, 6- and 8-arms echinopluteus larvae, competent larvae, and postlarvae), has not been explored. In the adult sea cucumber *Holothuria glaberrima*, a model echinoderm, it has been established that RGC generate new and functional neuron cells, and constitute the leader cells in CNS post-injury

regeneration. Research in this field will lead to important biomedical advances that improve the processes of neuronal repair in patients with neurodegenerative diseases such as Parkinson, Alzheimer or Huntington. In this context, the present research project aims to identify and characterize glial cells in the early development stages of echinoderms, to establish the time-frame it which these cells become functional, as assayed by the expression of the glial glutamate/aspartate transporter GLAST. Samples of either echinopluteus larvae or postlarvae of the echinoderm *Dendraster excentricus*, a sand dollar, were collected and GLAST was detected via Western blot. We were able to detect an increase in GLAST immunoreactivity at the beginning and at the end of echinopluteus development. Functional [^{3}H]D-aspartate uptake assays will be used to fully demonstrate the presence of glial glutamate transporters in larvae. Our results strengthen the notion of the involvement of radial glial cells in echinoderms neurogenesis.

Disclosures: A.B. Benítez-Mata: None. **R.A. Zúñiga-Astorga:** None. **F. Correa-Sandoval:** None. **A. Ortega:** None. **T.N. Olivares-Bañuelos:** None.

Poster

551. Neurogenesis and Gliogenesis: Glial Development and Interaction With Neurons

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 551.02/A30

Topic: A.01. Neurogenesis and Gliogenesis

Support: Department of Defense CDMRP Grant 11162432
NIH Grant ZIAHD000713-22

Title: Cholinergic signaling between axon and oligodendrocyte: Implications for myelin abnormalities in Gulf War illness

Authors: *J. BELGRAD[1], D. J. DUTTA[1,2], K. A. SULLIVAN[3], J. P. O'CALLAGHAN[4], R. D. FIELDS[1]
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Abstract: Cholinergic signaling has been recently implicated in myelination and as a promising target for demyelinating disorders. Despite established roles as a major neurotransmitter and source of choline metabolite, the contribution of acetylcholine (ACh) to oligodendrocyte development and myelin plasticity remains to be elucidated. Here, we show that oligodendrocytes express the receptors and enzymes necessary to engage in cholinergic signaling and respond to ACh with robust and heterogenous intracellular calcium kinetics. To investigate

the purpose of cholinergic signaling in oligodendrocytes, we studied the anticholinergic pathology associated with Gulf War Illness (GWI), the heterogeneous condition that afflicts 25% of US Veterans deployed in 1990-1991 Gulf War. Based on our previous studies, we hypothesized that the myelin abnormalities reported in GWI veterans, were due to atypical cholinergic signaling in oligodendrocytes. This hypothesis was tested using an animal model of GWI, mimicking the exposure to anticholinesterase agents (modeled by Sarin gas analog Diisopropyl fluorophosphate, DFP) and extreme stress (exogenous corticosterone, Cort). Western blot data reveals increased in myelin basic protein levels, a key protein in myelin production, in the combined Cort+DFP condition in whole brain homogenate at 24 hours and persisting through 21 days post exposure (One-way ANOVA, N=3, p=0.0141). At the molecular level, live cell calcium imaging data shows that pretreatment with DFP significantly increases the number of wild type oligodendrocytes that respond to ACh in vitro (two-tailed two-sample t-test, N= 5, p=0.005). The DFP-mediated increase in oligodendrocyte responsiveness is not due to acetylcholine produced by oligodendrocytes or astrocytes (N=3, T-test, p=0.55) suggesting GWI is a pathology of neuron-glia rather than a glia-glia cholinergic signaling. Taken together, this work demonstrates GW agents disrupt oligodendrocyte development in vivo and at the molecular level. These findings both reveal the importance of cholinergic signaling for proper myelin development and indicate that the anticholinergic and corticosterone mediated signaling by GW agents, and more generally by commercial-use pesticides, may be largely responsible for myelin changes in veterans with GWI and broader myelin-related pathologies.

Disclosures: D.J. Dutta: None. **K.A. Sullivan:** None. **J.P. O'Callaghan:** None. **R.D. Fields:** None.

Poster

551. Neurogenesis and Gliogenesis: Glial Development and Interaction With Neurons

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 551.03/A31

Topic: A.01. Neurogenesis and Gliogenesis

Support: NMSS RR-1512-07066

Title: Sox17 transgenesis reveals sonic hedgehog- and Gli2-mediated oligodendrocyte differentiation in adult white matter

Authors: *L.-J. CHEW[1], X. MING[1], J. L. DUPREE[2], B. MCELLIN[1], V. GALLO[1]
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Abstract: Sox17 is a Sox F transcription factor whose overexpression in CNPSox17 transgenic mice promotes postnatal oligodendrocyte development and prevents oligodendrocyte loss after

focal lysolecithin demyelination. Sox17 is expressed in remyelinating adult white matter lesions, but its function in oligodendrocyte generation is not understood. To investigate Sox17 function, we analyzed signaling mechanisms in intact and demyelinating white matter in CNPSox17 mice. Electron microscopy analysis confirms that Sox17 attenuates lysolecithin-induced demyelination, and increased BrdU+Olig2+ cells indicates de novo oligodendroglial production. Unlike in wild-type(WT) mice, activated beta-catenin (ABC) was not induced in CNPSox17 lesions, and fewer Iba1+ microglia indicates attenuated reactivity to tissue damage. Since elevated GLI2 and Sonic Hedgehog (SHH)-expressing cells were observed in CNPSox17 white matter, we determined their roles in oligodendrocyte regeneration. Targeted ablation of *Gli2* using PDGFRCreERT2 abolished Sox17-enhanced white matter thickness, suggesting Gli2 involvement in cellular homeostasis. Gli2 ablation showed that Gli2 represses CC1 cell formation in WT lesions but promotes CC1 accumulation in CNPSox17. This was accompanied by increased cells expressing beta-catenin in CNPSox17. Gli2 ablation in WT lesions instead decreased beta-catenin, indicating that Sox17 alters Gli2 function from a positive to negative regulator of beta-catenin, which in turn promotes lineage progression. Similar changes in CC1 were found following PDGFRCre-targeted Smo ablation, suggesting SHH-activated differentiation. Indeed, CNPSox17 lesions showed an increased percentage of Olig2 cells that colocalized with SHH, either from its expression or sequestration. Smo ablation also increased the number of proliferative GFAP+ cells in intact white matter, which may indicate a gliotic response. Finally, the stereotaxic application of the SMO agonist SAG in WT lesions prevents ABC increase and promotes OPC differentiation. Through increasing cell production and limiting glial reactivity, the application of Sox17 signaling targets, such as Smoothened activation with SAG, may be a viable therapeutic option for adult demyelinating disease.

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Poster

551. Neurogenesis and Gliogenesis: Glial Development and Interaction With Neurons

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 551.04/A32

Topic: A.01. Neurogenesis and Gliogenesis

Support: UNO GRACA to Candi Senior-Remsa
UNO FUSE to Wenxian Zhou

Title: The *Drosophila* ADAM protein MMD participates in the early formation of both epithelial and glial boundaries

Authors: *B. A. CHASE[1], C. SENIOR-REMSA[1], A. CASTRO[1], W. ZHOU[2], C. KERBER[1], G. E. GILSON[3], K. HIGGINS[1], E. KLUG[1]

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Abstract: Background: Interactions between the vertebrate proteins ADAM22, ADAM23, ADAM 11 and LGI are required in the developing peripheral nervous system for axonal sorting and myelination as well as in hippocampal neurons for trans-synaptic interactions and establishing synaptic strength. In *Drosophila*, *mmd (mind-meld*) encodes one secreted and multiple membrane-bound ADAM protein isoforms that are structurally similar to these ADAM proteins. Its transcripts are deposited maternally and become localized in the early embryo just beneath the plasma membrane. Later they become abundant in the developing CNS, indicating that *mmd* has two temporally and spatially distinct roles during development. To better understand these roles, we co-localized MMD proteins (1) in early embryos relative to cytoskeletal proteins important for the establishment of cell polarity and (2) in late embryos relative to extracellular matrix (ECM), neuronal and glial proteins important for nervous system development. **Results**: In the syncytial and cellularizing embryo, MMD is localized just beneath the apical surface in patterns indicating that it participates in the establishment of apical-basal epithelial cell polarity. During gastrulation, MMD is found along the outer epithelial surface of the embryo and remains there even as cells invaginate into the embryo. The intensity of the MMD signal in this region increases long before the cuticle is synthesized. Thus, MMD appears to be an early component of the ECM at the embryo's surface. While *mmd* mRNA expression is abundant in the developing CNS, MMD protein is not abundant within the CNS. Rather, MMD is found in the developing neural lamella that will envelop the CNS and peripheral nerves. **Conclusion:** These results suggest that MMD functions in the formation of both epithelial and glial boundaries. To test this, RNAi and the UAS-GAL4 and MARCM systems are being used to evaluate the effect of maternal and zygotic knock-down of *mmd* expression in specific tissues and cell types. Clear morphological phenotypes or effects on viability have not yet been identified. Experiments are in progress to test whether MMD interacts with other proteins involved in glial development and in establishing epithelial boundaries in the developing embryo.

Disclosures: C. Senior-Remsa: None. **A. Castro:** None. **W. Zhou:** None. **C. Kerber:** None. **G.E. Gilson:** None. **K. Higgins:** None. **E. Klug:** None.

Poster

551. Neurogenesis and Gliogenesis: Glial Development and Interaction With Neurons

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 551.05/A33

Topic: A.01. Neurogenesis and Gliogenesis

Support: The Adelson Medical Research Foundation

R01MH104701

Title: Single cell RNA sequencing of the humanized glial chimeric mouse brain identifies the transcriptional concomitants to human white matter maturation and myelination

Authors: *J. N. MARIANI[1], S. J. SCHANZ[1], K. M. CLARK[1], S. A. GOLDMAN[1,2,3]
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Abstract: The loss of myelin is observed in numerous devastating diseases of the CNS ranging from the hereditary leukodystrophies in children to multiple sclerosis and vascular white matter disease in adults. As such, acquisition of therapeutic targets stimulating myelin generation is of profound importance. However, neither rodent models nor in vitro studies of human cells accurately reflect the molecular regulation of human glial progenitor cell (hGPC) mobilization and myelination in vivo. To this end, in this study we used a humanized glial chimeric mouse model, allowed to develop a humanized white matter and then subjected to single cell RNA-Seq (scRNA-Seq), to begin to define the changes in oligodendroglial gene expression associated with human myelination in vivo. We established chimeric mice by neonatal transplantation of PDGFRA+ hGPCs, generated from human embryonic stem cells (WA09) and then purified by FACS, into neonatal immunodeficient and myelin-deficient shiverer mice. At 19 weeks of age, mice were sacrificed (n=3), their corpus callosa dissociated, and O4+ human and mouse oligodendroglial cells were isolated via FACS for scRNA-Seq. Expression profiling revealed the large majority of mouse cells to be oligodendrocytes (OLs). In contrast, following dimensionality reduction, human cells clustered into subpopulations consisting of PDGFRA+ GPCs, BCAS1+ immature OLs, MOBP+ mature OLs, and astrocytic progenitors upregulating GFAP and CD44. These GPCs were overall quite similar to FACS isolated human fetal and pre-transplant WA09 hGPCs with in vivo GPCs exhibiting higher degrees of lineage fidelity than their in vitro counterparts. Reconstruction of single-cell trajectories arranged cells logically in pseudotime from early GPCs to mature OLs, predicted an early branch point where GPCs opted towards astrocytic ends, and allowed for identification of genes changing as a function of time and fate. We next sought to identify transcription factors (TFs) active in subpopulations, through a combination of gene co-expression, motif enrichment, and extrapolation over cell-trajectories. This technique appropriately identified several lineage-specific TFs including SOX10 and NKX2-2 in OLs and SOX9 and HES1 in astrocytes. Through integration of these analyses, we generated a transcriptional network governing both early and late stages of myelination and astrocytic differentiation including novel TFs TFEB and ZIC1 respectively. By analyzing over 12,000 cells, we have identified pathways whose targeting may permit the therapeutic modulation of both the expansion and terminal maturation of human parenchymal glial progenitor cells.

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Poster

551. Neurogenesis and Gliogenesis: Glial Development and Interaction With Neurons

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 551.06/A34

Topic: A.01. Neurogenesis and Gliogenesis

Title: Unbiased stereological estimates of total number of astrocytes in the developing human cochlear nucleus

Authors: *S. SAINI[1], T. G. JACOB[2], A. THAKAR[3], K. K. ROY[4], T. S. S. ROY[1]
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Abstract: Introduction: Cochlear nucleus is the first central relay station in the auditory pathway that is responsible for transmitting and processing auditory information. Studies which have reported the morphological development of the cochlear nucleus in human lacks the description of glial cells. Glial cells are one of the major cell populations of the central nervous system (CNS). Astroglia, in the developing brain, help in guiding the migration of developing neurons and their processes. They also help in forming and pruning connections between neurons. Hence, these cells and their identification have key significance in any study on developing nervous tissue.A protein that is expressed early in these cells is Glial Fibrillary Acidic Protein (GFAP). Here, we have quantified astrocytes in the developing human cochlear nucleus by using unbiased stereology. **Materialsand Methods**: Twenty human brains were obtained from the departments of Obstetrics and Gynaecology and Forensic Medicine, with prior approval of the Institute Ethics Committee and informed consent from legal representatives. Sixteen were fetuses of 18-32 weeks of gestation WG; three were from the brainstems of neonates(two, three, five postnatal days- PND) and one was obtained from an infant of two-months of age. The brainstems were dissected, fixed in 4% buffered paraformaldehyde (0.1M phosphate buffer, pH 7.4), cryopreserved in 30% sucrose and sectioned on a cryomicrotome to obtain 40 µm thick serial sections. Using systemic random sampling, the sections were immunostainedfor the expression of GFAP (ab10062, 1:1000) using standard protocol. The total number of astrocytes were estimated by using the Optical Fractionator. Comparisons between the groups samples- 18-20, 21-25, 26-30 weeks of gestation and one group of postnatal samples were made using Kruskall-Wallis test. **Results:** The astroglial cells identified by GFAP contained round or elongated nucleus, outlined by a thin rim of chromatin with long processes and fine branching. The median value with interquartile range at 18-20; 21-25; 26-30 weeks of gestation and after birth were 32,087(27,399,39,033); 40,437(37,930, 45,348); 78,654(67,114, 94,614); 165,124(102,409, 182,867),respectively. A significant increase was observed in the number of astroglia in the postnatal samples versus the fetuses of weeks 18-20 ($p = 0.007$) and gestational age of 21-25 weeks ($p = 0.04$). This increase may explain the increasing size and

functioning of the cochlear nucleus with gestational age and that the gliogenesis continues postnatally too.

Disclosures: S. Saini: None. **T.G. Jacob:** None. **A. Thakar:** None. **K.K. Roy:** None. **T.S.S. Roy:** None.

Poster

551. Neurogenesis and Gliogenesis: Glial Development and Interaction With Neurons

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 551.07/B1

Topic: A.01. Neurogenesis and Gliogenesis

Support: 5R00HD058044-05

Title: FGF8 signaling plays an integral role during cuprizone-dependent astrocyte activation

Authors: *C. E. STEWART, W. C. CHUNG
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Abstract: Fibroblast growth factor (FGF) 8 signaling deficits delayed the maturation of anterior brain midline glial fibrillary acidic protein (GFAP) astrocytes. In contrast to perinatal development, the adult anterior brain midline GFAP astrocyte population did not exhibit marked deficits. Nonetheless, we cannot rule out the possibility that reduced FGF8 signaling may have disrupted adult astrocyte function. Especially in light of *in vitro* studies reporting that FGF8 increased cortical astrocyte branching complexity to facilitate wound healing. Here, we asked whether deficits in FGF8 signaling impair adult astrocyte activation. For this purpose, adult wildtype (WT) and *Fgf8* hypomorphic $^{(+/neo)}$ mice were fed with a 0.2% cuprizone (CPZ) diet for 2, 3, or 6 weeks or control chow. CPZ treatment increased GFAP expression in the medial corpus callosum (i.e., genu) in a non-genotype dependent fashion. In contrast, CPZ treatment increased GFAP expression in the lateral corpus callosum (i.e., cingulum) of $Fgf8^{+/neo}$ mice less compared to WT mice. Furthermore, we showed that after 2 weeks of CPZ treatment secondary branching was higher in WT mice than $Fgf8^{+/neo}$ mice, whereas tertiary branching was less in WT mice than $Fgf8^{+/neo}$ mice. To better understand this astrocyte activational mechanism, we used qPCR to examine CPZ effects on *Gfap*, *Stat3*, and *Fgf receptor (Fgfr) 1* mRNA expression. Prolonged CPZ exposure induced a more robust upregulation in *Stat3* and *Fgfr1* within $Fgf8^{+/neo}$ mice compared to WT mice. We then asked whether FGF8 signaling deficits impaired the CPZ-induced inflammatory response in the corpus callosum, and showed that callosal *Tnfα* mRNA production is FGF8-dependent. Together, our results showed that a developmental disruption in FGF8 signaling had long-term effects on the responsiveness of anterior brain midline astrocytes under demyelinating conditions.

Disclosures: C.E. Stewart: None. **W.C. Chung:** None.

Poster

551. Neurogenesis and Gliogenesis: Glial Development and Interaction With Neurons

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 551.08/B2

Topic: A.01. Neurogenesis and Gliogenesis

Support: Upstate Health Science Foundation

Title: Signaling pathways that regulate oligodendroglia migration and differentiation

Authors: *D. J. OSTERHOUT, B. BADILLO, H. BHATTI, I. GENEVA
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Abstract: Oligodendroglial progenitor cells (OPCs) are produced in the ventral neuroepithelium at later stages in the ontogenesis of the cortex. They migrate into the brain parenchyma, where they will contact axons and differentiate. As they undergo terminal differentiation, OPCs undergo prominent morphological changes, turning from a simple bipolar cell to a cell with multiple complex processes extending from the cell body. Once in contact with an axon, the oligodendrocyte process expands and begins to form the myelin membrane, which will wrap and ensheathe the axon.

Fyn tyrosine kinase is an important signaling pathway that can regulate both the migration and differentiation of oligodendroglial progenitor cells. Fyn activation occurs in progenitor cells before any changes in cellular morphology are observed. PDGF treatment will stimulate migration of OPCs and the activation of Fyn. Once active, Fyn also regulates the morphological differentiation of these cells, initiating process outgrowth and myelin sheet formation *in vitro.* In Fyn deficient mice, myelin formation is markedly reduced, demonstrating the importance of this kinase in myelination.

Fyn can interact with many downstream effectors, including molecular signaling pathways that interact with the cytoskeleton, regulating cell morphology and movement. One important interaction involves the adaptor protein Dab1. We have demonstrated that Fyn-Dab1 interactions are important for OPC migration, as animals deficient in either Fyn or Dab1 show reduced OPC migration from the subventricular zone *in vivo.* Further *in vitro* studies reveal more components of this pathway. Inhibition of Fyn-Dab1 interactions will reduce OPC migration and process outgrowth. Downstream targets of Fyn and Dab1 include Cdk 5, which may be important for migration, but not process outgrowth. Fyn interactions with additional cytoskeletal proteins influence the dynamic morphological changes during OPC differentiation.

Disclosures: D.J. Osterhout: None. **B. Badillo:** None. **H. Bhatti:** None. **I. Geneva:** None.

Poster

551. Neurogenesis and Gliogenesis: Glial Development and Interaction With Neurons

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 551.09/B3

Topic: A.01. Neurogenesis and Gliogenesis

Title: Rapid generation of mature cortical and spinal astrocytes from human iPSCs

Authors: *B. DUNGAR[1], Z.-W. DU[2]
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Abstract: Growing evidence implicates glia, particularly astrocytes, in neurological and psychiatric diseases. Astrocytes perform a variety of essential functions including glutamate regulation, axonal guidance, trophic support, inflammatory response, wound healing, formation of the blood-brain barrier, and neuronal synapse formation. Human cortical astrocytes are larger, structurally more complex and diverse, and respond differently to extracellular glutamate compared to their rodent counterparts. Given the unique biology of human astrocytes, it is critical that improved human-specific cell-based systems be established to enable the study of human astrocytes in health and disease. Because of the limited availability of primary human astrocytes, human iPSCs are currently used as a source of astrocytes. However, existing methods for astrocyte generation are slow (up to 6 months) or require additional selection to reduce heterogeneity. To rapidly generate mature astrocytes for disease modeling, we have developed a novel protocol that uses inducible expression of astrocyte differentiation master transcription factors NFIA and SOX9 and an optimized astrocyte differentiation medium. Human cortical or spinal astrocytes can be generated from normal or disease iPSCs in only one month. They express the key astrocyte markers GFAP and S100β at >90% and exhibit mature process-bearing morphologies. These astrocytes can promote neuron synapse formation and functional activity in MEA and calcium imaging applications and elicit a strong and rapid pro-inflammatory response. This protocol represents an important tool for modeling neurological diseases using a human iPSC-based astrocyte-neuron coculture platform, allowing the role of diseased astrocytes in neuronal degeneration to be probed.

Disclosures: **B. Dungar:** A. Employment/Salary (full or part-time):; BrainXell, Inc. **Z. Du:** A. Employment/Salary (full or part-time):; BrainXell, Inc.

Poster

551. Neurogenesis and Gliogenesis: Glial Development and Interaction With Neurons

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 551.10/B4

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant MH101188
MIND Institute IDDRC U54 HD079125

Title: Decreased adenosine alters microglial morphology and activation during embryonic cortical development

Authors: *J. '. KEITER[1], V. MARTINEZ-CERDENO[3], S. C. NOCTOR[2]
[1]UC Davis, Vacaville, CA; [2]Psych & Behavioral Sci., UC Davis, Sacramento, CA; [3]Pathology, UC Davis/Shriner's Hosp., Sacramento, CA

Abstract: Background: ATP, a strong pro-inflammatory signal, is released under normal conditions and is converted into adenosine, a potent anti-inflammatory signal, through a series of ectoenzymes, which balances the inflammatory effect of ATP. Adenosine is removed through re-uptake or the enzymatic action of adenosine deaminase (ADA), which reduces the anti-inflammatory signal. Regulating the level of ADA therefore, modulates the suppressive effects of adenosine on inflammation. We treated animals with ADA, through either direct injection into the cerebral ventricles of embryonic pups or IP injection into pregnant dams, to determine the effect of reducing the level of adenosine on microglial cells in the cerebral cortex.
Method: ADA or saline was injected into the cerebral ventricles of rat embryos at day E18 for the direct inject group, or injected IP into pregnant dams (E18) for the dam-injected group. Microglial cell morphology was assessed 24 hours later in the ventricular zone (VZ) and in the subventricular zone (SVZ). Microglial cell morphology was quantified across groups using a defined objective morphological index value (MI), that took into account cell soma size and process length, with a low MI value representing microglial cells with an amoeboid shape, and higher MI values representing microglial cells with complex morphology and increased ramification. CD68 expression by microglia was also quantified.
Results: ADA injected into the cerebral ventricle increased the MI value of microglia in both the VZ and SVZ, with the greatest increase in MI value occurring within the VZ. We also noted increased CD68 expression within the VZ after ADA injection. ADA dam injections increased MI values for microglial cells in the SVZ but produced no change in CD68 expression.
Conclusion: Decreasing the adenosine signal with direct application of ADA into the cerebral ventricles of E18 rat embryos increased the morphological complexity of cortical microglial cells over controls, and increased CD68 expression within the VZ. These data suggest that decreasing adenosine’s suppressive effect does not produce the same effect as ATP. We also found that

microglia in the VZ and SVZ responded differentially to ADA treatment, indicating a difference in the signaling milieu within these proliferative zones and potentially pointing to functional differences in VZ versus SVZ microglial cells. Maternal injection of ADA increased the morphological complexity of microglial cells within the SVZ over the VZ, demonstrating that maternal immune perturbations can influence embryonic microglia in a region specific manner, potentially through intermediaries.

Disclosures: J.'. Keiter: None. **V. Martinez-Cerdeno:** None. **S.C. Noctor:** None.

Poster

551. Neurogenesis and Gliogenesis: Glial Development and Interaction With Neurons

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 551.11/DP01/B5

Topic: A.01. Neurogenesis and Gliogenesis

Support: Wenner Gren Hunt Postdoctoral Fellowship
NIH Grant MH101188
MIND Institute: IDDRC; U54 HD079125

Title: Microglia and their associations with neural stem cells vary spatiotemporally in fetal human and nonhuman primate neocortical neurogenesis

Authors: *N. BARGER[1], S. C. NOCTOR[2]
[1]Univ. of California, Davis - MIND Inst., Sacramento, CA; [2]Psych & Behavioral Sci., UC Davis, Sacramento, CA

Abstract: In large-brained primate species, the cellular generation of the neocortex is a complex, protracted process that occurs primarily in utero. Microglia, brain immune cells, are increasingly recognized as critical participants in this process. We have shown that microglia interact with neural stem cells and regulate their numbers, while others suggest they contribute to wiring the fetal neocortex. Yet, little is known about microglial distributions across the primate brain during the neocortical neurogenic period, especially in the context of neural stem cells. To fill this gap, we present a comprehensive analysis of microglia and neural progenitor interactions in the developing fetal macaque neocortex at four age points through early and late neocortical neurogenesis and a preliminary comparison with human neocortex at multiple points in the second trimester. We used multiple immunofluorescence to label 10 or more coronal sections through the full rostrocaudal extent of the macaque neocortex with the general nuclear marker DAPI and antibodies against iba1, a microglial marker, and EOMES (Tbr2), a marker of cortical progenitor cells committed to a neuronal fate. We also incorporate these labels and the stem cell marker, Pax6, in a series of macaque and human cases. This design has a twofold advantage. First, it enables us to use an explicit molecular label, EOMES, to identify the ventricular, inner

subventricular, and outer subventricular zones to accurately assess microglial distribution in discrete germinal niches. Second, it provides a window into microglial interactions with different stem cell types. To assess cellular distributions, scalable images of entire coronal sections were constructed from images taken at 20x magnification on a Keyence BZ-X700 microscope. Cell-cell interactions were visualized with an Olympus FV1000 confocal microscope. We show that microglial distribution in the germinal zones changes dramatically over gestation, increasing in complexity as the primate cortex begins to differentiate. Additionally, we illustrate that stem cell-microglial interactions increase in number and complexity through human and macaque neocortical development. Given the reported role for microglia in shaping the developing cortex, this data can provide important information about the processes contributing to neocortical expansion and elaboration in human and nonhuman primates. Additionally, it may prove critical to understanding how immune disruption at specific developmental time-points contributes to lifetime susceptibility to neurodevelopmental disorders, as has been reported in the literature on maternal immune activation.

Disclosures: N. Barger: None. **S.C. Noctor:** None.

Poster

551. Neurogenesis and Gliogenesis: Glial Development and Interaction With Neurons

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 551.12/B6

Topic: A.01. Neurogenesis and Gliogenesis

Title: The retinoic acid-synthesizing enzyme retinaldehyde dehydrogenase 2 is required for normal oligodendrogenesis and myelination in the developing postnatal brain

Authors: *V. MORRISON, J. K. HUANG
Biol., Georgetown Univ., Washington, DC

Abstract: The vitamin A-derived signaling molecule retinoic acid (RA) has been shown to affect oligodendrocyte lineage cell (OLC) progression *in vitro*. It remains unknown, however, if endogenously synthesized RA in the central nervous system (CNS) has a role in OLC progression, and therefore myelination, *in vivo*. This study uses a cell-specific knockout of the key RA-synthesizing enzyme retinaldehyde dehydrogenase 2 (Raldh2) to test the hypothesis that RA is necessary for correct OLC progression and myelination in the early postnatal period. Cell-specific excision of loxP-flanked Raldh2 was achieved using Cre recombinase under the control of the neural/glial antigen 2 (Ng2) promoter, Ng2 being largely co-expressed with Raldh2 in CNS cells. Conditional Raldh2 knockout (cKO) mice were compared to control littermates at four time points during developmental myelination (postnatal days 2, 7, 14, and 21). Using immunofluorescence microscopy, we examined differences in OLC number, maturational state, proliferation, and death, as well as the degree of myelination in the corpus callosum. We found

that cKO mice had a persistent deficit in OLCs compared to controls. Of those OLCs, more of them were oligodendrocyte precursors cells (OPCs) in cKO mice when compared to controls, while, conversely, the proportion of mature oligodendrocytes (OLs) was decreased in cKO mice. Also, in cKO mice, more OPCs were dividing, but also, more cells were undergoing cell death. Finally, cKO mice had less myelin in the corpus callosum. Together, these findings support the hypothesis that RALDH2 and the RA it creates are necessary for normal OLC progression and myelination. These findings shed light on the role of RA in the postnatal brain, and in particular, its role in regulating myelination.

Disclosures: V. Morrison: None. **J.K. Huang:** None.

Poster

551. Neurogenesis and Gliogenesis: Glial Development and Interaction With Neurons

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 551.13/B7

Topic: A.01. Neurogenesis and Gliogenesis

Support: Bridgewater State University ATP Research Grant

Title: Identification of the Nab2 nuclear localization signal

Authors: J. M. LAVALLEE, T. GRANT, N. BERRY, S. KLETSOV, K. M. ABT, *K. W. ADAMS
Biol. Sci., Bridgewater State Univ., Bridgewater, MA

Abstract: NGFI-A binding protein 2 (Nab2) is a transcriptional coregulator that modulates gene expression through protein-protein interactions with early growth response proteins 1-3 (Egr1-3), which play roles in a wide variety of cell behaviors. Egr1 and 2 contribute to the transcriptional program underlying neuronal differentiation of PC12 cells in response to nerve growth factor, while Nab2 plays a role in a negative feedback loop to repress their transactivation. Essential roles for Nab2 and Egr2 in development and maintenance of peripheral nerve myelination are also well established, whereas roles for Egr1 and 3 are better documented in the central nervous system, where they contribute to learning and memory through regulation of genes that mediate synaptic plasticity and long-term potentiation. Nab2 acts largely as a transcriptional repressor, at least in part by recruiting the nucleosome remodeling and deacetylase (NuRD) complex upon binding Egr1-3. However, much remains unknown about the molecular mechanisms that regulate Nab2. This study expands our knowledge of Nab2 by identifying its nuclear localization signal (NLS). More specifically, we generated an expression construct encoding a Nab2-GFP fusion protein, which localized to the nucleus following transfection. Analysis of the Nab2 sequence identified two putative NLS sequences, one spanning amino acids 263-277 that match the bipartite NLS consensus sequence ($RKX_{10}KRR$) and a second site spanning 343-346 (KKXK).

K/R-to A mutation of the site spanning 343-346 resulted in predominantly cytoplasmic localization, indicating it represents the functional Nab2 NLS. Evaluation of Nab2-GFP truncation mutant providing corroborating data; Nab2-GFP truncations that retained amino acids 343-346 all retained the nuclear localization pattern, while truncations lacking 343-346 did not. Lastly, fusion of Nab2 amino acids 340-350 to the cytoplasmic protein eIF2Bε resulted in nuclear localization. Altogether, this study used multiple approaches to determine that amino acids 343-346 (KKLK) of Nab2 function as its NLS.<!--EndFragment-->

Disclosures: J.M. Lavallee: None. **T. Grant:** None. **N. Berry:** None. **S. Kletsov:** None. **K.M. Abt:** None. **K.W. Adams:** None.

Poster

551. Neurogenesis and Gliogenesis: Glial Development and Interaction With Neurons

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 551.14/B8

Topic: A.01. Neurogenesis and Gliogenesis

Support: Major State Basic Research Program of China 2016YFA0501000
National Natural Science Foundation of China 31490590
National Natural Science Foundation of China 31490592
National Natural Science Foundation of China 31501128

Title: Hemocyte regulates the development of neuromuscular junctions in *Drosophila*

Authors: *D. DUAN[1], J. CHU[2], C. WU[2], Z. TING[2], S. LU[2], Y. LIU[2], S. DUAN[2]
[1]Zhejiang Univ., Zhejiang, China; [2]Zhejiang Univ. Med. Sch., Zhejiang, China

Abstract: The development of nervous system requires precise control to establish functional neuronal connectivity. Although several related processes in central nerve systems (CNS), such as synapse formation and subsequent pruning, have been well documented, the underlying regulatory mechanism in periphery nerve system (PNS) remains elusive. Here, we report that *Drosophila* macrophages, also called hemocytes, orderly migrate followed the route of ventral nerve cord (VNC) development. Localized with the marker of neuromuscular junctions (NMJs), HRP, hemocytes were observed engulfing NMJ structures spontaneously. Ablation of hemocyte decreases the number of large synaptic boutons, while increases immature ghost boutons and active zones in large boutons. Meanwhile, depletion of hemocyte also decreases amplitude of excitatory functional potentials (EJPs) while increases the frequency of miniature EJPs (mEJPs) without affect its amplitude, accompanied by an ascent in the paired-pulse ratio. Furthermore, loss of hemocytes induces locomotion behavior defects in third instar larva. Taken together, our results suggest that hemocytes regulate the morphologic and functional development of

Drosophila NMJs by controlling the ratio of large synaptic boutons and associated electrophysiological activities.

Disclosures: D. Duan: None. **J. Chu:** None. **C. Wu:** None. **Z. Ting:** None. **S. Lu:** None. **Y. Liu:** None. **S. Duan:** None.

Poster

551. Neurogenesis and Gliogenesis: Glial Development and Interaction With Neurons

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 551.15/B9

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH grant R01NS072427
CHARGE syndrome Foundation

Title: A critical role of Autism-related chromatin remodeler CHD8 for oligodendrocyte development and remyelination

Authors: *C. ZHAO, C. DONG, R. LU
Brian Tumor Center, Cancer & Blood Dis. Inst., Cincinnati Children'S Hosp. Med. Ctr., Cincinnati, OH

Abstract: Disruptive mutations in chromatin remodeler CHD8 cause autism spectrum disorders, a heterogeneous disease with significant phenotypic complexity, exhibiting widespread white matter abnormalities; however, the underlying molecular and cellular mechanisms remain elusive. We show that cell-type specific deletion of Chd8 in oligodendrocyte progenitors, but not in neurons, results in myelination defects, revealing a cell-intrinsic dependence on CHD8 for oligodendrocyte lineage development, myelination and post-injury re-myelination. CHD8 activates expression of BRG1-associated SWI/SNF complexes that in turn activate CHD7, thus initiating a successive chromatin remodeling cascade that orchestrates oligodendrocyte lineage progression. Genome-wide occupancy and accessibility analyses reveal that CHD8 establishes an accessible chromatin landscape, and recruits KMT2 histone methyltransferase complexes distinctively around proximal promoters to promote oligodendrocyte differentiation. Inhibition of histone demethylase activity partially rescues myelination defects of CHD8-deficient mutants. Our data indicate that CHD8 exhibits a dual function through inducing a cascade of chromatin reprogramming and recruiting H3K4 histone methyltransferases to establish oligodendrocyte identity, suggesting potential strategies of therapeutic intervention for CHD8-associated white matter defects.

Disclosures: C. Zhao: None. **C. Dong:** None. **R. Lu:** None.

Poster

552. Stem Cells and Reprogramming: Neural Lineage Reprogramming

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 552.01/B10

Topic: A.03. Stem Cells and Reprogramming

Support: NRF Grant 2016R1A6A3A11936076

Title: A noble finding of miRNAs in neurogenic differentiation of human adipose tissue derived mesenchymal stem cells

Authors: *S. JANG, J.-S. PARK, S.-H. PARK, H.-S. JEONG
Chonnam Natl. Univ. Med. Sch., Gwangju, Korea, Republic of

Abstract: MicroRNAs (miRNAs) are small noncoding RNAs that emerge as regulators of stem cell lineage such as proliferation, development, differentiation, and apoptosis. We hypothesized that miRNA was involved in the neurogenic differentiation of mesenchymal stem cells. Here, the role of miRNAs in the neurogenic differentiation of human mesenchymal stem cells (MSCs) is investigated. By performing a miRNA-mRNA paired microarray screening, we identified miR-4650-5p and miR-3146 among the most upregulated miRNAs during neurogenic differentiation. After selection of the miRNAs, we investigated the ability of neurogenic differentiation of miRNAs in human adipose tissue-derived MSCs (hADSCs). We found that miR-4650-5p or miR-3146 was increased the most of neuronal gene expressions by a quantitative PCR. Using bioinformatics and functional assay, we confirmed that miR-4650-5p and miR-3146 potentially targeted on JNK and GSK3β to regulate Wnt signaling pathway. Overall comparative analysis revealed that Wnt signaling was enhanced more potently and played a more important role in neurogenic differentiation of hADSCs. These findings suggest that the miR-4650-5p and miR-3146 expression contributes the neurogenic differentiation of MSCs by increasing the neuronal genes and Wnt signaling pathway. The miRNAs regulation and downstream pathway network suggested the important role of miRNAs and Wnt signaling in the neurogenic differentiation of MSCs.

Disclosures: S. Jang: None. **J. Park:** None. **S. Park:** None. **H. Jeong:** None.

Poster

552. Stem Cells and Reprogramming: Neural Lineage Reprogramming

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 552.02/B11

Topic: A.03. Stem Cells and Reprogramming

Support: NRF Grant 2016R1A6A3A11936076

Title: The influence of Wnt5a activator and antagonist in neurogenic differentiation of mesenchymal stem cell *in vitro*

Authors: *H.-S. JEONG[1], S.-H. PARK[1], J.-S. PARK[1], H.-H. CHO[2], B.-C. KIM[3], S. JANG[1]
[1]Chonnam Natl. Univ., Jeollanam-Do, Korea, Republic of; [2]Otolaryngology Head and Neck Surgery, [3]Neurol., Chonnam Natl. Univ. Med. Sch., Gwangju, Korea, Republic of

Abstract: Mesenchymal stem cells (MSCs) have an ability to differentiate into multiple lineages, therefore, the possibility of neurogenic differentiation is important as a target for the clinical field. Wnt signaling, which is one of the remarkable regulators, plays a role in the development of the central nervous system and regulates the controlling neuronal differentiation. We hypothesized that regulating of Wnt signaling both activation and inhibition participated in the neurogenic differentiation of human adipose tissue-derived MSCs (hADSCs). In the present study, we developed the neurogenic differentiation of cells using an Anandamide, a Wnt5a activator, and Box5, a Frizzled-5-dependent Wnt5a antagonist, and studied the mechanisms for further differentiation *in vitro*. We treated Anandamide or Box5 and found that the Anandamide-treated cells have features such as neuron-like cells; exhibited distinct bipolar or multipolar morphologies with branched processes. Following PCR and quantitative PCR experiments, neuronal gene expressions were increased with Anandamide treatment; it was the same result; the protein levels of NFL and Tuj1 were highly expressed by immunofluorescence staining. We studied mechanisms of differentiation and found that Wnt signaling and downstream MAP kinase, especially GSK-3β pathway, were involved in neurogenic differentiation following Wnt5a activator. Especially, Wnt4 and Wnt11, which are a group of non-canonical Wnts, protein levels were highly increased after treatment of Anandamide; the Wnt5a activator could regulate the non-canonical Wnts signaling broadly. In addition, Anandamide activated through regulating Dvl2 and Dvl3 and resulted in expression of Axin level following highly increasing phosphorylated-JNK. Taken together, Wnt5a activator regulated the most of non-canonical Wnt signaling and the downstream pathway, especially controlling GSK-3β and JNK levels in the neurogenic differentiation of MSCs.

Disclosures: H. Jeong: None. **S. Park:** None. **J. Park:** None. **H. Cho:** None. **B. Kim:** None. **S. Jang:** None.

Poster

552. Stem Cells and Reprogramming: Neural Lineage Reprogramming

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 552.03/B12

Topic: A.03. Stem Cells and Reprogramming

Support: Glenn Center for Aging Research at the Salk Institute
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Title: Differentiation of suprachiasmatic nucleus (SCN) neurons from mouse and human fibroblasts

Authors: *M. HIRAYAMA, H. D. LE, L. S. MURE, S. PANDA
Salk Inst. For Biol. Studies, LA Jolla, CA

Abstract: The suprachiasmatic nucleus (SCN) in hypothalamus composed of heterogeneous population of 20,000 neurons acts as a master oscillator to orchestrate the approximately 24 hour cycle of behavior and physiology, which is essential for an organism to optimally adapt to environmental changes accompanying the 24 hours day/night cycle. The SCN is composed of neuropeptide expressing cells including VIP and AVP. The neuropeptides released from the SCN neurons play important roles in synchronizing circadian oscillations among SCN neurons and for communication with extra-SCN neurons. During embryonic hypothalamus ontogenesis, SCN differentiates from neuro-epithelium caudal to the optic recess. Distinct spatial and temporal patterns of expression of a small set of transcription factors (TFs) are presumed to mediate SCN development. In this study, we generated the induced SCN neurons (iSCN) from mouse and human fibroblasts by a direct conversion method with a small subset of TFs, which promotes neuronal conversion of fibroblasts into neurons with SCN neuronal phenotypes. We have identified TFs that are enriched in SCN tissue, many of which have been implicated in the differentiation and function of two principal subpopulations of SCN neurons - VIP and AVP neuropeptide expressing GABAergic neurons. We used a modified protocol of the induction method of neurons (iN) with pro-neuronal TFs of Ascl1 (Mash1) and Ngn2 (AN) with small molecule-based inhibition of glycogen synthase kinase 3β and SMAD signaling. The iN protocol in combination with a cocktail of SCN enriched TFs successfully differentiated SCN neurons (iSCN) from mouse embryonic fibroblasts (MEF). The iSCN showed efficient neuronal conversion from MEF in the neuronal conversion and maturation culture environment into

bipolar / multipolar GABAergic neurons, co-expressing VIP, which is the post-mitotic SCN enriched neuropeptides critical for the rhythmic circadian oscillation. The expression of VIP, AVP or Rorα was verified in the iSCN. The calcium transient in the iSCN showed spontaneous activities characteristic of mouse SCN neurons. Using the similar protocol we could also differentiate human fibroblasts to iSCN neurons containing GABAergic neurons with VIP or AVP expression. Our results suggest a possibility to generate principal subpopulations of SCN neurons from patients with suspected circadian rhythm disruption.

Disclosures: M. Hirayama: None. **H.D. Le:** None. **L.S. Mure:** None. **S. Panda:** None.

Poster

552. Stem Cells and Reprogramming: Neural Lineage Reprogramming

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 552.04/B13

Topic: A.03. Stem Cells and Reprogramming

Support: Intramural award to CJM
Intramural award to JCW

Title: Neocortical projection neurons instruct inhibitory interneuron circuit development in a lineage dependent manner

Authors: *J. C. WESTER[1], D. CALVIGIONI[5], S. HUNT[2], X. YUAN[3], C. J. MCBAIN[4]
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Abstract: In the cerebral cortex, excitatory pyramidal cells (PCs) can be segregated based on the target of their long-range axonal projection: intratelencephalic (IT) PCs target cortex/striatum while pyramidal tract (PT) PCs target the midbrain, brainstem, and spinal cord. Their output is regulated by inhibitory interneurons (INs), which can be segregated into two non-overlapping subgroups based on their embryonic lineage from either the caudal or medial ganglionic eminences (CGE and MGE). Interestingly, PCs and INs are biased in their laminar distributions: superficial layers contain exclusively IT PCs and the majority of CGE INs, while all PT PCs and the majority of MGE INs reside in deep layers. Here, we show that IT PCs influence the radial migration, molecular expression profile, and circuit integration of CGE INs during postnatal development. We used a Cre-Lox strategy in mice to conditionally knockout (KO) the transcription factor Satb2 from PCs during embryogenesis to induce them to adopt a PT-type identity. These mice were further crossed to the 5HT3A-GFP line to selectively label CGE INs. Loss of IT PCs disrupted CGE IN radial migration, such that a higher percentage of these cells settled in deep layers relative to controls. In contrast, the laminar positioning of MGE INs (PV and SOM+) was not affected. In cortex, CGE INs can be broadly parsed into VIP and Reelin

expressing subtypes. In KO mice, VIP INs were mislaminated, while the Reelin cohort was not and remained confined primarily to layer 1. VIP and Reelin expression remained non-overlapping. We probed for a third marker, CCK, which labels a small subset of superficial CGE INs. Surprisingly, the density of CCK+ CGE INs was dramatically increased in KO mice and they were found ectopically in deeper layers. We next performed dual whole-cell patch clamp recordings between PCs and CGE INs in superficial layers of KO and control mice to test for synaptic connections. We found that the probability of finding a connection from a PC to CGE IN (but not IN to PC) was significantly reduced in KO mice, suggesting IT PCs selectively target these INs. To confirm this, we injected retrograde tracers into the contralateral visual cortex or ipsilateral superior colliculus of control mice to target IT or PT PCs, respectively. In deep layers, where these types are intermingled, IT PCs made excitatory connections on to CGE INs whereas no connections from PT PCs to CGE INs were found. This connectivity bias was confirmed using combinations of transgenic mouse lines and viral vectors to drive channelrhodopsin-mediated input selectively from populations of PCs of either type. Our data show a selective influence of IT PCs on CGE IN circuit development.

Disclosures: J.C. Wester: None. **D. Calvigioni:** None. **S. Hunt:** None. **X. Yuan:** None. **C.J. McBain:** None.

Poster

552. Stem Cells and Reprogramming: Neural Lineage Reprogramming

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 552.05/B14

Topic: A.03. Stem Cells and Reprogramming

Support: DFG SFB 870
DFG SPP1557
EXC1010 Synergy

Title: Region and layer specific differences in astrocyte to neuron reprogramming after brain injury

Authors: *N. MATTUGINI[1,2,3], C. LAO[4,2], O. TORPER[4,2,5], M. GÖTZ[4,2,6]
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Abstract: Direct reprogramming of local glial cells into neurons is a promising approach for brain repair. A key question is which glial cells to target. Astrocytes are promising candidates as they retain expression of patterning transcription factors from their ancestors in development, the radial glial cells. Thus, astrocytes may be best specified to generate neuronal subtypes appropriate for the respective brain region. However, proliferating astrocytes perform beneficial functions after brain injury. We therefore decided to target non-proliferating astrocytes using AAVs that have a slow onset of expression peaking when astrocyte proliferation is over. The neurogenic factors were cloned in flexed orientation, so they are reverted only in GFAP-Cre expressing astrocytes.
Using this system we compared direct neuronal reprogramming in different positions in the Grey Matter (GM) and White Matter (WM) of the cerebral cortex after injury. We discovered a novel combination of either proneural factors (Neurog2 or Ascl1) with a transcription factor repressing oxidative stress that allows highly efficient neuronal reprogramming in GM astrocytes. Surprisingly, this very same combination did not reprogram WM astrocytes. We further show that the reprogrammed neurons in the cortex GM acquire different identities at different layer positions, demonstrating for the first time the profound influence that the region- and layer-specific identity of astrocytes has on the neuronal subtype generated in direct reprogramming in vivo.

Disclosures: N. Mattugini: None. **C. Lao:** None. **O. Torper:** None. **M. Götz:** None.

Poster

552. Stem Cells and Reprogramming: Neural Lineage Reprogramming

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 552.06/B15

Topic: A.03. Stem Cells and Reprogramming

Support: ERC-2014-CoG-647012
BFU2015-64432-R

Title: Clonal lineage determines the direct conversion of thalamic astrocytes into subtype-specific thalamocortical neurons

Authors: *A. HERRERO NAVARRO[1], V. MORENO-JUAN[1], A. SEMPERE[1], R. SUSÍN[1], B. ANDRÉS-BAYÓN[1], M. FIGUERES-OÑATE[3], J. LÓPEZ-ATALAYA[2], M. KAROW[4], L. LÓPEZ-MASCARAQUE[3], B. BERNINGER[5], G. LÓPEZ-BENDITO[1]
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Abstract: Forced expression of defined transcription factors leads to the direct conversion of various cell types into induced neurons (iNs). Specifically, successful reprogramming of resident brain astrocytes *in vitro* as well as *in vivo* represents a great advantage for the generation of neurons and derived circuits. However, whether astrocytes from distinct brain regions might show a reprogramming specificity towards a unique iN type remains largely unknown. Here, we use direct reprogramming of thalamic astrocytes by *Neurog2* to generate specific excitatory sensory-modality thalamocortical neurons. Moreover, we show that the origin, but not the environment of the astrocytes determines the fate of the iNs after direct reprogramming. Indeed, clonal analysis in the thalamus shows that astrocytes from the distinct thalamic nuclei are clonally related determining the specificity of the iNs generated from those astrocytes. We also found that the potential of the same transcription factor to reprogram nuclei-specific thalamic astrocytes into precise subsets of thalamocortical neurons depends on particular epigenetic modifications. In sum, our study provides novel insights into the mechanisms that control the specification of thalamic neurons and importantly those that are required for direct programming of sensory neurons. Generation of specific sensory brain circuits might be an approach for future rehabilitation strategies.

Disclosures: A. Herrero Navarro: None. **V. Moreno-Juan:** None. **A. Sempere:** None. **R. Susín:** None. **B. Andrés-Bayón:** None. **M. Figueres-Oñate:** None. **J. López-Atalaya:** None. **M. Karow:** None. **L. López-Mascaraque:** None. **B. Berninger:** None. **G. López-Bendito:** None.

Poster

552. Stem Cells and Reprogramming: Neural Lineage Reprogramming

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 552.07/B16

Topic: A.03. Stem Cells and Reprogramming

Support: NIH AG045656
Alzheimer's Association ZEN-15-321972
Charles H. "Skip" Smith Endowment Fund at Pennsylvania State University

Title: Molecular mechanisms of direct astrocyte-to-neuron conversion revealed by transcriptome analysis

Authors: *N. MA, B. PULS, J. YIN, G. CHEN
Biol., Pennsylvania State Univ., University Park, PA

Abstract: Our lab has previously demonstrated direct astrocyte-to-neuron conversion through either overexpression of a single neural transcription factor NeuroD1 (Guo et al., 2014, Cell Stem Cell) or a cocktail of small molecules (Zhang et al., 2015, Cell Stem Cell). Such direct

reprogramming technology represents a potential remedy for neuronal loss in neurodegenerative diseases and brain injuries. Despite the high conversion efficiency and fast procedure, the molecular events and downstream effectors during the astrocyte-to-neuron conversion are not well understood. To tackle these questions, we used time series analysis of RNA-seq data to characterize the transcriptome dynamics before, during, and after conversion, in order to understand the critical factors mediating such cell transition process. Administration of small molecules (core drugs: CHIR99021, DAPT, SB431542, LDN193189) significantly modified signaling pathways such as hedgehog, Wnt / β-catenin, SMAD and JAK / STAT. These signals rapidly elicited the neurogenic transcription factor network, including the members of bHLH family, and activated both excitatory and inhibitory neuronal genes. Meanwhile, converted cells exhibited a metabolic transition from glycolysis to oxidative phosphorylation, and reduced proliferation rate. Within two weeks, the gene ontology terms associated with neuronal functions became highly expressed. Moreover, we investigated the similarity and difference between chemical reprogramming mediated by core drugs and transcription factor NeuroD1 mediated cell conversion. Although both schemes turned on neurogenic programs, NeuroD1 overexpression showed more specific targeting and expedited conversion process, while core drugs had much broader effects. Together, these findings provide insights into the molecular mechanism of astrocyte-to-neuron reprogramming and may help develop efficient therapy for clinical applications. This work is supported by Charles H. Skip Smith Endowment Fund to Gong Chen.

Disclosures: N. Ma: None. **B. Puls:** None. **J. Yin:** None. **G. Chen:** None.

Poster

552. Stem Cells and Reprogramming: Neural Lineage Reprogramming

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 552.08/B17

Topic: A.03. Stem Cells and Reprogramming

Support: NSF IGERT Award

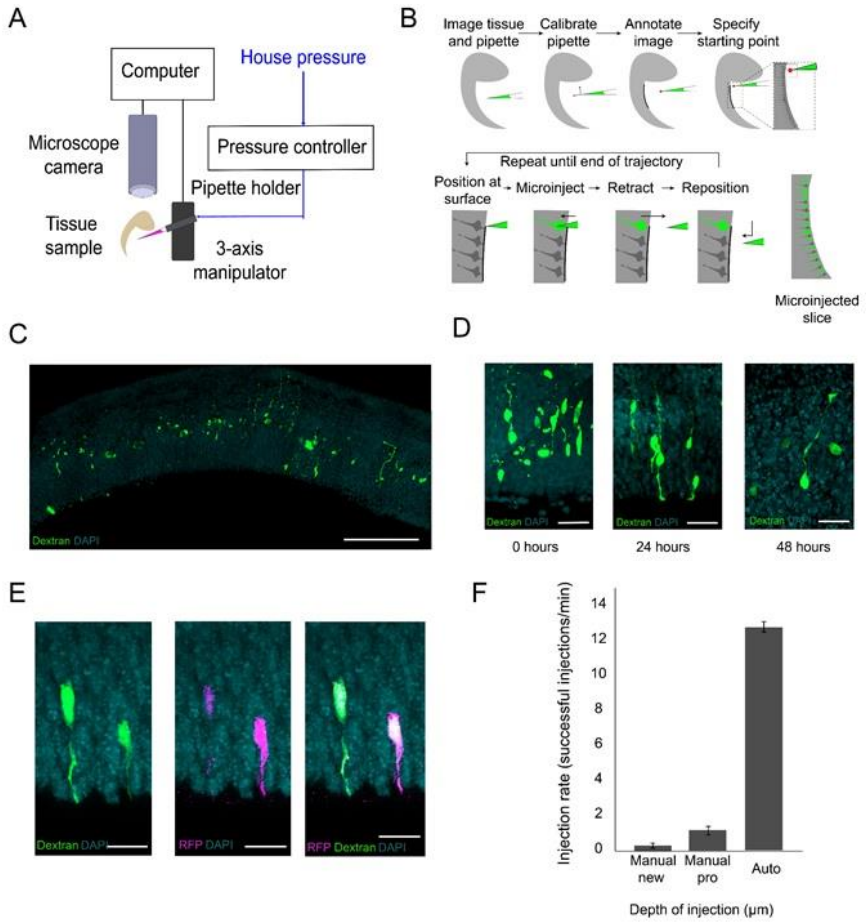
Title: The Autoinjector: An image guided microinjection platform for injecting progenitors in the mouse telencephalon

Authors: *G. SHULL[1], C. HAFFNER[3], W. HUTTNER[3], E. TAVERNA[4], S. B. KODANDARAMAIAH[2]
[2]Mechanical Engin., [1]Univ. of Minnesota, Minneapolis, MN; [3]Max Planck Inst. of Mol. Cell Biol. and Genet., Dresden, Germany; [4]Max Planck Inst. for Evolutionary Anthrop., Leipzig, Germany

Abstract: Understanding the genetic basis for the unique power of the human brain is a fundamental goal of neuroscience and has direct implications for eradicating neural pathologies.

An approach to understand the function of genes in brain development is to genetically manipulate progenitors using single cell manual microinjection of mRNA or dye in brain models and observe the effects on neural proliferation, migration, and differentiation. A limitation of manual microinjection is that it is time consuming, results in low yield, and requires expertise which has limited its adoption as a tool for investigating cell fate. We developed a computer vision guided robot, termed the 'Autoinjector', to overcome limitations of manual microinjection (Figure 1A, 1B). We used the Autoinjector to inject neural progenitors with mRNA, and/or dye and investigated the efficiency of the process using organotypic slices of the E14.5 mouse telencephalon. We demonstrated that the Autoinjector increases yield of injection relative to manual use (10-46 fold increase, Figure 1F), allows for targeting of large (800 μm) regions of tissue (Figure 1C), does not affect viability over 0, 24, and 48 hours in culture (Figure 1D), and enables mRNA translation of injected RFP after 24 hours in culture (Figure 1E). The autoinjector platform can thus open the door to new types of experiments investigating effects of mRNA concentration, composition on cell fate and tracking these effects on cell reprogramming and lineage using fluorescent dyes.

Figure 1: The Autoinjector. A. Hardware schematic of the Autoinjector. B. Algorithm procedure of microinjection. C. Cells injected with fluorescent dye and cultured for 24 hr. Scale bar is 200 μm. D. Images of cells injected with dye and cultured for 0, 24, or 48 hours. Scale bars are 50 μm. E. Cells injected with dye (green) and mRNA of RFP translate RFP after 24 hours of culture. Scale bar 25 μm. F. Injection yield for manual microinjection of a new user, experienced user, and automated platform.



Disclosures: G. Shull: None. **C. Haffner:** None. **W. Huttner:** None. **E. Taverna:** None. **S.B. Kodandaramaiah:** None.

Poster

552. Stem Cells and Reprogramming: Neural Lineage Reprogramming

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 552.09/B18

Topic: A.03. Stem Cells and Reprogramming

Support: NJCSCR16ERG019

Title: A bio-inspired artificial transcription factor for effective cellular reprogramming

Authors: *K. LEE[1], D. CHUENG[2], W. YOUNG[1], X. QIU[1], D. SUN[1]
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Abstract: This presentation will focus on the interface between nanoscience and cellular reprogramming. Even though it is well-established that stem cell fate and cellular reprogramming are regulated by interactions that occur between microenvironmental cues and intrinsic cellular programs, our understanding of the function of the microenvironment and gene expression during the aforementioned process is hampered by the limitations of conventional methods and the lack of extensive knowledge of multiple regulatory signals. For example, the devastation associated with spinal cord injury (SCI) causes severe and permanent neurological loss. Given the intrinsically limited regenerative potential of the central nervous system (CNS) and the complex inhibitory SCI environment, there is an urgent need for effective strategies towards robust axon regeneration and neurite outgrowth of neurons to re-establish the damaged neural circuitry. In this collaborative project, we have integrated several fields of research, Nanotechnology, Biomaterials, Chemical Biology, Neuroscience, and Stem Cell Biology, to develop a novel nanomaterial-based platform that induces axon regeneration and neurite outgrowth that are safe for *in vivo* transplantation and potential clinical applications. To address the fundamental impediment of regeneration associated with SCI, we propose to develop a non-viral delivery method of axon regeneration promoting transcription factor to the injured neurons. ***NanoScript***, ***nano****particle-based artificial tran****script****ion factor protein capable of efficiently and selectively regulating genes in a non-viral manner*, is a novel synthetic transcription factor platform suited for regulating transcriptional activity and targeted gene expression (e.g., PTEN, which has been identified as a targeted protein that modulates the PTEN/mTOR pathways regulating axon growth and regeneration). In this presentation, a summary of the most updated results from these efforts and future directions will be discussed.

Disclosures: K. Lee: None. **D. Chueng:** None. **W. Young:** None. **X. Qiu:** None. **D. Sun:** None.

Poster

552. Stem Cells and Reprogramming: Neural Lineage Reprogramming

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 552.10/B19

Topic: A.03. Stem Cells and Reprogramming

Support: CRACKIT23 Challenge Phase 1 award (NC/CO16206/1)
European Research Council (#614620)
MRC Confidence in Concept award (MC_PC_15030)
RP Fighting Blindness Innovation grant (GR584)

Title: Light responsive human stem cell derived retinal organoids for pharmacology and drug screening purposes

Authors: *E. SERNAGOR[1], D. HALLAM[2], G. HILGEN[3], B. DORGAU[2], M. FELLENBAM[2], L. ARMSTRONG[2], M. LAKO[2]

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Abstract: The availability of viable *in vitro* models of the human retina is crucial to investigate potential therapeutic approaches and perform toxicological studies. An essential step for developing such models is the ability to generate laminated, physiologically functional and light-responsive retinal organoids from renewable and patient specific sources. We investigated different human embryonic stem cell (hESC) and human induced pluripotent stem cell (iPSC) lines and found that there is significant variability in their efficiency to generate retinal organoids. Despite such variability, by month 5 of differentiation, the organoids were able to generate light responses, albeit immature, comparable to the earliest light responses recorded from the neonatal mouse retina, around the time of eye opening. By that time, all lines exhibited laminated retinal organoids with well-formed outer nuclear like layers containing photoreceptors with inner segments, connecting cilium and outer like segments. The differentiation process was highly dependent on seeding cell density and nutrient availability. We adopted the differentiation protocol to a multiwell plate format which enhances generation of retinal organoids with retinal pigmented epithelium and improves ganglion cell development and the response to physiological stimuli. We tested the response of iPSC-derived retinal organoids to Moxifloxacin and showed that similarly to *in vivo* adult mouse retina, the primary affected cell types were photoreceptors. Together our data indicate that light responsive retinal organoids derived from carefully selected and differentiation efficient human stem cell lines can be generated at the scale needed for pharmacology and drug screening purposes.

Disclosures: E. Sernagor: None. **D. Hallam:** None. **G. Hilgen:** None. **B. Dorgau:** None. **M. Fellenbam:** None. **L. Armstrong:** None. **M. Lako:** None.

Poster

553. Sensory Circuit Assembly and Reorganization

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 553.01/B20

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

Support: National Taiwan University
the Ministry of Science and Technology

Title: Exploring the interaction between stage II retinal waves and the glutamate release from retinal ganglion cells during development

Authors: *S.-P. HSU[1], C.-Y. YANG[1], H.-Y. CHEN[1], C.-T. WANG[1,2,3,4]
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Taiwan Univ., Taipei, Taiwan; [4]Genome and Systems Biol. Program, Natl. Taiwan Univ. and Academia Sinica, Taipei, Taiwan

Abstract: During a critical period of visual circuit refinement, stage II retinal waves are initiated by the release from starburst amacrine cells (SACs), propagating to retinal ganglion cells (RGCs). We previously found that overexpressing a calcium sensor triggering exocytosis, synaptotagmin I (Syt I), in RGCs enhances wave frequency and this effect can be abolished by iGluR antagonists, suggesting that RGCs may release glutamate to modulate stage II retinal waves. However, how the glutamate release from RGCs affects stage II retinal waves remains unknown. Here, we explore the interaction between stage II retinal waves and the glutamate release from RGCs. First, to determine the presence of glutamate in developing retinas, we performed immunofluorescence staining and found that glutamate was present in RGCs and inner plexiform layer in developing retinas. Second, to detect whether glutamate release is the volume release, we applied the cell-based glutamate optical sensor in developing retinas. In retinas overexpressing Syt I in RGCs, we found that the glutamate volume release was increased in the presence of CGS 21680, a selective agonist of adenosine A2AR shown to increase wave frequency via SACs. By contrast, the glutamate volume release cannot be increased by CGS 21680 in retinas overexpressing the Syt I dominant-negative mutant in RGCs, suggesting that increasing wave frequency further enhances the glutamate volume release from RGCs. Third, to determine the causal relation between wave frequency and glutamate transmission, we bath-applied CGS, iGluR antagonists, or both. We found that the CGS-mediated increase in wave frequency was abolished by iGluR antagonists, suggesting that glutamate transmission acts at the downstream of CGS-regulation of wave frequency. Further, to determine if glutamate acts in an autocrine/retrograde manner, we transfected the glutamate optical sensor in RGCs/SACs and found that glutamate was detectable by RGCs/SACs, suggesting that glutamate acts in an autocrine or retrograde manner. Moreover, intraocular injection of iGluR antagonists diminished the eye-specific segregation of dorsal lateral geniculate nucleus. Together, our data suggest that the glutamate release from RGCs is important for regulating stage II retinal waves.

Disclosures: S. Hsu: None. **C. Yang:** None. **H. Chen:** None. **C. Wang:** None.

Poster

553. Sensory Circuit Assembly and Reorganization

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 553.02/B21

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

Support: Fondecyt 1170027
Fondecyt 1151432

Title: Effects of the early eye removal on the visual DVR (molecular and neural) organization in chicks

Authors: R. REYES, C. NORAMBUENA, C. WEISS, G. MARIN, *J.-C. LETELIER, J. MPODOZIS
Univ. of Chile, Santiago, Chile

Abstract: One of the main components of the avian pallium is a dorsal intraventricular protrusion termed dorsal ventricular ridge (DVR), which is constituted by two apposed cellular masses: the internal nidopallium (N) and the more external mesopallium (M). The internal most aspect of the N contains discrete areas receiving auditory, visual and trigeminal ascending projections. Of particular interest to us is the visual DVR, which can be regarded as a complex composed of three highly interconnected layers: an internal nidopallial layer, the entopallium (E), in receipt of visually driven afferents from the thalamic nucleus rotundus (Rt); an overlaying nidopallial layer, the intermediate nidopallium (NI), which serves an associative role connecting to other pallial areas; and a more external mesopallial layer, the ventral mesopallium (MV) which participate in the local interlaminar circuitry. 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. Previously, we have found that the establishment of the highly organized Rt- E projection, as well as that of the E-MV reciprocal connectivity, occurs very early in chick development and before the retinal fibers had reached their central targets. In the present study we investigated the possible influence of the establishment of retino-central synapsis in the development and maintenance of this intrapallial circuitry. To that end we performed monocular and binocular enucleations in chick embryos early in development in order to analyze the neural arrangement as well as the molecular profile of the visual DVR at different developmental stages, from E6 to E18. We found, as classic works did, that these manipulations altered the cytoarchitecture of retinorecipient structures such as the ventral lateral Geniculate Nucleus (GLv) and the Tectum opticum (TeO), which is the source of visually driven afferents to the Rt. Even more, these enucleations also altered the structure of second order visual centers, such as the isthmic nuclei. However, neither mono nor binocular enucleations modified the pattern of connectivity and molecular pattern expression of the visual DVR at any stage of the developmental series analyzed. These results indicate that, unlike mammals, in birds the establishment and maintenance of a highly organized pallial visual circuitry is independent of the retinal afferent influences.

Disclosures: R. Reyes: None. **C. Norambuena:** None. **C. Weiss:** None. **G. Marin:** None. **J. Letelier:** None. **J. Mpodozis:** None.

Poster

553. Sensory Circuit Assembly and Reorganization

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 553.03/B22

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

Title: Cross-hierarchical corticothalamic plasticity following sensory deprivation in the mouse visual system

Authors: *C. GIASAFAKI, E. GRANT, S. HAYASHI, A. HOERDER-SUABEDISSEN, Z. MOLNAR
Dept. of Physiology, Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom

Abstract: The thalamus is considered the relay center for sensory information to the cortex and has an essential role in the regulation of fundamental brain processes, including sleep, alertness, consciousness and cognition, via various distinct nuclei. However, the mechanisms governing corticothalamic connectivity and plasticity are still largely unknown. In this study, we examine neuronal rewiring of cortical Layer 5 (L5) projections and the plasticity of these axons to structurally rearrange following visual deprivation. This compensatory mechanism is observed in the absence of visual input as a result of congenital blindness. After visually depriving L5-labelled (Rbp4-Cre::tdTomato) transgenic mice at birth by performing monocular enucleation (MoE), we study the effects on the ingrowth of corticofugal projections into visual thalamic nuclei, the first-order dorsal lateral geniculate nucleus (dLGN) and the higher-order lateral posterior nucleus (LP). L5 fibres do not normally innervate dLGN, but only higher order thalamic nuclei; however, they rewire to innervate dLGN after MoE (Grant et al., 2016 *Cereb Cortex* 26(3):1336-48). In order to investigate the origin of aberrant L5 projections in the sensory deprived dLGN (only receiving ipsilateral retinal input after enucleation), we performed injections of Cre-dependent, GFP-expressing adeno-associated virus 2 (AAV2) in the primary visual (V1) and somatosensory (S1) cortices of adult Rbp4-Cre mice (n=5 for V1, n=2 for S1) that had been monocularly enucleated at birth. Our findings indicate innervation of GFP positive axons in dLGN and LP only from V1 with formation of aberrant side branches and vesicular glutamate transporter 1 (VGluT1) positive boutons in the deprived dLGN. No alterations in the S1 projections to thalamus were observed following MoE. Additionally, we examine the molecular changes induced by MoE in the thalamus at postnatal days 6 and 8 as previous microarray and real-time quantitative PCR data from our lab demonstrated changes in gene expression in dLGN upon MoE (Grant E., 2017, unpublished data). We performed *in situ* hybridization for validating gene expression of cell signaling and extracellular matrix molecules, and confirmed differential expression in control and deprived dLGN (n=4), suggesting a potential functional role of these genes in L5 aberrant ingrowth. These findings provide opportunities for further investigation of the cellular and molecular factors implicated in the

rewiring of L5 projections in dLGN after visual deprivation, which could potentially give insights into the mechanism of cross-hierarchical corticothalamic plasticity in the mouse visual system.

Disclosures: C. Giasafaki: None. **E. Grant:** None. **S. Hayashi:** None. **A. Hoerder-Suabedissen:** None. **Z. Molnar:** None.

Poster

553. Sensory Circuit Assembly and Reorganization

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 553.04/B23

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

Support: Welcome Trust Grant 204788/Z/16/Z

Title: The organisation, dynamics and development of neural assemblies in the tectum

Authors: *T. SAINSBURY[1], G. DIANA[2], M. MEYER[2]
[2]Dept. of Developmental Neurobio., [1]King's Col. London, london, United Kingdom

Abstract: Neural assemblies (or ensembles) are groups of coactive neurons whose activity may be triggered spontaneously, by sensory stimuli or behaviour. Such assemblies are therefore likely to constitute the building blocks of brain function, but little is known about their structure, organization and dynamics. Such descriptions will provide insight into circuit connectivity, constraints and preferred states and will be crucial for determining how brain function emerges from assembly firing sequences. Here we use functional imaging in larval zebrafish to describe the structure and dynamics of spontaneous activity in the optic tectum. Using 2-photon volumetric imaging we can capture the activity of between 8,000-10,000 neurons throughout both tectal hemispheres at 4.8Hz. Using Bayesian inference techniques we are able to make probabilistic estimates of assembly number, three-dimensional structure, and within- and between-assembly dynamics. We are also using these methods to determine how tectal assemblies emerge over the course of development and how their development is shaped by activity-dependent plasticity. Specifically, we are testing how developmental shifts in the subunit composition of the NMDA receptor contribute to the developmental refinement of tectal assemblies.

Disclosures: T. Sainsbury: None. **G. Diana:** None. **M. Meyer:** None.

Poster

553. Sensory Circuit Assembly and Reorganization

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 553.05/B24

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

Support: Whitman College Perry and Abshire Awards
A gift from the family of Dr. R.F. Welty

Title: Profiling synapse density changes across development in the visual cortex of rats using quantitative immunofluorescence

Authors: *G. S. WITHERS, M. B. LAWRENCE, H. B. FADENRECHT, J. C. HODGSON, C. S. WALLACE
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Abstract: Classic neuroanatomical studies have shown that the net number of synapses in the visual cortex of rats increases dramatically for a period after eye opening and thereafter plateaus into adulthood. More recently, live imaging studies have demonstrated that filopodia and spines are dynamic over this entire time course, but the relative rates of addition and removal shift in ways that appear to reflect developmental status, and are likely to be predictive of synapse dynamics. Combined, these studies show an initial developmental phase that involves a higher rate of addition than removal (consistent with a net increase in synapse number), and a plateau in adulthood that suggests the rates of addition and removal attain equilibrium, and increased synapse stability. Live imaging has also revealed an intermediate period in rodent cortex when removal outpaces addition. This stage of "pruning" predicts a net reduction in synapse number. In mouse visual cortex, the temporal parameters from peak to adult levels vary by cortical region and method of imaging, but in visual cortex, it is around one month. Similar data on dynamics are not available for the rat, but determining the time course of synapse density changes could help reveal if the patterns observed in mice can be generalized to rats. Here, we used quantitative immunofluorescence and stereology to analyze how synapse and neuron density changed across development in the primary visual cortex of the Long Evans hooded rat. Littermates were housed socially, under controlled, identical conditions. Brain tissue was collected at postnatal days 7, 14, 21, 24, 30, 45, 60 and 90. Synapses were detected using an automated image analysis algorithm for coincident staining of both pre- and post-synaptic markers (antibodies for Synapsin I, or VGLUT1/2 for presynaptic sites; PSD95 was used to identify postsynaptic sites). To minimize potential variability associated with the stratified organization of cortex, we restricted our analyses to layers II/III of the primary visual cortex, and distinguished between monocular and binocular regions. The highest density of synapses was between P30 and P45, and was significantly greater than at P90. These data fit well with critical periods for visual system

plasticity in the rat, but also help to identify strategic time points for analysis of mechanisms of synapse formation and pruning associated with developmental plasticity in the rat.

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Poster

553. Sensory Circuit Assembly and Reorganization

Location: SDCC Halls B-H

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Program #/Poster #: 553.06/B25

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

Support: NIH Grant DC013304 to D.D.S.
Fulbright Scholar to D.D.S.
Wellcome Trust to W.M. (102892)

Title: Mutant mouse models with reduced ionic signaling pathways have cochlear outer hair cells with disrupted efferent and afferent innervation patterns

Authors: *D. D. SIMMONS[1,2], A. COX[1], J. MCCLUSKEY[1], F. CERIANI[2], A. HENDRY[2], J.-Y. JENG[2], W. MARCOTTI[2]
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Abstract: In the mammalian cochlea, outer hair cells (OHCs) amplify vibrations of the cochlear partition that directly enhance sensitivity and frequency selectivity. The onset of OHC function depends on a variety of ionic signaling mechanisms and is associated with major changes in both afferent and efferent connections to the OHCs. Prior to the onset of hearing, afferent Type II spiral ganglion fibers and terminals form extensive arbors with OHCs, and efferent cholinergic olivocochlear fibers form terminals below OHCs.
We investigated whether disruption of either non-sensory GAP junctions or Ca^{2+} membrane channels alter afferent or efferent OHC innervation in pre-hearing mice. We used connexin 30 $(Cx30)^{-/-}$ mice to investigate disruption of GAP junctions in non-sensory cells, and voltage-gated Ca^{2+} channel 1.3 $(Cav1.3)^{-/-}$ mice to investigate disruption of Ca^{2+} entry through voltage-gated channels. Compared to wild-type controls, $Cx30^{-/-}$ mice had fewer peripherin-labeled outer spiral fibers (OSFs), reduced peripherin-labeled OSFs crossing the tunnel of Corti, fewer choline acetyltransferase (ChAT)-labeled efferent fibers crossing the tunnel of Corti, and absent or irregular efferent terminals. Compared to wild-type controls, $Cav1.3^{-/-}$ mice demonstrated a similar reduction in peripherin-labeled OSFs but unlike either $Cx30^{-/-}$ or wild-type controls, OSFs in $Cav1.3^{-/-}$ mice spiraled in both apical and basal directions. Also compared to wild-type controls, $Cav1.3^{-/-}$ mice had fewer ChAT-labeled tunnel crossing fibers, highly disorganized efferent terminal patterns, and very dense ChAT labeling below inner hair cells. For both $Cx30^{-/-}$

and Cav1.3$^{-/-}$ mice, these innervation abnormalities were more severe in the apex. Furthermore, we found presynaptic ribbons (e.g., CtBP2) and postsynaptic proteins (e.g., SK2) altered in Cav1.3$^{-/-}$ mice, but only presynaptic ribbons altered in Cx30$^{-/-}$ mice. We conclude that disruption of ionic signaling mechanisms via the absence of connexins in non-sensory cells or the lack of Ca^{2+} voltage-gated membrane channels severely alters afferent and efferent innervation patterns. Thus, both of these signaling paths are critical for the maturation of normal cochlear OHC innervation.

Disclosures: D.D. Simmons: None. **A. Cox:** None. **J. McCluskey:** None. **F. Ceriani:** None. **A. Hendry:** None. **J. Jeng:** None. **W. Marcotti:** None.

Poster

553. Sensory Circuit Assembly and Reorganization

Location: SDCC Halls B-H

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Program #/Poster #: 553.07/DP02/B26

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

Support: NIH Grant DC005798 to GS
CoBRE Grant P30 GM103503 to GS
P41 GM103412 to ME
Howard Hughes Medical Institute
Gordon and Betty Moore Foundation

Title: Rapid terminal reorganization within local target territories during formation of the giant nerve terminal, the calyx of Held

Authors: *D. R. JACKSON[1], J. M. HEDDLESTON[2], M. MOOREHEAD[1], T.-L. CHEW[2], S. PIDHORSKYI[1], P. S. HOLCOMB[1], S. SIVARAMAKRISHNAN[1], S. M. YOUNG, JR[3], S. RAY[1], T. DEERINCK[4], M. H. ELLISMAN[4], G. A. SPIROU[1]
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Abstract: The large CNS nerve terminal, the calyx of Held (CH), provides strong and temporally precise excitation to targeted principal cells of the medial nucleus of the trapezoid body (MNTB). CHs exhibit hallmark developmental features of strengthening and pruning necessary to yield monoinnervation of MNTB neurons. Using serial block-face scanning electron microscopy (SBEM) collected from neonatal littermate mice (24-48 hr sampling), we previously found that between postnatal (P) days 2 and 4, most of 10-20 small pioneering inputs on each cell are pruned while 1-3 terminals grow at rates of 200 μm^2 per day. By P6, 75% of principal cells are monoinnervated. During this period, collateral processes of variable length extend from

the edges of the CH and are known sites of Ca entry. Although we found no evidence of direct interaction between CHs along the principal cell soma, collaterals largely occupy shared territories. However, little is known about their function in this system. Our lab is interested in the competition that mediates this competitive process. Here, we sought to examine the temporal dynamics of synaptic organization in the MNTB as it relates to the ultrastructure revealed with SBEM. We employed lattice light-sheet (LLS) microscopy which offers rapid and high-resolution image acquisition with minimal bleaching. Acute coronal brainstem slices (300-600 μm thickness) were collected in neonatal mice ranging from P0-14. Following 4D image acquisition, data was imported into syGlass, a custom software package designed in-house, for immersive virtual reality (VR) aided-analysis. Processes were manually tracked in syGlass to reveal growth dynamics of CHs and their associated processes, both filopodia- and growth cone-tipped terminal arbors. We found these collaterals form a dynamic field around each CH. Peak motility coincided with CH growth and peak dynamics rival or exceeded rates of axonal extension described elsewhere in the CNS. Growth cones, perhaps the most dynamic feature of this system, extended fastest during the ages of CH expansion, yet slowed as monoinnervation was established (P2: 21±18 μm/hr; P3: 43±20 μm/hr; P4: 58±24 μm/hr; P5: 58±21 μm/hr). Moreover, live imaging and ultrastructural analysis presented here established CH arbors repeatedly form transient associations with other CHs, neurons and glia within the neuropil. Thus, physical interaction may serve an instructive role in circuit formation within the MNTB. In summary, these data are effective in monitoring the navigation patterns of assembling neural circuits in an intact system and reveal the dynamic nature of growth and retraction of developing neurite reorganization.

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Poster

553. Sensory Circuit Assembly and Reorganization

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 553.08/B27

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

Support: UCR SEED funds

Title: Contributions of peri-neuronal nets to parvalbumin-positive interneuron excitability in developing mouse auditory cortex (A1)

Authors: S. MAPLES[1], J. KOKASH[2], K. RAZAK[1], T. A. FIACCO[3], *P. W. HICKMOTT[1]
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Cellular, Mol. and Systems Biol. & Interdpartmental Neurosci. Pgm., Univ. California, Riverside, Riverside, CA

Abstract: Cellular and circuit properties of primary auditory cortex (A1) change during postnatal development. In particular, by approximately P21 in mice and rats, the response properties and the balance of excitation and inhibition in A1 are similar to those found in adults. This maturation of the A1 circuit also approximately coincides with the closure of the critical period for changes in tonotopy in A1. Thus, the period from P14-P21 in mice is a time of considerable change in the properties of A1.

One important population of inhibitory interneurons in neocortex are parvalbumin-positive (PV) cells. PV cells are known to participate in the proper development and regulation of the balance of excitation and inhibition in a variety of cortical areas. An interesting feature of these PV cells is that most of them are surrounded by an extracellular matrix structure referred to as a peri-neuronal net (PNN). PNNs are known to regulate the excitability of neurons that they surround, and the number of PV cells that are surrounded by PNNs increases from P14 to P21 in mouse A1. Therefore, we hypothesize that at least some of the changes in A1 circuit properties from P14-P21 are regulated by the emergence of PNNs, due to their influence on PV neuron excitability.

We have performed whole-cell recordings *in vitro* from slices of A1 in mice that express the fluorophore tdTomato specifically in PV cells. This preparation allows us to specifically target only PV neurons. We have analyzed data from P14-P21 mice because it is during this developmental period that the PNNs are developing in A1. In order to assess their intrinsic excitability, we have determined their responses to hyperpolarizing and depolarizing steps of current (500 msec duration). We have also examined synaptic excitability onto these cells by analyzing spontaneous synaptic events. After these recordings the presence or absence of PNNs around the PV cells was determined using staining for wisteria floribunda lectin (WFA), which labels PNNs, and confocal microscopy. We will present data comparing the excitability of PV neurons that are surrounded by PNNs and those that are not. We hypothesize that PV neurons without PNNs will exhibit reduced excitability (either intrinsic or synaptic) as compared to those that express PNNs.

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Poster

553. Sensory Circuit Assembly and Reorganization

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 553.09/B28

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

Title: Clustered protocadherins regulated high reciprocal connectivity between clonal cortical neurons are selectively modified by short sensory deprivation in mouse barrel cortex

Authors: *E. TARUSAWA[1], M. SANBO[2], M. HIRABAYASHI[2], T. YAGI[1], Y. YOSHIMURA[3]
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Abstract: The specificity of neural connections in the sensory cortex is fundamental for the proper processing of sensory information. We previously have shown that high reciprocal connectivity is established between clonal cortical neurons and it's regulated by clustered protocadherins in mouse barrel cortex. In this study, we analyzed the effect of sensory experience on the establishment of the cell-lineage-dependent reciprocal connectivity. To visualize clonal neurons, we generated chimeric mice by injecting a small number of induced pluripotent stem cells (iPS cells) marked with GFP into blastocysts. We conducted dual whole-cell recordings from GFP-positive neuron pairs (presumed clonal pairs) or GFP-positive and negative neuron pairs (non-clonal pairs) within a layer 4 barrel in cortical slices prepared from the chimeric mice. Sensory deprivation was produced by whisker trimming from postnatal day 13 (P13) to a day before recording.
In normal development, there was no significant difference in the connection probability (the number of detected connections/the number of tested connections) between clonal and non-clonal pairs at P9-11. The probability increased significantly from P9-11 to P15-16 and then decreased only in clonal pairs, resulting in the same connection probability in clonal and non-clonal pairs at P18-20. Sensory deprivation completely prevented the temporal increase in the connection probability only in clonal pairs. Therefore, cell-lineage-specific neural connections seem selectively modified by sensory experience.
We next analyzed the reciprocity, the proportion of reciprocally connected pairs among connected pairs. The reciprocity was not significantly different between clonal and non-clonal neuron pairs at P9-11. Then the reciprocity continued to increase significantly in clonal pairs until P18-20, whereas it showed only an insignificant increase in non-clonal pairs during that period. Sensory deprivation prevented almost completely the increase in the reciprocity in clonal pairs, while it did not affect the reciprocity in non-clonal pairs. These results suggest that the sensory inputs are required for the proper function of clustered protocadherins leading to establishment of cell-lineage-dependent reciprocal connections.

Disclosures: E. Tarusawa: None. **M. Sanbo:** None. **M. Hirabayashi:** None. **T. Yagi:** None. **Y. Yoshimura:** None.

Poster

553. Sensory Circuit Assembly and Reorganization

Location: SDCC Halls B-H

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

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"Dynamic regulation of Brain Function by Scrap & Build System" (JP16H06459)

Title: Dynamics of dendritic tree selection revealed by long-term *in vivo* imaging of neonatal barrel cortex layer 4

Authors: *S. NAKAZAWA[1,2], H. MIZUNO[1,2,3], T. IWASATO[1,2]
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Abstract: Proper neuronal circuit function relies on precise dendritic projection, which is established through activity-dependent refinement during early postnatal development. Here we revealed the dynamic mechanisms associated with dendritic refinement in the mammalian brain by conducting long-term in vivo imaging of the neonatal mouse barrel cortex. In the mature mouse barrel cortex, spiny stellate (SS) neurons and thalamocortical (TC) axon termini form “barrels” that are morphologically and functionally distinct modules corresponding to individual whiskers on the face. In each barrel, SS neurons are located around the barrel edge and extend the basal dendrites (BDs) selectively toward the barrel center to make synapses with TC axons. We visualized SS neurons in vivo by using in utero electroporation-based Supernova labeling (Mizuno et al., 2014; Luo et al., 2016) and TC axons by using the TCA-GFP Tg mouse (Mizuno et al., 2014). By “retrospective” analyses, we identified “prospective” barrel-edge SS neurons in early neonates, which had an apical dendrite and primitive BDs. These neurons retracted the apical dendrite gradually and established strong BD orientation bias through continuous “dendritic tree” turnover. A greater chance of longevity was given to BD trees emerged in the barrel-center side, where TC axons cluster. Additionally, we conducted long-term in vivo imaging and in vivo calcium imaging of infraorbital nerve cut mice to investigate the impact of the neural activity on the dendritic refinement. Our in vivo imaging system contributes to understanding of developmental mechanisms of cortical maturation in neonates.

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

Poster

553. Sensory Circuit Assembly and Reorganization

Location: SDCC Halls B-H

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Program #/Poster #: 553.11/B30

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

Support: Kakenhi: 15KK0318 to CI
KawanoMasanori Memorial Public Interest Incorporated Foundation to CI
Kakenhi: 17K07057 to FK

Title: Effects of exogenously administered cannabinoids on axonal projections of L4 neurons in the mouse barrel cortex

Authors: *C. ITAMI[1], J.-Y. HUANG[2], H.-C. LU[3], F. KIMURA[4]
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Abstract: Recent studies revealed that cannabinoid CB_1 receptors (CB_1Rs) play important roles in the development of neural circuit formation and plasticity. In the rodent barrel cortex, CB_1Rs at the thalamocortical terminals causes a developmental switch from long-term potentiation (LTP) to long-term depression (LTD) both in a spike timing dependent manner (tLTP, tLTD) during the second postnatal week. In addition, endogenous cannabinoid ligands help regulate the thalamocortical termination within layer 4 (L4) barrel areas since disorganized thalamocortical termination was observed in CB_1R-KOs (Itami, 2016). Subsequently, CB_1Rs appear at L4 terminals from the beginning of the third postnatal week (P13-15), as we showed previously, which again causes a switch from tLTP to standard STDP with LTP and LTD components. We also showed that L4 axon morphology is attenuated in mutant mice lacking an endogenous ligand, 2-arachidonoylglycerol (2-AG) synthesizing enzyme, diacylglycerol lipase α (DGLα). In the present study, we asked whether administration of cannabinoid agonists causes any effects on L4 axon morphology. We injected either Δ^9-tetrahydrocannabinol (THC, 2mg/Kg body weight, i.p.), or WIN, a CB1R agonist (5mg/Kg, i.p.) from postnatal day 12 (P12) to P22. At P18-22, thalamocortical slice were made and whole-cell patch recordings were performed from L4 spiny stellate neurons using neurobiotin in the recording pipettes. Slices were fixed with PFA, then observed under confocal microscopy, and axon morphology was analyzed with Image-J and Neurolucida. There were significant reduction in total axon length of L4 spiny stellate neurons in CB1R agonist-treated animals. In control, total axon length was 9037±716 μm (n=12), but it was 8237±444 μm in THC ($p<0.05$, n=13), and 6272±531 μm in WIN ($p<0.01$, n=5). Similarly, axon length in L2/3, home column in L2/3 were also significantly reduced in THC and WIN treated

animals. These results, together with DGLα-KO study, indicate that cannabinoid signalling plays an important role in regulating L4 axon morphology during development.

Disclosures: C. Itami: None. **J. Huang:** None. **H. Lu:** None. **F. Kimura:** None.

Poster

553. Sensory Circuit Assembly and Reorganization

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 553.12/B31

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

Support: Machaon Foundation

Title: Single-cell molecular connectomics of somatosensory cortex circuit assembly

Authors: *E. KLINGLER[1], J. PRADOS[1], J. KEBSCHULL[2], A. ZADOR[3], A. DAYER[1], D. JABAUDON[1]
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Abstract: Intracortically projecting neurons are a heterogeneous population, which send their axon across cortical areas, both within and across hemispheres. Understanding the precise connectivity and diversity of these neurons is important, because intracortical projections allow coordination of neuronal activity across cortical areas and behaviourally critical sensorimotor transformation. Although population-based intracortical wiring diagrams have been identified, the single-cell connectivity of these neurons and their corresponding developmental transcriptional programs remain unknown. Here, we address this question by combining a barcoding strategy to identify single-cell connectomics (Kebschull et al., Neuron, 2016), with single-cell RNA sequencing to identify the developmental gene expression programs of hodologically-defined single neurons. By combining these two scalable single-cell resolution approaches, we showed developmental dynamics of primary somatosensory intracortical projections and found transcriptional programs defining specific connectivity patterns.

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Poster

553. Sensory Circuit Assembly and Reorganization

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Program #/Poster #: 553.13/B32

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

Support: MOST Grant 2016YFA0501000
NNSFC Grant 31530030

Title: Multiple morphological factors underlie experience-dependent crossmodal plasticity in the sensory cortices

Authors: M. WANG, Z. YU, *X. YU
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Abstract: During the early postnatal period, neural activity, both in the form of spontaneous electrical activity and sensory stimulation, is critical to the formation of functional neural circuits. A large body of work using unimodal sensory deprivation manipulations has shown that depriving the appropriate inputs during early development reduced responsiveness in the corresponding cortical region. Previous work in our laboratory showed that whisker deprivation (WD) during early development not only reduced the excitatory synaptic transmission of the correspondent cortical region, but also crossmodally reduced synaptic transmission in other sensory cortices (Zheng et al., Nat. Neurosci., 2014, doi:10.1038/nn.3634). Here, we investigate the morphological basis of this crossmodal plasticity. We found that WD from P0 to P14 reduced presynaptic bouton density, and possibly also spine density, of L2/3 pyramidal neurons in the primary somatosensory cortex (S1), as well as crossmodally in the primary auditory cortex (Au1). Combining *in utero* electroporation with an optimized optical clearing agent for high-resolution fluorescence imaging (SeeDB2), we identified various changes in dendrite and axon arborization the S1 and Au1 of WD mice. Increasing sensory experience by rearing mice in an enriched environment significantly rescued the effects of sensory deprivation, providing evidence for directional regulation of structural plasticity by sensory experience. Together, these results demonstrate that multiple morphological factors contribute to experience-dependent structural plasticity during early neural circuit development.

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Poster

554. Rett Syndrome

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 554.01/C1

Topic: A.07. Developmental Disorders

Title: Integrative behavioral, molecular and electrophysiological analyses of female Mecp2$^{tm1.1\ Bird}$ Rett syndrome model

Authors: *J. A. SANCHEZ, J. PALMA, K. KRETSCHMANNOVA, J. BELTRAN, M. KWAN, L. THIEDE, T. HANANIA, A. GHAVAMI
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Abstract: Rett Syndrome is a neurodevelopmental disorder caused by mutations in the *Mecp2* gene encoding for the methyl-CpG-binding protein 2 (MeCP2). While most studies have analyzed male *Mecp2* mice, analysis of female mice is clinically relevant to the female population of Rett syndrome patients. A combination of behavioral, molecular, and electrophysiological techniques has been employed here in the female Mecp2$^{tm1.1Bird}$ model (CreLox-deletion of exon3-4 deletion). Behavioral studies in female Mecp2$^{tm1.1Bird}$ mice show that the heterozygous mice have motor imbalance, gait deficits, breathing abnormalities, and impaired cognitive function. Extracellular field recordings in hippocampal slices from 6-month old female Mecp2$^{tm1.1Bird}$ mice displayed a reduction in long-term potentiation (LTP) at the Schaffer collateral-CA1 synapse. Given that MeCP2 protein regulates gene expression, quantitative polymerase chain reaction (qPCR) analysis was employed here using hippocampal tissue from 4 and 10-month old female Mecp2$^{tm1.1Bird}$. qPCR analysis revealed a reduction in three known genes regulated by MeCP2: *Bdnf*, *Sapap3*, and *Kir4.1*. A reduction in mRNA coding for synaptic markers *Psd95* and *synaptophysin* was also detected along with upregulated mRNA levels for glutamate receptors (*Glur1*, *Glur2*, *Nr2a*, and *Nr2b*). Altogether, this integrative analysis suggests that female *Mecp2* mice displayed significant behavioral and synaptic plasticity deficits, along with robust alterations in gene expression that can be utilized as disease readouts for preclinical testing.

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Poster

554. Rett Syndrome

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 554.02/C2

Topic: A.07. Developmental Disorders

Support: CNMPB
SFB1286

Title: Neuronal redox-imbalance in Rett syndrome affects mitochondria as well as cytosol, and is accompanied by intensified mitochondrial O_2 consumption and ROS release

Authors: *M. MUELLER, K. CAN, C. MENZFELD, P. REHLING, S. KUEGLER, J. DUDEK
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Abstract: Rett syndrome (RTT), an X chromosome-linked neurodevelopmental disorder affecting almost exclusively females, is associated with various mitochondrial alterations. Mitochondria are swollen, show altered respiratory rates, and their inner membrane is leaking protons. To advance the understanding of these disturbances and to clarify their link to redox impairment and oxidative stress in RTT, we assessed mitochondrial respiration in defined brain regions and cardiac tissue of male wildtype (WT) and MeCP2-deficient (*Mecp2*$^{-/y}$) mice. Also, we quantified for the first time neuronal redox-balance with subcellular resolution in cytosol and mitochondrial matrix. Quantitative roGFP1 redox imaging revealed more oxidized conditions in the cytosol of *Mecp2*$^{-/y}$ hippocampal neurons than in WT neurons. Furthermore, cytosol and mitochondria of *Mecp2*$^{-/y}$ neurons showed clearly exaggerated redox-responses to hypoxia and cell-endogenous reactive oxygen species (ROS) formation. Biochemical analyzes exclude a disease-related increase in mitochondrial mass in *Mecp2*$^{-/y}$ hippocampus and cortex. Protein levels of complex I core constituents were slightly lower in *Mecp2*$^{-/y}$ hippocampus and cortex than in WT; those of complex V were lower in *Mecp2*$^{-/y}$ cortex. Respiratory supercomplex-formation did not differ among genotypes. Yet, due to reverse electron flow into complex I, mitochondria of *Mecp2*$^{-/y}$ cortex and hippocampus consumed more O_2 than WT. Furthermore, mitochondria from *Mecp2*$^{-/y}$ hippocampus released more ROS. In conclusion, we further advanced the molecular understanding of mitochondrial dysfunction in RTT. Intensified mitochondrial O_2 consumption, increased mitochondrial ROS generation and disturbed redox balance in mitochondria and cytosol represent a causal chain, which provokes dysregulated proteins, oxidative tissue damage, and finally neuronal network dysfunction in RTT.

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Poster

554. Rett Syndrome

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 554.03/C3

Topic: A.07. Developmental Disorders

Support: International Rett Syndrome Foundation
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Title: Vagus nerve stimulation therapy to restore auditory processing in a rat model of Rett syndrome

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Abstract: Rett syndrome is a rare neurological disorder associated with a mutation in the X-linked gene MECP2. This disorder mostly affects females, who typically have normal early development followed by a regression of skills. The Mecp2 transgenic rat model of Rett syndrome exhibits similar symptoms shown in patients such as seizures, anxiety, breathing abnormalities, motor and auditory deficits. Individuals with Rett syndrome and Mecp2 heterozygous rats both exhibit atypical neural and behavioral processing of auditory stimuli, which likely impacts effective speech processing. The development of therapies that can enhance plasticity in auditory networks and improve speech processing has the potential to impact the lives of individuals with Rett syndrome.
Here, we tested two potential strategies, Insulin-like growth factor 1 (IGF-1) or vagus nerve stimulation (VNS) paired with auditory stimuli, to restore auditory processing in MeCP2 transgenic rats. IGF-1 has been successfully utilized in both human clinical trials and in rodent models, with improvements in apnea, anxiety, and restoration of plasticity deficits. Similarly, evidence suggests that precisely-timed VNS-sound pairing can drive robust neuroplasticity and enhance the benefits of rehabilitation.
Following 2 weeks of IGF-1 or saline therapy during development, heterozygous Mecp2 and WT rats were trained to discriminate speech sounds in quiet and in various levels of background noise to assess speech discrimination abilities. IGF-1 therapy did not improve speech discrimination performance in Mecp2 rats. In a separate experiment, auditory cortex responses were examined in heterozygous Mecp2 rats following 20 days of VNS-tone pairing or sham therapy. Preliminary results suggest that VNS may improve abnormal auditory cortex responses

in Mecp2 rats. These studies could lead to the development of novel adjunctive therapies that could enhance auditory functioning, and ultimately improve the quality of life of individuals with Rett syndrome.

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Poster

554. Rett Syndrome

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Topic: A.07. Developmental Disorders

Support: Loulou Foundation
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Title: Programmable transcription of MECP2 and CDKL5 suggests limited binding of dCas9 to the inactive X chromosome

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Abstract: Neurological diseases are a heterogeneous group of disorders caused by alterations in nervous system function and many of these disorders can be attributed to genetic factors such as chromosomal aberrations or gene mutations. The neurodevelopmental disorders Rett Syndrome (RTT) and CDKL5 deficiency disorder (CDD) are caused by de novo mutations in MECP2 and CDKL5 on the X-chromosome, respectively. Females with RTT or CDD undergo X-chromosome inactivation (XCI) forming a mosaic of cells expressing mutant and wild type alleles. Our research is focused on methods to specifically reactivate the healthy CDKL5 and MECP2 allele on the silenced X-chromosome in human neuronal-like cell lines. Despite the availability of small molecule drugs that can globally reactivate XCI-silenced genes, locus specific approaches remain elusive. Our group has been the first to identify proximal cis regulatory elements in the CDKL5 and MECP2 core promoter regions using CRISPR/dCas9 fused to effector domains for programmable transcription in several neuronal-like cells, including the male U87 as well as in the female SH-SY5Y and LUHMES cell lines. The observed increase in gene expression and protein levels could be due to superactivation of the

active allele, activation of the silenced allele, or a combination of the two. We sought to investigate if the preferred superactivation of CDKL5 and MECP2 expression is due to limited binding of dCas9 to the inactive X chromosome. Overlay of >80,000 SNPs in SH-SY5Y cells with ATAC-seq data sets further allowed us to investigate the accessibility of the inactive versus active X-chromosome with our dCas9 approach. Synergistic approaches using targeted DNA demethylation of CDKL5 and MECP2 paired with LwCas13a RNA targeting of the XCI key player long-non coding RNA XIST suggest increased accessibility by ATAC-seq and induction of locus-specific escape from XCI. This approach holds great potential for individuals suffering from RTT and CDD.

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Poster

554. Rett Syndrome

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Program #/Poster #: 554.05/C5

Topic: A.07. Developmental Disorders

Support: Rett Syndrome.org Basic Research Award #3211

Title: Characterization of mammalian target of rapamycin (mTOR) pathway alterations in Rett syndrome mice model

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Abstract: Rett syndrome (RTT), an X-linked dominant neurological disorder, is caused by *MECP2* gene mutations. Although multiple neurological abnormalities characterize RTT, reduced brain and neuronal soma size are the most consistent neuropathological findings observed in human brain and Mecp2 mutant mouse models. Studies in several organisms have shown that mTOR signaling pathway regulates cell size. Emerging evidence indicates the general dysfunction of the Akt/mTOR pathway connected to neuronal soma size in RTT models. The mTOR pathway operates through two different complexes: i) mTORC1, linked to RAPTOR, that response to signals, including growth factors and nutrients, and ii) mTORC2, connected to RICTOR, responds primarily to growth factors. The relative contribution of mTORC1 and mTORC2 signaling modifications in the pathogenesis of RTT remains still elusive. In this study, we explored the role of mTORC1 and mTORC2 signaling pathway alteration in the *Mecp2 A140V* and *Mecp2*$^{-/-}$ mouse models. Using qPCR, western blot and immunofluorescence assays, we quantified the relative levels of mTOR pathway molecules from brain and tissue sections of

male and female mice of age-matched *Mecp2* mutant and wild type. We found that mTORC2 pathway is considerably downregulated in *Mecp2 A140V* mice. Furthermore, comprehensive protein analysis revealed alterations overlapping both the mTORC1 and mTORC2 signaling pathway in the *Mecp2* mouse models. We further tested if mTOR activation rescues biochemical deficits in the Mecp2 mutant animals by crossing female carriers *(Mecp2$^{-/+}$)* with *Tsc2 (Tsc2$^{-/+}$)* mutant males. Genetic rescue reverses some of the biochemical abnormalities in mTOR signaling, including Akt activity. Akt-T308 phosphorylated form is essential for the Akt activation and the downstream function. In our study, we found that the phosphorylation of Akt-T308 was significantly downregulated, and this was rescued in *Mecp2 A140V-Tsc2$^{+/-}$* model (TSC2-A140V). We also found a significant reduction in the phosphorylation of S6K1 in A140V mice, which was rescued in the TSC2-A140V brain. We provide here direct biochemical evidence supporting the role of downregulated mTOR signaling pathway, which can be salvaged in Rett syndrome model. Our current studies defining the role of mTOR activation in the reversal of RTT phenotype may serve as a central strategy for the development of novel therapeutics to treat Rett syndrome.

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Poster

554. Rett Syndrome

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Topic: A.07. Developmental Disorders

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Title: Glial-targeted glutamate antagonism for the treatment of Rett Syndrome phenotype in Mecp2-deficient mice

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Abstract: Glutamate dysregulation plays a prominent role in the neuropathology in Rett Syndrome (RTT) and in mouse models of RTT. Patients show increased presence of glutamate and glutamate metabolites in CSF. Work in *Mecp2*-null mouse models has shown that this increase in glutamate may be specific to microglia and can contribute to excitotoxicity and cellular injury. This combined with other coinciding neuropathology (i.e. oxidative stress, altered

NMDA receptor expression) could potentially contribute to cell stress and toxicity as well as neurobehavioral consequences associated with RTT. Blockade of glutamate synthesis via inhibition of the enzyme glutaminase is a potential therapeutic avenue for targeting diseases where glutamate levels are excessive. However, known glutaminase inhibitors are poor drug candidates for RTT due to limited brain penetration. Furthermore, global/universal inhibition of glutamate production throughout all cells in the nervous system would not be desirable in a developing brain, as neurons require glutamate for proper functionality. Our data demonstrate elevated levels of glutamine in both hippocampus and striatum of *Mecp2*-deficient mice suggesting that targeting glutamine may be a more effective route. Glutamine antagonists such as 6-diazo-5-oxo-L-norleucine (DON) have a structure similar to L-glutamine and broadly inhibit glutamine-utilizing pathways including glutaminase, but their clinical application has been limited due to toxic side effects. Thus we chose to investigate the utility of PAMAM dendrimers to deliver a glutamine antagonist to the brain to decrease glutamate production. PAMAM dendrimers are selectively taken up in 'activated' microglia and astrocytes making them an ideal candidate for targeted inhibition of glutamate formation in these cells. We conjugated a prodrug of DON to the dendrimer and evaluated the efficacy in a *Mecp2*-null mouse model of RTT. Preliminary findings indicate that weekly dendrimer-delivered DON prodrug administration beginning at postnatal day 21 improves the neurobehavioral phenotype including paw clench and gait abnormalities in *Mecp2*-null mice. Further work is being done to characterize the impact of systemic injection of the dendrimer-drug conjugates on microglia health and phenotype as well as glutamate production. Our preliminary results indicate that dendrimer-mediated inhibition of glutamate production may be a viable treatment approach for reducing glutamate-related neuropathology in RTT.

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Poster

554. Rett Syndrome

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Support: NIH Grant 1R01-NS073875

Title: Effects of the antitussive cloperastine on a rett syndrome mouse model

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Abstract: Rett Syndrome (RS) is a neurodevelopmental disorder caused mostly by mutations in the *MECP2* gene. RS patients show characteristic breathing abnormalities that respond to GABA receptor agonists, and are likely to be a result of increased brainstem neuronal excitability. GIRK channels play a role in regulation of membrane potentials, and thus may be a potential therapeutic target for RS symptom release. GIRK channels have previously been shown to act on brainstem neurons. Indeed, the GIRK channel inhibitor Cloperastine is currently available as an over-the-counter antitussive in several Asian and European countries. In this study, we tested whether Cloperastine had effects on breathing abnormalities in *Mecp2*-deficient mice as well as potential mechanisms. We found that Cloperastine reduced apnea counts in *Mecp2*-null mice. Significant reduction in apnea counts started 0.5 hours after Cloperastine administration (30mg/kg, ip), and lasted ~4 hours. Similar inhibition of breathing frequency variability was also seen. In the heterologous HEK expression system, Cloperastine potently inhibited GIRK1-GIRK2 channels with an IC50 ~2 μM. In whole-cell current clamp, 10 μM Cloperastine had both inhibitory and excitatory effects on norepinephrinergic neurons in the locus coeruleus and GABAergic neurons in the dorsal tegmental nucleus. Because these opposite effects could be produced by pre- and postsynaptic mechanisms, we studied GABAergic inhibitory postsynaptic currents (IPSCs) in locus coeruleus neurons in voltage clamp. The predominant effect of Cloperastine was an increase in GABAergic IPSC frequency as well as IPSC amplitude to a lesser degree, which was inhibited in the presence of the $GABA_B$ receptor inhibitor Phaclofen. These results suggest that Cloperastine seems to have beneficial effects on breathing abnormalities in the RS model, which has fast onset lasts 4 h, and involves inhibition of GIRK channel-dependent presynaptic $GABA_B$ receptors.

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Poster

554. Rett Syndrome

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Title: Reduction of aberrant NF-kB signaling and vitamin D supplementation ameliorate Rett syndrome cortical phenotypes in Mecp2-null mice

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Abstract: Rett syndrome (RTT) is a severe, progressive X-linked neurodevelopmental disorder caused by mutations in the transcriptional regulator *MECP2*. We previously identified an aberrant up-regulation of the NF-κB pathway in the cortex of *Mecp2*-null mice and demonstrated that genetically attenuating NF-κB signaling rescues some of the well characterized neuronal phenotypes in RTT, such as the reduced dendritic complexity of layer II/III neocortical callosal projection neurons (CPN). These results raised the intriguing question of whether NF-κB pathway inhibitors could provide a therapeutic avenue in RTT, at least in part. Among the many known inhibitors of the NF-κB pathway are vitamin D and its analogues, and, strikingly, vitamin D deficiency is prevalent in RTT patients. We find that *Mecp2*-null mice similarly have significantly reduced total serum levels of 25(OH)D compared to wildtype littermates. Further, treating cortical neurons *in vitro* with calcitriol, the activated form of vitamin D, increases the reduced neurite outgrowth observed after *Mecp2* knockdown. Thus, to investigate whether vitamin D supplementation reduces the aberrant NF-κB activity in *Mecp2*-null cortex *in vivo*, and might have therapeutic benefit, we treated both male *Mecp2* hemizygous null and female *Mecp2* heterozygous mice and wild-type littermates with vitamin D supplemented chow, beginning at an early symptomatic stage. We found that this simple, cost-effective dietary supplement ameliorates neocortical dendritic morphology and soma size phenotypes in a dose-dependent manner, although it only modestly improves the reduced lifespan of *Mecp2*-null mice. In addition, vitamin D supplementation rescues immature spine morphology in *Mecp2*-null mice. These results provide new insight into the fundamental neurobiology of RTT and could provide critical information about vitamin D dietary supplementation as a potential cost-effective partial therapeutic intervention for RTT.

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Poster

554. Rett Syndrome

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Title: Identification of post-translational regulators of MeCP2 protein levels as treatment targets

Authors: *M. ZAGHLULA[1], J.-Y. KIM[1], C. E. ALCOTT[1], H.-H. JEONG[1], Z. LIU[1], W. KIM[1], S. J. ELLEDGE[2], H. Y. ZOGHBI[3,1]
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Abstract: Advances in clinical sequencing continue to highlight the involvement of dosage-sensitive genes in the pathogenesis of neurological disorders. Proper brain function depends on the maintenance of the levels of these proteins within a narrow range. Methyl-CpG-binding protein 2, *MECP2*, is one such gene: loss-of-function mutations in *MECP2* cause Rett syndrome (RTT) while duplications spanning *MECP2* cause *MECP2* duplication syndrome (MDS). Normalization of protein levels has been shown to ameliorate disease phenotypes in mouse models of both disorders. Therefore, our goal is to identify post-translational modifiers of MeCP2 that can be targeted therapeutically to normalize MeCP2 levels. To this end, we performed arrayed siRNA and pooled CRISPR screens in a reporter cell line that allows us to monitor changes in MeCP2 levels. From these screens we obtained hundreds of hits, which we are currently validating by evaluating their effects on endogenous MeCP2 in HEK293T cells. In parallel, we have also selected two promising candidates, *RIOK1* and *USP1*, to perform mechanistic and genetic interaction studies. We have previously shown that shRNA-mediated knockdown of *RIOK1* reduces MeCP2 levels in cells; to test the *in vivo* effects of RIOK1 reduction, we generated a null allele in the mouse using CRISPR-Cas9 editing. We found that *Riok1*$^{+/-}$ mice have a 15% decrease in MeCP2 protein levels in whole brain lysate. However, RIOK1 does not exhibit kinase activity towards MeCP2 *in vitro* and the two proteins also do not interact directly, leading us to believe that MeCP2 regulation is occurring via an intermediate interactor. Given that reducing MeCP2 by a mere 15% is unlikely to significantly improve behavioral abnormalities, we selected another candidate that we validated in HEK293T cells, *USP1*. To assess the effect of *Usp1* knockdown on MeCP2 levels *in vivo*, we delivered AAV-shRNA viruses targeting *Usp1* by intraventricular injection into P0 mouse pups. At 8 weeks of age, we harvested the posterior cortex of these mice and found a 30% decrease in MeCP2 levels *in vivo.* We are now focused on elucidating the molecular mechanisms by which USP1 and RIOK1 regulate MeCP2 levels, and evaluating whether combinatorial targeting of these genes may have additive effects. Overall, this screening approach is proving to be a powerful tool to identify post-translational regulators of MeCP2 and potentially druggable targets for *MECP2* duplication syndrome.

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Poster

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Program #/Poster #: 554.10/C10

Topic: A.07. Developmental Disorders

Support: NIH Grant NS094178

Title: Pontine modulation of laryngeal adductor reflex is suppressed in a Mecp2 mutant mouse model of Rett syndrome

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Abstract: Respiratory disturbances with repetitive apnea (breathholding) during wakefulness is a hallmark of patients with Rett syndrome, a neurologic disease in females often caused by mutation of the *MECP2* gene. The repetitive breathholding phenotype is recapitulated in *Mecp2* mutant mice. Under anesthesia these mutant mice exhibit hypersensitivity of efferent modulation of laryngeal adductor activity when premotor neurons in the pontine Kölliker-Fuse nucleus (KFN) are stimulated by glutamate. In previous study[1] we have shown that laryngeal adductor activity is driven by a specific population of premotor neurons in KFN that are characterized by their critical dependence on NMDA receptor and a decrementing activity pattern during the postinspiratory (post-I) phase of the respiratory rhythm. These previous findings led to our hypothesis that the repetitive breathholding phenotype in *Mecp2* mutant mice might represent a peculiar form of wake state-dependent recurrent laryngospasm caused by abnormalities of post-I driver neurons in the KFN. In healthy humans and animals, laryngospasm may also result from the classic laryngeal adductor reflex (LAR) evoked by activation of irritant receptors in the laryngeal mucosa or electrical stimulation of the superior laryngeal nerve (SLN). In the present study, we found that low-intensity short-train electrical stimulation of the SLN (0.1-0.2 s at 50 Hz during the expiratory phase) in WT female mice evoked both ipsilateral short-latency (~8 ms) and bilateral long-latency (~60 ms) excitations of recurrent laryngeal nerve discharge that are characteristic of the biphasic LAR response, along with simultaneous inhibition of phrenic discharge that is consistent with a concurrent activation of post-I activity. Interestingly, microinjection of the NMDA receptor antagonist AP5 at bilateral KFN significantly attenuated the long-latency component of the LAR response without affecting the short-latency component. In contrast, in *Mecp2* heterozygous (female) mutant mice at a similar age (110 ± 7 days old), the same SLN stimulus evoked a similar short-latency component of the LAR response but the long-latency component became much weaker or abolished. These data suggested that modulation of the LAR by post-I driver neurons in KFN was suppressed in *Mecp2* mutant mice. Finally, pre-

treatment of the *Mecp2* mutant mice with the Rett syndrome drug candidate rhIGF-1 (1 mg/kg, i.p., daily for 3 weeks) did not restore the long-latency component of the LAR or mitigate the breathing abnormalities in these mice.

1. Song G, Tin C, Poon CS (2015) Multiscale fingerprinting of neuronal functional connectivity. Brain Struct Funct. 220:2967-82.

Disclosures: G. Song: None. **C. Poon:** None.

Poster

554. Rett Syndrome

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Support: NIH Grant NS094178

Title: Local CRISPR knockout of Mecp2 at Kölliker-Fuse nuclei produces Rett-like respiratory abnormalities in adult rats without anxiety symptoms

Authors: G. SONG, A. CAO, *C.-S. POON
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Abstract: Breathing dysfunction with repetitive apnea (involuntary breathholding) and other respiratory abnormalities during wakefulness is a hallmark of patients with Rett syndrome, a neurologic disease in females often caused by mutation of the *MECP2* gene. Previous studies have indicated that malfunction of neurons in the dorsolateral pontine Kölliker-Fuse nucleus (KFN) may underlie similar respiratory abnormalities observed in animal models of Rett syndrome. However, because of the whole-body *Mecp2* mutation in these animal models, influences from other brain regions cannot be ruled out. In particular, *Mecp2* mutant mice and patients with Rett syndrome often exhibit heightened anxiety which may contribute to the breathing abnormalities. To eliminate this possibility, we have employed a brain site-specific CRISPR gene editing technique[1] to selectively knockout the *Mecp2* gene in the adult rat KFN. 4-8 weeks after injection of a mixture of two AAV vectors respectively encoding SpCas9 (AAV9-pMecp2-SpCas9-spA, Addgene plasmid #60957) and Mecp2 sgRNA (AAV9-U6-rMecp2-gRNA-hSYN1-eGFP) at bilateral KFN where electrical stimulation caused apnea, the KFN-Mecp2 knockout rats (of either sex) exhibited breathing disturbances similar to those observed in *Mecp2* mutant mice, including significant increases in the incidences of apnea, sighs and respiratory variability as compared with normal rats. By comparison, injection of a mixture of AAV9-SpCas9 and AAV9-LacZ (AAV9-U6-LacZ-sgRNA-hSYN1-eGFP) at bilateral KFN (LacZ control) did not cause similar breathing disturbances as in the KFN-Mecp2 knockout rats. As with *Mecp2* mutant mice, the breathing disturbances in the KFN-Mecp2 knockout rats were

mitigated by i.p. injection of a 5-HT1A receptor agonist (8-OH-DPAT) or a GABA reuptake inhibitor (NO-711). In contrast to *Mecp2* mutant mice, however, both the KFN-Mecp2 knockout rats and KFN-LacZ control rats did not exhibit increased anxiety (as determined by the open-field test) compared with normal rats. Post-mortem immunohistology showed that the number of neurons expressing Mecp2 was significantly reduced in the dorsolateral pons of KFN-Mecp2 knockout rats but no significant changes were observed in the KFN-LacZ control rats. These results demonstrated that the breathing disturbances observed in *Mecp2* mutant mice could result from abnormalities of neurons in the KFN alone that are responsive to drug therapies. Behavioral disturbances such as increased anxiety are not necessary for the induction of such respiratory abnormalities.

1. Swiech L et al. (2015) In vivo interrogation of gene function in the mammalian brain using CRISPR-Cas9. Nat Biotechnol. 33(1):102-6.

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Poster

554. Rett Syndrome

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Title: Characterization of the adenosinergic system and the BDNF-mediated signalling in heterozygous females of a Rett syndrome model

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Abstract: Rett syndrome is a neurodevelopmental disorder characterized by an apparently normal development during the first 6-18 months followed by a regression period. It is known that at least 90% of the cases of Rett syndrome are caused by mutations in the *MECP2* gene which is located in the X-chromossome. The MeCP2 protein is able to regulate genetic expression through its role as an activator and repressor of transcription. BDNF is an important neurotrophic factor that has its expression controlled by MeCP2. BDNF activates TrkB-FL

receptor to promote neuronal differentiation and survival and also synaptic plasticity. It has been shown that the BDNF-mediated signalling in RTT animal models is impaired. On the other hand it has been seen that the increase of the BDNF expression leads to an augmentation of the lifespan and an improvement in motor skills. In spite of these promising results the inability of BDNF to cross the blood-brain barrier makes it difficult to use it as a therapeutic strategy. Alternatively the activation of adenosine A_{2A} Receptors ($A_{2A}R$) potentiates the BDNF synaptic actions. The characterization of the adenosinergic system in *KO* mice (male hemizygous mice knock-out for *MECP2* gene) was previously accomplished, in our lab, and results revealed an increase in the protein expression level of A_1R and a decrease in the protein expression level of $A_{2A}R$ in the cortex and diminished endogenous adenosine levels in the hippocampus. Lower levels of TrkB-FL were also observed in the hippocampus and in the cortex of male *KO* mice. Considering that the severity of this disorder is very variable and that females are the most affected it is mandatory to evaluate if the alterations found in *KO* mice can also be found in heterozygous females as they have a less severe phenotype. Thus the aims of this work were to characterize the adenosinergic system and the BDNF-mediated signalling in heterozygous females of the Rett syndrome model. The results obtained through Western Blot revealed diminished BDNF and MeCP2 levels in the cortex and diminished $A_{2A}R$ levels in the hippocampus of 26 weeks old heterozygous symptomatic females (n=5-6). Overall the results observed are similar to those obtained in *KO* mice. This points to an impairment in the adenosinergic system of heterozygous females which, in turn, can somehow clarify the BDNF-mediated signalling dysfunction. The fact that in a less severe phenotype, such as the one presented by heterozygous females, adenosinergic system is also significantly affected demonstrates that potentially the adenosinergic system can be used as a therapeutic target for Rett syndrome.

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Poster

554. Rett Syndrome

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Title: Adenosinergic system dysfunction in Rett syndrome

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Abstract: Rett Syndrome (RTT) is a neurodevelopmental disorder primarily caused by mutations in the methyl-CpG binding protein 2 (*MECP2*) gene. MeCP2 is known to modulate the expression of brain-derived neurotrophic factor (BDNF), a neurotrophin with essential functions in cell differentiation, synaptic plasticity and survival. BDNF signalling is impaired in RTT. However, the therapeutic use of BDNF is a challenge due to its inability to cross the blood-brain barrier. Adenosine (ADO) is a neuromodulator that acts mainly through A_1 and A_{2A} receptors (A_1R, $A_{2A}R$). The activation of $A_{2A}R$ potentiates BDNF synaptic actions, important to overcome cognitive deficits presented by RTT patients. On the other hand, A_1R activation has antiepileptic effect important to ameliorate epilepsy in RTT patients. Thus, activation of both ADO receptors could be a potential therapeutic strategy. To overcome the lack of knowledge about ADO system in RTT we developed a new line of research on this topic by using: **i)** a well-established animal model of RTT: male hemizygous mice knock-out for Mecp2 gene (KO) and **ii)** *post-mortem* human brain samples from a RTT patient. The results obtained, by binding assays, revealed that the protein expression level of A_1R, is significantly increased in the cortex of *Mecp2 KO* mice (n=5-6, $p<0.05$), while protein expression level of $A_{2A}R$, evaluated by western blot, is decreased when compared with *WT* (n=5-6, $p<0.05$). The levels of ADK, the most relevant enzyme for the regulation of ADO levels, are significantly decreased in the hippocampus from KO mice at pre-symptomatic stage when compared to wild type (WT) mice (n=4-5, $p<0.05$). Hippocampal electrophysiological recordings of field excitatory post-synaptic potentials (fEPSPs), revealed that the inhibitor of ADK, ITU, and the selective agonist of A_1R, DPCPX, induce a significantly higher disinhibition of synaptic transmission in hippocampal slices from *WT* mice, suggesting lower ADO levels in *KO* mice (n=4-10, $p<0.05$). In addition, changes in TrkB-FL protein levels were found in cortex and hippocampus of KO mice at symptomatic stage when compared to the age matched WT (n=13-14, $p<0.05$). In one *post-mortem* human cortical brain sample, an increase in A_1R mRNA expression levels and a decrease in $A_{2A}R$ mRNA expression levels were detected. Overall, the results show a dysfunction in the adenosinergic system, which could explain, at least in part, BDNF dysfunction and epilepsy in RTT. This data could, therefore open a new avenue in the treatment of RTT considering ADO receptors as new therapeutic targets.

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Poster

554. Rett Syndrome

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 554.14/C14

Topic: A.07. Developmental Disorders

Support: Telethon Grant GGP15098
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Albero di Greta
IFCR

Title: Pre-clinical study on CDKL5 deficiency disorder: Class I metabotropic glutamate receptors as a promising therapeutic target

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Abstract: CDKL5 deficiency disorder is a rare disease without a cure, caused by mutations in the cyclin-dependent kinase-like 5 gene, that is characterised by severe cognitive, sensorimotor and autonomic dysfunctions. CDKL5 is a serine/threonine kinase that can localize at excitatory synapses and it participates in the regulation of dendritic spines as well as synaptic transmission and plasticity. However, how CDKL5 intervenes in the mechanisms underlying the molecular organization of synaptic contacts and what are the consequences of its loss remains obscure. We believe that answering to these questions will help uncovering druggable targets for this disease. We identified Shank1, a synaptic scaffolding-protein required for both maturation and stabilization of dendritic spines, as a novel interactor of CDKL5 using both in-vitro and in-vivo assays. Our data indicated that Shank1 may form a bridge between CDKL5 and Homer1bc, a protein scaffold that regulates the synaptic expression of Class I metabotropic glutamate receptors (mGluR). Accordingly, we found a reduction of the synaptic expression of both Homer1bc and mGluR5, but surprisingly not Shank1, in primary sensory cortices of CDKL5 KO mice. This altered molecular organization of excitatory synapses was associated with a decreased expression of Arc, a protein downstream of mGluR5-mediated activity, and atypical NMDA receptors currents. Because mGluR5 is crucial for synaptic contacts maturation occurring during the critical period of cortical plasticity, we then followed the expression and activity of this receptor in the developing visual cortex of CDKL5 KO mice. Our data showed a sharp decrease of both the synaptic localization of mGluR5 and Arc expression in this cortical area, indicating

that CDKL5 loss could hamper the functional refinement of visual cortical connections at crucial developmental phases by altering the correct expression/localization of postsynaptic receptors. Finally, we explored the therapeutic potentials of targeting mGluR5 activity for CDKL5 deficiency disorder by administering to mutant mice CDPPB, a positive allosteric modulator of this class of receptors. Interestingly, our results showed that, one hour after an acute injection with CDPPB (i.p.; 3mg/Kg), the deficits shown by CDKL5 KO mice in both sensory (adhesive removal) and cognitive (Y-maze) tests were rescued. In conclusion, our study discloses novel molecular interactors of CDKL5 that are crucial for dendritic spines formation, maintenance and plasticity. Moreover, we unveiled a promising druggable pathway that we are extensively exploring for its therapeutic efficacy and translational potentials.

Disclosures: M. Giustetto: None. **A. Gurgone:** None. **R. Pizzo:** None. **A. Raspanti:** None. **A. Alfieri:** None. **N. Morello:** None. **F. Pilotto:** None. **F. Gardoni:** None. **P. Defilippi:** None. **E. Turco:** None. **T. Pizzorusso:** None.

Poster

554. Rett Syndrome

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Program #/Poster #: 554.15/C15

Topic: A.07. Developmental Disorders

Support: LouLou Foundation Pilot Grant CDKL5 - 17 - 106 - 01
International Foundation for CDKL5 research "Uncovering synaptic deficits of the cerebral cortex underlying CDKL5 Disorder: The AKT/mTOR pathway as a therapeutic target"

Title: Visual phenotypes of a mouse model of CDKL5 disorder: Neuroplasticity, behavioral correlates and therapeutic approaches

Authors: *T. PIZZORUSSO[1,2,3], L. LUPORI[2], R. MAZZIOTTI[3], G. SAGONA[3], V. MARTINI[3], E. PUTIGNANO[1], M. GIUSTETTO[4]
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Abstract: CDKL5 deficiency disorder (CDD) is a neurodevelopmental disorder still without a cure. The devastating symptoms comprise seizures, impairment of motor skills, lack of language and a substantial delay in many aspects of development. In order to develop and test preclinical treatments and to understand the biological processes underlying the disease, our lab has previously established the analysis of cortical responses to visual stimuli as a precision tool to probe cortical circuits function. This experimental strategy has proven to be successful in discriminating mutant from wild type mice with remarkably high accuracy, and to predict

amelioration of other anatomical and behavioral deficits after experimental treatments. We are currently expanding this research along three lines: first, since CDKL5 null mice show a decreased signalling of the metabotropic glutamate receptor mGLuR5, we are testing the effect of a single dose of an mGluR5 agonist drug (CDPPB) on visual responses. Preliminary data showed a remarkable recovery of normal visual processing in treated animals. Second, we are investigating if neuroplasticity, another fundamental feature deeply studied in the visual system, is impaired when CDKL5 is missing. We analyzed Ocular Dominance Plasticity (ODP) after 3 days of Monocular Deprivation (MD) beginning at P27-P28, a protocol that mainly results in the depression of cortical inputs coming from the deprived eye. We found no differences of ODP between wt and mutants. Finally, due to the importance of behavioral phenotyping, we are investigating behaviors that are tightly coupled with visual function but still reflects integrated functions. We have started this analysis by developing a custom fully automated setup for Appetitive Conditioning (AC). In this setup, mice are trained to press a button in response to a visual stimulus to get a reward in a Skinner-like manner. Mice performance is translated into various behavioral parameters including real-time tracking, number of trials initiated and latency to response. AC showed that CDKL5 null mice display an hyperactive behavior: they complete more trials for each session and the stimulus to button-press latency is shorter. Strikingly, we found that the general performance of mice is tightly correlated with the amplitude of visual responses measured by intrinsic signal optical imaging, thus establishing AC as an effective test to probe integrated behaviors directly coupled to the visual biomarker in CDKL5 null mice.

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Poster

554. Rett Syndrome

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Program #/Poster #: 554.16/C16

Topic: A.07. Developmental Disorders

Support: BCH pilot grant
Loulou Foundation
MH111647
ETH Career See No. SEED-42 16-1
SFARI #400101

Title: Testing functional and structural connectivity in CDKL5 disorder as novel biomarkers

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Abstract: De novo mutations in the X-linked gene *CDKL5* are associated with a rare neurodevelopmental disorder characterized by early neonatal/infantile onset of epilepsy, developmental delays and cortical visual impairment. CDKL5 is expressed from late gestation into early postnatal life and likely contributes to the assembly of neuronal circuits and their experience-dependent refinement during critical neurodevelopmental periods. How CDKL5 affects such complex processes is still largely unknown.
Through an RNA Seq screen, we have identified CDKL5 as a potential signaling molecule that may be involved in activity-dependent callosal synapse refinement. Callosal connections are the major connection across the cerebral hemispheres and mediate the integration of information and acquisition of a functionally lateralized brain. The disruption of their development has been linked to neurodevelopmental disorders. To assess the impact of CDKL5 deficiency on callosal connectivity, we performed *in vitro* recordings from cortical sections of CDKL5 KO and WT mice at postnatal day P5 and P15. We found that while no differences were present at P5, there were significantly more callosal synaptic inputs at P15 in the KO mice compared to WT, suggesting these connections may fail to refine in the absence of CDKL5.
In order to evaluate whether such abnormal functional connectivity would persist in adulthood and impact network activity across brain regions, we performed resting-state fMRI and ex-vivo DTI in CDKL5 mutant and control adult mice (n=10-11 each). BOLD time series were extracted using the Allen Reference Atlas ontology and their connectivity couplings were measured using Pearson's correlation coefficients across 65 regions of interest. We discovered that mice lacking CDKL5 exhibited robust over-connectivity between retrosplenial, anterior cingulate and somatomotor cortices, and across inter-hemispheric posterior associative, entorhinal hemispheres and between the retrosplenial cortex and the anterior portion of anterior commissure/motor cortex when analyzed by voxel-wise (network analysis) or roi-roi analysis (connectome analysis). These default networks are involved in motor and visual areas. Consistent with these findings, when we recorded VEP from visual cortex, we found a significant decrease of both amplitude of response to low spatial frequency, as well as spatial resolution. Our results indicate that CDKL5 is necessary for the proper refinement of callosal projections, and point at the use of DTI, rs-fMRI and VEP as syndrome-specific translational biomarkers, which may be employed to predict progression of the disorder and response to treatment.

Disclosures: P.N. Awad: None. **E. Johnson-Venkatesh:** None. **M. Markicevic:** None. **E. Centofante:** None. **V. Zerbi:** None. **A. Gozzi:** None. **H. Umemori:** None. **M. Fagiolini:** None.

Poster

555. Mechanisms of Developmental Disorders

Location: SDCC Halls B-H

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Program #/Poster #: 555.01/C17

Topic: A.07. Developmental Disorders

Support: NIH Grant U01MH103346A
NIH Grant R01CA136924

Title: A 3D epigenomic map of olfactory neuronal cells reveals schizophrenia-associated genes

Authors: *S. K. RHIE[1,2], S. SCHREINER[1], H. WITT[1], C. ARMOSKUS[3], F. D. LAY[1], A. CAMARENA[3], V. N. SPITSYNA[3], Y. GUO[1], B. P. BERMAN[4], O. V. EVGRAFOV[5], J. A. KNOWLES[5], P. J. FARNHAM[1,2]
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Abstract: Cultured Neuronal cells derived from Olfactory Neuroepithelium (CNON) display transcriptomic patterns similar to neural progenitors, a type of cell that plays a key role in neurodevelopment. Not only can these cells be obtained from large numbers of individuals via nasal biopsy, they are renewable via growth in culture. As part of PsychENCODE, we have developed comprehensive 3-dimensional epigenomic profiles of CNON using biopsies from 63 individuals. To identify regulatory elements, nucleosome positioning, and transcription factor binding sites in CNON, we used chromatin immunoprecipitation (ChIP-seq) and nucleosome occupancy and DNA methylome (NOMe-seq) assays. We also performed in situ Hi-C looping assays to detect chromatin interactions, including large active and inactive topological associating domains (TADs), high-resolution enhancer-promoter loops, and repressive loops. We identified hundreds of thousands of regulatory elements in CNON and mapped transcription factor binding platforms within these elements. We identified 6,800 TADs, which include inactive TADs enriched with genes involved in sensory reception of smell, and hundreds of thousands of intra-chromosomal loops, with the majority being anchored by regions of repressed or heterochromatic chromatin. Using NOMe-seq, we characterized nucleosome positioning at promoters, enhancers, and insulators, as well as a novel category of nucleosome-depleted regions (NDRs) that do not have marks of active chromatin. Comparison of CNON active enhancers (the epigenetic state most closely linked to cellular identity) to active enhancers in a hundred different cell types revealed that CNON cluster with neuroblastoma cells and that thousands of CNON enhancers are active in the brain. Also, CNON active enhancers are enriched with motifs associated with cells of neuronal origin. Schizophrenia is a neurodevelopmental psychiatric disorder with 81% heritability and has been associated with deficits in olfactory perception. Therefore, we used CNON as a model to identify and characterize regulatory elements linked to schizophrenia. We identified 147 TADs harboring ~1,000 variants in regulatory elements active in CNON, including one TAD at chr17p11 with hundreds of schizophrenia risk-associated variants. Finally, we predicted enhancer:target gene interactions linked to increased risk for schizophrenia. Our results suggest that CNON is a useful model for epigenetic studies of mechanisms underlying neurodevelopmental components of psychiatric disorders.

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Poster

555. Mechanisms of Developmental Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 555.02/C18

Topic: A.07. Developmental Disorders

Support: FDA Protocol E0752801

Title: Ketamine-induced mitochondrial toxicity in zebrafish embryos

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Abstract: Ketamine, a phencyclidine derivative, is an antagonist of the calcium-permeable N-methyl-d-aspartate (NMDA)-type glutamate receptors. A pediatric anesthetic implicated in developmental neurotoxicity, ketamine has been shown to deplete ATP in mammalian cells. Based on our previous studies showing acetyl L-carnitine (ALCAR) prevented ketamine-induced cardiotoxicity and neurotoxicity in zebrafish embryos, the effect of which was blunted by oligomycin A, an inhibitor of ATP synthase, we further investigated the effects of ketamine and ALCAR on ATP levels, mitochondria and ATP synthase in zebrafish embryos. Embryos at 28 h post fertilization (hpf) were treated with 2 mM ketamine (equivalent to an internal concentration of 8.4 μM) for 20 h. Analyses of the 48 hpf embryos post-exposure demonstrated that ketamine reduced ATP levels in the embryos but not in the presence of ALCAR. Ketamine also reduced total mitochondrial protein levels and mitochondrial potential, which were prevented with ALCAR co-treatment. To determine the cause of ketamine-induced ATP deficiency, we explored the status of ATP synthase. The results showed that a subunit of ATP synthase, atp5alpha1, was transcriptionally down-regulated by ketamine, but not in the presence of ALCAR, although ketamine caused a significant upregulation in another ATP synthase subunit, atp5beta, and total ATP synthase protein levels. In addition, ketamine-treated embryos developed an abnormal heart structure. In these embryos, with an enlarged heart, the atrioventricular (AV) valve separating the auricle and ventricle did not develop. ALCAR co-treatment, however, prevented ketamine-induced defects in the heart structure. This study suggests that ketamine's adverse effects could be mediated by ATP deficiency due to mitochondrial dysfunction.

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Poster

555. Mechanisms of Developmental Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 555.03/C19

Topic: A.07. Developmental Disorders

Support: NS090160

Title: Exploring mechanisms behind zdhhc9 mutations that cause x-linked intellectual disability

Authors: K. S. SERRANEAU[1], *L. N. KIROUAC[2], K. REDDY[2], R. DESCHENES[2]
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Abstract: zDHHC9 is a protein acyltransferase (PAT) that enzymatically adds palmitate to cysteine residues on specific protein substrates. This modification results in increased protein hydrophobicity and membrane association and is reversible by the action of depalmitoylating thioesterases. This dynamic process plays a key role in the spatiotemporal distribution of proteins within the neuron. zDHHC9 is abundant in the brain and while there are over 300 potential substrates, only H- and N-Ras are known substrates of the enzyme. Recently, three loss of function mutations in the zDHHC9 gene have been identified in individuals with X-Linked Intellectual Disability (XLID). Our lab has previously determined that two missense point mutations, P150S and R148W, result in a enzymatically deficient zDHHC9. Here, we characterize the zDHHC9 R298* nonsense mutation that results in the expression of a C-terminal truncated protein, in the context of the mature hippocampal neuron. Using primary rat hippocampal neurons, we perform techniques such as subcellular fractionation, immunofluorescence and live cell imaging to understand the underlying pathophysiology that this specific mutation imparts. From our observations, we find that the nonsense R298* zDHHC9 mutation associated with XLID results in restricted trafficking of the mutant protein. While WT-zDHHC9 is trafficked through the axons and dendrites, the c-terminal truncated R298* mutant is restricted to the Golgi within the cell body. This finding suggests that the subcellular mislocalization of R298* zDHHC9 mutant potentially limits its access to specific protein substrates involved in maintaining synaptic function. Additionally, we examine how loss of function mutations in zDHHC9 might affect downstream signaling. Palmitoylation of zDHHC9 substrates, H- and N-Ras, dictates activity by changing the localization of the protein to the plasma membrane where it can interact with its effectors. In the brain, Ras signaling is an important event involved in synaptic plasticity and dendritic morphogenesis. Utilizing control or zDHHC9 knock out human chronic myelogenous leukemia cell lines, we examine Ras-dependent signaling cascades using Western blot analysis. Additionally, we examine the activity

of Ras in these cell lines utilizing a Ras activation pull-down assay. These experiments are novel and largely unexplored. We believe our data will be insightful to understanding the pathophysiology in the brains of XLID patients.

Disclosures: K.S. Serraneau: None. **L.N. Kirouac:** None. **K. Reddy:** None. **R. Deschenes:** None.

Poster

555. Mechanisms of Developmental Disorders

Location: SDCC Halls B-H

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Program #/Poster #: 555.04/C20

Topic: A.07. Developmental Disorders

Support: KBRI basic research : 18-BR-02-02
National Research Foundation of Korea(NRF) : 2015M3C7A1029037

Title: Exosome-derived mitochondrial components as a potential diagnostic/therapeutic markers for neurodevelopmental and neurodegenerative disorders

Authors: *B. HA, J. HEO, Y.-J. JANG, T.-S. PARK, J.-Y. CHOI, J. JOO, S.-J. JEONG
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Abstract: Exosomes are cell-derived nanoscale size vesicles, playing roles with a paracrine messenger affecting nearby recipient cells as well as presenting a systemic messenger in the all eukaryotic fluids, including blood, urine, and cultured medium of cell cultures. Exosomes include tissue-specific and disease-related molecules such as lipids, proteins and RNAs. In central nervous system, recent evidences show that exosomes are remarkably stable in body fluids proving their utility as disease biomarkers. Exosomes can transfer pathogens such as prion protein (PrP), responsible for Creutzfeldt-Jakob disease; α-synuclein, involved in the pathogenesis of Parkinson's disease; amyloid β (Aβ) and phosphorylated tau deposited in the brain of Alzheimer's disease (AD). In contrast, exosomes may have a protective function by relieving the cells from toxic accumulation of these pathogens or transferring beneficial molecules. Intact mitochondria can be transferred between cells in disease conditions such as cancer, stroke, and lung injury, but the details on the mechanism of transfer remains elusive. Recently, extracellular vesicles (EVs) from mesenchymal stem cells (MSCs), were reported to contain some mitochondrial components, including proteins and mtDNA. These studies suggest that mitochondrial components are secreted from the cells in the form of EVs. However, it is still unknown whether mitochondrial proteins are secreted as exosomes. In this study, we investigated the expressions of mitochondrial components in exosomes isolated from brains, plasma, and primary neuron/astrocyte of neurodevelopmental and neurodegenerative disorders mouse model. Our findings show that mitochondrial components were decreased in disease mouse models,

compared with wild type mouse models. In conclusion, these results suggest that mitochondria and exosome biogenesis pathway are interconnected and exosomes-derived mitochondrial components have a possibility as potential diagnostic/therapeutic targets.

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Poster

555. Mechanisms of Developmental Disorders

Location: SDCC Halls B-H

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Program #/Poster #: 555.05/C21

Topic: A.07. Developmental Disorders

Title: PQBP1 promotes protein translation through suppressing EEF2 phosphorylation

Authors: *S. Y. QIAN, Z. Z. CHAO
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Abstract: Polyglutamine binding protein-1 (PQBP1) is a splicing factor whose mutations have been associated with Renpenning syndrome, a type of X-linked intellectual disability. Recent studies find that cytoplasmic PQBP1 may be involved in protein translation but the underlying mechanism is unclear. Here, we identify PQBP1 as a ribosome binding protein that directly binds with ribosomal proteins on the 80S ribosome. Furthermore, we reveal that PQBP1 interacts with non-phosphorylated eukaryotic elongation factor 2 (eEF2) and suppresses its phosphorylation through blocking the phosphorylation site of eEF2. These findings identify PQBP1 as a novel translational regulator and indicate that PQBP1 promotes protein translation through suppressing eEF2 phosphorylation.

Disclosures: S.Y. Qian: None. **Z.Z. chao:** None.

Poster

555. Mechanisms of Developmental Disorders

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Topic: A.07. Developmental Disorders

Support: UTMB Presidential Scholars Program (CT, KM)

NIH/NIEHS-T32ES007254 (CT)
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Title: Acute and early-life exposure effects of the pyrethroid insecticide deltamethrin on medium spiny neurons of the nucleus accumbens

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Abstract: Deltamethrin (DM), a commonly used pyrethroid insecticide, exerts its effect on insects by delaying onset of inactivation in voltage gated sodium (Nav) channels fundamental for neuronal excitability. Epidemiological data showed a correlation between pyrethroid metabolites in urine and increased risk of ADHD diagnosis in children. In rats, exposure to DM results in behavioral phenotypes that mimic aspects of ADHD and are associated with the dopaminergic (DA) reward pathway in the nucleus accumbens (NAc). Dysregulation of DA medium spiny neurons (MSNs) in the NAc is thought to play a critical role in neuropsychiatric disorders like ADHD, anxiety, and depression. The Nav 1.6 channel, critical in synaptic transmission, is abundant in the MSNs. Here, we investigate the mechanism of MSNs dysfunction due to both acute and developmental DM exposure. For the acute model, rodent brain slices containing the NAc were incubated in 10uM DM. Using whole-cell patch clamp electrophysiology, we assessed changes to intrinsic excitability of MSNs. An increase in the instantaneous firing frequency and the total number of action potentials and a decrease in the peak amplitude was observed at multiple injected current steps (n=7-8, data was normal with equal variance, two-sample t-test, $p<0.05$). For the early-life exposure model, pregnant female B6 mice were exposed to 3.0 mg/kg of DM throughout pregnancy and lactation. Then, male mice litter-mates 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. A decrease in the total number of action potentials and instantaneous firing frequency was observed (n=7-12, data was normal with equal variance, two-sample t-test, $p<0.05$). 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 and potential increased vulnerability to neurodevelopmental disorders.

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Poster

555. Mechanisms of Developmental Disorders

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Topic: A.07. Developmental Disorders

Support: NIH Grant ES025585

Title: Environmental contribution to transcriptome and methylome dynamics of excitatory neurons in the maternal immune activation model of autism spectrum disorder

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Abstract: Maternal immune activation (MIA) in rodents during early embryonic development causes profound neurodevelopmental alterations in the offspring. This leads to neurotransmitter system and behavioral abnormalities that resemble those of human autism spectrum disorder (ASD). How activation of the maternal immune response interacts with underlying genetic factors during early development to influence ASD phenotypes is still largely unknown. Recent evidence suggests that dysregulation of epigenetic pathways, and ensuing altered gene expression, could cause the neurodevelopmental alterations observed in the offspring. Our previous analysis showed intricate dynamics of methylation and transcriptional changes during embryonic and early postnatal development, suggesting this period is highly vulnerable to disruption by environmental insults. To address this hypothesis, we performed MIA by injecting polyinosinic:polycytidylic acid (PolyI:C) in pregnant mice at embryonic day 12.5. We measured the transcriptome in mouse frontal cortex of MIA and control offspring by mRNA sequencing (RNA-Seq) at embryonic day 14.5 (E14) (n=4/group), postnatal day 0 (P0) (n=4/group), and in adults at 10 weeks of age (n=6/group). PolyI:C exerted profound effects on gene expression in offspring at P0. However, these transcriptome data at the whole tissue level may reflect a complex pattern of gene expression regulation across multiple cell types. To further investigate neurodevelopmental alterations in a cell-type specific manner, we used INTACT to label nuclei in excitatory neurons using the ClSun-Nex-Cre mice. We generated transcriptomes (nuclear RNA-Seq, n=9-11 mice from 3 litters/group/time point) and single base resolution methylomes in frontal cortex excitatory neurons (n=6 mice from 3 litters/group/time point) at P0 and P13. Consistently, PolyI:C exerted a strong effect on excitatory neurons in MIA offspring at P0. Specifically, 38 synapse-related genes were down-regulated in MIA, including ion transporters (*Nkcc1)*, ion channel subunits (*mGlur5/mGlur7, Cacna1b*), and cell adhesion molecules (*Nrxn3)*.

Importantly, at P0 the ratio of mRNA expression of Nkcc1 to Kcc2 was 70% higher in MIA offspring in comparison with the control group. A higher Nkcc1/Kcc2 ratio in immature neurons suggests that MIA may delay the excitatory-to-inhibitory GABA switch in MIA offspring. These differences were not observed at P13. This study identified transcriptome as well as methylome dynamics at key developmental time points that can further our understanding of the underlying contribution of environmental factors to autism spectrum disorder.

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Poster

555. Mechanisms of Developmental Disorders

Location: SDCC Halls B-H

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Program #/Poster #: 555.08/C24

Topic: A.07. Developmental Disorders

Support: NIH grant R01NS094597
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Title: Human brain lysosomal cathepsin gene expression profiles during normal development from prenatal to infant, childhood, adolescent, and young adult

Authors: *V. Y. HOOK[1,3,4], A. HSU[2], S. P. PODVIN[2]
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Abstract: Cathepsin protease genes are necessary for protein homeostasis in normal brain development and function, and numerous brain disorders of development. Cathepsins are present in lysosomes that participate in protein degradation and cellular proteostasis, and are composed of fifteen cathepsins consisting of cysteine, aspartyl, and serine protease subtypes. The diversity of cathepsin proteolytic activities raises the question of what are the human brain expression profiles of the cathepsin genes during development from early prenatal to infant, childhood, adolescence, and young adult stages. This question was addressed by evaluating the gene expression profiles of the cathepsin genes in sixteen human brain regions during normal developmental periods by quantitative RNA-sequencing data obtained from the Allen Brain Atlas resource. The novel finding was the remarkable consistency in relative proportions of cathepsin gene expression levels among brain regions during the developmental stages of prenatal, infancy, childhood, adolescence, to young adult. Expression of the cathepsin genes in brain regions among the ages showed (a) high expression of cathepsins B, F, and D, (b) moderate expression of cathepsins A, L, and Z, (c) low expression of cathepsins C, H, K, O, S, and V, and

(d) very low expression of cathepsins E, G, and W. It is of interest that widely different cathepsin expression profiles among brain regions and ages were not observed. These findings demonstrate that the lysosomal cathepsin genes display similar rank orders of expression during human brain development. The consistent pattern of these expression profiles suggests that human brain developmental functions utilize well-defined, balanced profiles of cathepsin gene expression. Knowledge of the normal expression profiles of lysosomal cathepsin proteases during human brain development provides an important basis for future investigation of lysosomal cathepsin protease dysregulation occurring in traumatic brain injury, lysosomal storage disease, schizophrenia, and many related brain disorders.

Disclosures: V.Y. Hook: None. **A. Hsu:** None. **S.P. Podvin:** None.

Poster

555. Mechanisms of Developmental Disorders

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Topic: A.07. Developmental Disorders

Support: NIH R01NS092062

Title: Regulation of filamin and Fmn2 on proliferation and differentiation of neural progenitor cells

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Abstract: Neural progenitor proliferation and cell fate decision from self-renewal to differentiation are crucial factors in determining brain size and morphology. The cytoskeletal dependent regulation of these processes is not entirely known. The actin-binding filamin A (FlnA) and Fmn2 were shown to regulate proliferation of progenitors by transducing upstream Wnt signals through b-catenin to downstream changes in cell cycle proteins such as Cdk1. Here, we report that activated RhoA-GTPase disengages Fmn2 N- to C-terminal binding to promote Fmn2 activation and redistribution into lysosomal vesicles. Fmn2 colocalizes with β-catenin in lysosomes and promotes its degradation. Further, Fmn2 binds the E3 ligase Smurf2, enhances Smurf2-dependent ubiquitination and degradation of Dishevelled-2 (Dvl2), thereby initiates β-catenin degradation and impairs cell proliferation. Moreover, functional loss of FlnA not only affects the rate of proliferation by altering cell cycle length but also causes a defect in early differentiation through changes in cell fate specification. FlnA interacts with Rho GTPase RhoA, and FlnA loss impairs RhoA activation. Disruption of either of these cytoskeletal associated proteins delays neurogenesis and promotes neural progenitors to remain in proliferative states. Inhibition of FlnA or RhoA impairs Aurkb degradation and alters its localization during mitosis.

Our findings suggest that shared cytoskeletal processes can direct neural progenitor proliferation by regulating the expression and localization of proteins that are implicated in the cell cycle progression and cell fate specification.

Disclosures: G. Lian: None. **V. Ekuta:** None. **V. Sheen:** None.

Poster

555. Mechanisms of Developmental Disorders

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Topic: A.07. Developmental Disorders

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Shriners Hospital Grant 85300-NCA

Title: Mechanisms of glutamate release during neural tube formation

Authors: R. GOYAL, *L. N. BORODINSKY
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Abstract: Failure of neural tube closure leads to one of the most common human birth defects, known as neural tube defects (NTDs), which can have serious neurological consequences or be lethal. The use of antiepileptic drugs (AEDs), during pregnancy increases the incidence of NTDs. Our previous studies have shown that glutamate signaling through NMDA receptors is important for the formation of the neural tube and that the AED valproic acid perturbs this signaling which induces an increase in neural plate cell proliferation and impairs neural plate cell migration, resulting in NTDs. The mechanism of glutamate release by neural plate cells is unclear since synapses are not assembled yet at these early stages of development. In this study we investigate the molecular mechanisms by which glutamate is released and signals in the folding neural plate of *Xenopus laevis* embryos. To determine whether vesicular release of glutamate occurs in the neural plate we first assessed the expression of the vesicular glutamate transporter 1 (VGluT1) during neurulation and found that VGluT1 transcripts are present at these developmental stages. Through whole-mount immunostaining we found that VGluT1 protein localizes to medial regions of the neural plate. Knocking down VGluT1 expression by injecting a specific VGluT1 translation-blocking morpholino in 2-cell stage embryos leads to NTDs, indicating that VGluT1 expression in neural plate cells is necessary for neural tube formation. In order to determine the source of glutamate and the dynamics and mechanisms of its release during neural plate folding we expressed the genetically-encoded, glutamate-sensor, iGluSnFR. *In vivo* imaging of neurulating embryos reveals that the fluorescent signal from iGluSnFr is selectively brighter in the neural plate compared to the non-neural ectoderm, thereby suggesting that glutamate is

released from neural plate cells. In turn, released glutamate may recruit calcium dynamics in neural plate cells. We found that unilateral knockdown of VGluT1 decreases the number of spontaneous calcium transients in the affected half neural plate and impairs its folding. Moreover, exogenous addition of ionomycin enhances the fluorescence intensity of iGluSnFr in neural plate cells, which suggest that glutamate is released by calcium-dependent vesicular exocytosis. Altogether these findings suggest that vesicular glutamate release occurs in the neural plate, elicits calcium dynamics, and is necessary for the formation of the neural tube. Elucidating the mechanisms of neurotransmitter signaling during neurulation may contribute to identify antiepileptic drugs that are safe during pregnancy.

Disclosures: R. Goyal: None. **L.N. Borodinsky:** None.

Poster

555. Mechanisms of Developmental Disorders

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Topic: A.07. Developmental Disorders

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Title: βIV spectrinopathies cause profound intellectual disability, congenital hypotonia, and motor axonal neuropathy

Authors: *C.-C. WANG[1], X. R. ORTIZ-GONZALEZ[2], S. W. YUM[2], S. M. GILL[2], A. WHITE[2], E. KELTER[3], L. H. SEAVER[4], S. LEE[5], G. WILEY[6], P. M. GAFFNEY[6], K. J. WIERENGA[7], M. N. RASBAND[1]
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Abstract: βIV spectrin functions together with ankytinG to cluster Na+ and KCNQ2/3 K+ channels at axon initial segments (AIS) and nodes of Ranvier. These channels are necessary for the initiation and propagation of action potentials in the nervous system. Pathogenic variants of αII spectrin (SPTAN1) cause severe infantile epilepsy including seizures, hypomyelination and brain atrophy; pathogenic variants of βI (SPTB) and βIII (SPTBN2) spectrin lead to hereditary spherocytosis and spinocerebellar ataxia type 5, respectively. Although a variety of *quivering* mice bearing mutations in βIV spectrin (*Sptbn4*) have been reported and studied, our understanding of human pathogenic variants in βIV spectrin (SPTBN4) is limited to one single

case report of an individual with congenital myopathy, neuropathy and deafness. However, the pathogenic mechanism still remains elusive. Here, we report five family cases of bi-allelic pathogenic variants (three homozygous and two compound heterozygous) in SPTBN4 that cause profound intellectual disability, congenital hypotonia, and motor axonal neuropathy. We show that 5/7 are loss-of-function variants that disrupt AIS localization or phosphoinositide binding. Nerve biopsies from a proband with a loss-of-function variant also showed reduced nodal Na+ channels and no nodal KCNQ2 K+ channels. We also demonstrate that although ankyrinR/βI spectrin can partially compensate for the clustering of Na+ channels upon the loss of ankyrinG/βIV spectrin, ankyrinR/βI spectrin cannot recue the clustering of KCNQ2/3 K+ channels. In summary, our studies reveal the molecular pathologies of variants in SPTBN4 and define a new class of spectrinopathies.

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Poster

555. Mechanisms of Developmental Disorders

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Program #/Poster #: 555.12/C28

Topic: A.07. Developmental Disorders

Support: 5R01AA021402-06

Title: Single-cell genomic analyses of somatic mosaicism in fetal alcohol spectrum disorders

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Abstract: Fetal alcohol spectrum disorders (FASD) collectively classify neurodevelopmental and psychiatric problems attributed to maternal ingestion of alcohol. Previous genetic studies of FASD have primarily focused on variants associated with risk, but have not investigated genomic alterations that may result from ethanol exposure to the developing brain. Genomic mosaicism, or cell-to-cell DNA variability, has been established as a feature of the normal cerebral cortex, with significant genomic variations occurring during development. We worked to determine if ethanol exposure during neurogenesis in the developing brain could alter the rate of genomic variation in neurons. We developed a novel, single-cell whole genome analysis approach to assess these somatic genomic changes in the developing cortex. Integration of multiple approaches allowed us to determine the effects of *in utero* ethanol exposure in both

dividing and interphase cells. Analysis of embryonic mouse brain cells after *in utero* ethanol exposure during neurogenesis showed altered genomic variations when compared to control mice. Results from this study reveal lasting genomic alterations resulting from ethanol exposure during development and suggest that the aberrant genomic changes observed contribute to the range of neurological defects present in FASD.

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Poster

555. Mechanisms of Developmental Disorders

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Topic: A.07. Developmental Disorders

Support: R01AA013440
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Title: A novel pseudogene-encoded long noncoding RNA mediates fetal alcohol effects

Authors: *N. A. SALEM[1,2], A. TSENG[1], A. H. MAHNKE[1], C. GARCIA[1], H. KOLAHI-JAHROMI[1], R. C. MIRANDA[1,2]
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Abstract: Prenatal alcohol exposure is a leading non-genetic cause of neurodevelopmental disability. Neural stem cells (NSCs) that give rise to most neurons of the adult brain during the first and second trimester are particularly vulnerable. We previously found that ethanol exposure did not result in NSC death, but rather, the loss of NSCs due to premature maturation. This effect was mediated in part by the loss of specific miRNAs in NSCs. Here, we investigate whether ethanol also specifically prevents NSC renewal. We assessed the regulation of the homeobox transcription factor, Oct4/POU5F1, which is important for maintaining stem cell renewal and pluripotency. The Oct4 family includes several long non-protein coding RNAs (lncRNA) transcribed from pseudogene loci. We identified Octpg9 as one pseudogene-derived lncRNA transcript that was expressed in NSCs at significantly higher levels than the parent Oct4 mRNA transcript. Ethanol exposure results in elevated levels of Oct4pg9, whereas Oct4 protein levels are reduced. We studied the effect of ethanol exposure on the expression of Oct4 and Oct4pg9. Ethanol decreased Oct4 protein levels, but increased Oct4pg9 lncRNA. We assessed the effects of elevated Oct4pg9 on stem cell fate markers in NSCs, compared to the effects of ethanol. Oct4pg9 overexpression increased DCX, NeuN and GFAP mRNA transcripts, an effect that was mimicked by ethanol exposure. In contrast, siRNA-mediated Oct4pg9 knockdown resulted in downregulation of DCX and MAP2 mRNA. These data suggest that ethanol-mediated elevation

of Oct4pg9 shifts NSCs towards a neuronal/oligodendrocytic fate. Moreover, we found that CRISPR mediated knockdown of Oct4pg9 disrupts the correlated expression of stemness and differentiation markers. We show that siRNA mediated Oct4 knockdown, mimicking the effect of ethanol, resulted in an increased rate of DNA synthesis rate, an effect which can be reversed by knocking down Oct4pg9.Our results suggest that a novel OCT4-related lncRNA regulates NSC renewal and mediates some of the teratogenic effects of ethanol. Manipulating this lncRNA may be an interventional approach to reverse some of ethanol effects on neural stem cells.

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Poster

555. Mechanisms of Developmental Disorders

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Title: Regulation of the basic helix-loop-loop transcription factor TCF4 activity in neuronal cells

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Abstract: Transcription factor 4 (TCF4) belongs to a family of basic helix-loop-helix transcription factors also known as E-proteins. TCF4 has been associated with several mental disorders such as schizophrenia, intellectual disability, bipolar disorder and a very rare disease known as Pitt-Hopkins syndrome (PTHS). Furthermore, expansion of trinucleotide repeats in an intron of TCF4 have been shown to be responsible for the development of Fuchs' endothelial corneal dystrophy. We have previously demonstrated that human TCF4 gene is transcribed using numerous 5' exons potentially yielding in TCF4 protein isoforms with different N-termini that vary in their subcellular distribution and ability to regulate transcription. Additionally, we have found that PTHS-associated mutations impair the functions of TCF4 by diverse mechanisms ranging from hypomorphic to dominant-negative effects. Our recently published data show that neuronal activity and protein kinase A lead to phosphorylation of TCF4 and activation of its transcriptional activity indicating that synaptic activation of nerve cells, that is the basis of brain

function, regulates TCF4 function. We have further investigated regulation of TCF4 function in neurons by studying how various sequence variations, mutations and interaction partners modulate the activity of TCF4. Most recent results obtained in these studies will be presented.

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Poster

555. Mechanisms of Developmental Disorders

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Topic: A.07. Developmental Disorders

Support: NIDDK Intramural Research Program

Title: Analyzing pathogenic missense variants in GNB5

Authors: *C. KITTOCK, J. ZHANG, P. ADIKARAM, M. PANDEY, W. F. SIMONDS
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Abstract: The refinement of high-throughput sequencing technologies has led to an increase in the application of Whole Genome Sequencing and Whole Exome Sequencing (WES) to the diagnosis of human diseases. Recently, through WES analysis, Loss of Function (LoF) and missense variants in the GNB5 gene have been identified as causative in patients with a novel syndrome called intellectual developmental disability with cardiac arrhythmia (IDDCA), characterized by cognitive disability, cardiac abnormalities, and other neurological phenotypes. The GNB5 gene encodes Gβ5, a structurally and functionally divergent isoform of the Gβ family of Gα/Gβ/Gγ heterotrimeric G proteins, and is primarily expressed in neural, neuroendocrine, and endocrine tissues. Gβ5 binds with regulator of G protein signaling (RGS) proteins from the R7-RGS sub-family to hasten the inherent GTPase activity of the Gαi/o subunit causing a faster turn over in G protein signaling. Uncovering the mechanism by which missense variants impair Gβ5 function can help elucidate the role of Gβ5 in this syndrome. We hypothesized that these missense variants could alter Gβ5 structure, causing a disruption of function in one of two ways: by decreasing Gβ5 stability or by impacting the interaction between Gβ5 and its binding partners. We conducted various experiments to investigate Gβ5 carrying the missense variants found in patients. First, we employed the Iterative Threading ASSEmbly Refinement (iTASSER) server to model the potential structural impacts of these missense variants on the Gβ5 molecule. We found perturbations in the predicted structures of these variants when compared to the wild-type structure. Next, we performed *in vitro* experiments to assess the stability Gβ5. While initial data do not suggest that Gβ5 is destabilized with these missense variants, it cannot yet be ruled out as a mechanism for Gβ5 dysfunction. Lastly, experiments probing the interactions between

RGS7/Gβ5 and their binding partner R7BP were performed. Data from these experiments do not suggest that these variants have significant impacts on these interactions. More studies will be necessary to understand the mechanisms of LoF of these missense variants in IDDCA.

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Poster

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Support: NIH Grant R01HD092593
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Title: Placental allopregnanolone loss alters fetal GABAergic signaling

Authors: *J. J. O'REILLY[1,2], D. BAKALAR[2], J. ABBAH[2], C. M. VACHER[2], J. SALZBANK[2], H. LACAILLE[2], V. GALLO[2,3], A. A. PENN[1,2,4]
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Abstract: A major consequence of preterm birth is the loss of the placenta and the support it provides. The placenta supplies the developing fetus with critical hormones, including the neurosteroid allopregnanolone (ALLO). ALLO is a progesterone metabolite, synthesized by 3α-hydroxysteroid dehydrogenase (3αHSD; in mouse encoded by the *Akr1c14* gene). ALLO is a potent positive allosteric modulator of $GABA_A$ receptors ($GABA_A$-Rs) which also regulates $GABA_A$-R subunit expression. In the immature cortex, GABA acts an excitatory signal, due to the expression of the ion transporters NKCC1 and KCC2, which create a high-chloride environment inside the cell. This excitatory GABAergic signaling is critical for neuronal development and maturation. Importantly, high levels of placental ALLO coincide with a predominance of GABAergic (vs glutaminergic) synapses in the cortex. To directly test the hypothesis that placental ALLO loss disrupts the development of the GABAergic system, we utilize our $Akr1c14^{Cyp19a}$KO mouse model (KO), in which placental ALLO production is reduced. These mice have cortical changes and behavioral deficits that mirror those seen in human preterm survivors. Here, gene expression, protein quantification, and in-situ hybridization were used to assess molecular changes in the cortical GABAergic system during development in the absence of placental *Akr1c14*. KO mice had long-lasting, sex-specific alterations in $GABA_A$-R subunit expression and developmentally disrupted NKCC1 and KCC2 expression. These molecular changes correlate with electrophysiological changes: at P30, KO pyramidal cells had

IPSCs with faster decay rates, without changes in IPSC frequency, consistent with the changes in $GABA_A$-R subunits and ion transporters. Experiments are now focused on determining the mechanistic links between ALLO loss, GABAergic alterations and the loss of upper layer cortical neurons that we previously described. This is a novel and key link between placental function and long-term neurological outcomes, emphasizing the importance of the growing field of neuroplacentology.

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Poster

555. Mechanisms of Developmental Disorders

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Australian NHMRC Senior Principal Research Fellowship 1041920
NHMRC Program Grant 628952

Title: E3 ubiquitin ligase mutations in X-linked intellectual disability

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Abstract: Intellectual disability (ID), which affects 1-2% of the general population, is a devastating neurodevelopmental disorder with the most lifetime costs of all diagnoses in the U.S. However, males are more susceptible to ID than females and are often found to have severe outcomes. Mutations in X-chromosomal genes are thought to account for this male-biased phenomenon. KLHL15 was recently identified as a novel XLID gene. It encodes Kelch-like protein 15 (KLHL15), a substrate adaptor of a Cullin-3 (CUL3)-based E3 ubiquitin ligase complex that targets proteins, including the brain-enriched B′β regulatory subunit of protein phosphatase 2A (PP2A), for degradation by the ubiquitin/proteasome system (UPS). Several KLHL15 mutations have been found in the poorly characterized BACK domain, which is a "hotspot" for many deleterious variants of the other KLHL family members resulting in either Mendelian diseases or human cancers. We identified both loss-of-function (ΔFY241, ::ACOT9)

and gain-of-function (R249H) alleles, and we hypothesize that small deletions and point mutations in KLHL15's BACK domain lead to structural rearrangement that change the alignment between bound substrates and the ubiquitin-transfer (E2/E1) complex to either slow or accelerate substrate ubiquitination and degradation, causing dysregulated protein turnover of $CUL3^{KLHL15}$-targeted substrate(s) and eventually pathogenesis of ID.

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Poster

555. Mechanisms of Developmental Disorders

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Title: Determining pathogenesis of a rare pediatric intellectual disability and progressive microcephaly syndrome

Authors: *K. JOHNSON[1], L. SCHAFER[3], A. SCHAFFER[2], G. YEO[3]
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Abstract: Human mutations in nuclear proteins that regulate mRNA export have been shown to be causative for multiple rare pediatric intellectual disability and progressive microcephaly syndromes. These proteins are conserved among higher organisms and are known to facilitate nuclear to cytoplasmic mRNA export in addition to regulating the cellular processes of transcription elongation and genome stability; however, the function of each protein in the complex remains unknown. We aim to resolve the temporal and spatial expression of these proteins and determine their requirement during neurogenesis. In addition, we will investigate the mechanism of disease for the known pathogenic human variants using in-vitro and in-vivo approaches.In order to determine protein expression during neurogenesis, we have developed a mouse model harboring a proximal V5 tag. We will characterize expression of V5 in conjunction with brain specific cell-type markers to identify potentially vulnerable cell types to loss of this complex during brain development. To test the requirement for these proteins during neurogenesis, we will characterize a knockout mouse model we recently developed. Based on the patient phenotype of progressive microcephaly suggestive of postnatal neurodegeneration, we will characterize brain morphology and assess changes in proliferation, R-loop formation (DNA

damage), and apoptosis during embryogenesis. To create a cellular model for the disease to study the molecular mechanism of this syndrome, we have also generated primary mouse neuronal progenitor cells (NPC) lines from multiple V5/- and V5/+ embryos at e12.5. Of note, the null embryos are embryonic lethal prior to e12.5, thus NPC lines from knockout mice can not be developed. First, we will validate our in-vitro system is representative of the in vivo phenotype by assessing for cell proliferation, R-loop formation, and apoptosis. Following validation, we will assess levels of mRNA export by collecting whole cell lysate RNA, as well as nuclear and cytoplasmic fractions of RNA for sequencing. If mRNA export is defective in mutant cells, we predict we will see retention of cytoplasmic RNAs within the nucleus compared to controls. Since this protein complex is also known to play a role in transcriptional elongation and RNA splicing, we may observe changes in mRNA expression levels and splicing as another possible mechanism of disease. Overall, we aim to characterize the role of this complex in brain development, as well as characterize the pathogenic mechanisms leading to pediatric brain disease.

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Poster

555. Mechanisms of Developmental Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 555.20/C36

Topic: A.07. Developmental Disorders

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Board of Visitors Perinatal Neuroprotection Program (formerly Board of Visitors Cerebral Palsy Prevention Program), Children's National Medical Center

Title: Placental allopreganolone loss alters postnatal cerebellar development and long-term function

Authors: *J. SALZBANK[1,2], C.-M. VACHER[2], H. LACAILLE[2], D. BAKALAR[2], J. O'REILLY[1,2], A. A. PENN[3,2,1]
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Abstract: Preterm birth is a substantial risk factor for autism and related disorders. A major consequence of premature birth is early loss of the key endocrine organ of pregnancy, the placenta. Placental endocrine dysfunction or loss may place many thousands of fetuses at risk of

life-long neurodevelopmental impairments each year. We have been investigating the contribution of a neuroactive steroid, Allopregnanolone (ALLO), primarily synthesized by the placenta during late gestation, to neurodevelopmental impairments. ALLO exerts neurotrophic and neuroprotective effects in neurons and glial cells through allosteric activation of the GABA-A receptor suggesting that its loss could substantially alter the normal developmental GABAergic milieu. To assess the impact of placental ALLO deficiency, we generated a transgenic mouse line (AKR1c14^{CYP19a}KO) in which the gene encoding the enzyme responsible for ALLO production is specifically deleted by Cre-Lox recombination in the placenta. We examined cerebellar development because its rapid 3rd trimester growth makes it particularly vulnerable in preterm birth. Here we report three key cerebellar findings in our model. First, there are significant, sex-specific anatomical and molecular alterations in maturing cerebellar white matter. Second, social-cognitive cerebellar function is impaired but motor function is largely intact. Third, genes dysregulated in the KO compared to littermate controls overlap significantly with autism-linked genes from the SFARI database, particularly in myelin-related genes. White matter injury, a primary cause of deficits in preterm birth survivors, is also commonly seen in autism, particularly in the human cerebellum and cerebellar circuits. However, cerebellar white matter development is primarily a postnatal phenomenon, so placental endocrine alteration leading to this change is a particularly striking result. We are now investigating the mechanism by which placental ALLO loss leads to cerebellar white matter differences in an autism-like behavioral phenotype. The concept that compromised placental function may program lifelong mental disorders is a promising angle from which to approach their etiology and to identify new therapeutic targets that could decrease risk even before birth.

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Poster

555. Mechanisms of Developmental Disorders

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Topic: A.07. Developmental Disorders

Support: NIH Grant HD083157

Title: Ranbp1 mutations disrupt development of cranial neural crest

Authors: *E. M. PARONETT, C. A. BRYAN, B. A. KARPINSKI, A. S. LAMANTIA, T. M. MAYNARD
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Abstract: 22q11.2 Deletion Syndrome (22q11 DS) is a neurodevelopmental disorder that impacts 1 in 4,000 live births. Craniofacial anomalies associated with multiple neural crest-derived tissues, including structural defects of the palate and cranial bones, as well as defects in sensory/motor coordination that impair speech and swallowing, are apparent in most individuals carrying 22q11.2 deletions. We have found that *Ranbp1,* a 22q11.2 DS candidate gene, is a key regulator of multiple aspects of craniofacial development. Mice with homozygous null mutations of *Ranbp1* have a severe, strongly penetrant cleft palate phenotype, with a complete failure of palatal closure, and a concomitant failure to form key neural-crest derived palatal bone structures including the palatal processes of the maxilla and premaxilla. Conditional neural crest-specific knockout of *Ranbp1* yields a highly-penetrant but less-severe phenotype: *Wnt1*-Cre::*Ranbp1* null embryos have closed but highly dysmorphic palatal structure. Other crest-derived cranial bones show dysmorphology; in particular the structure of the vomer is altered such that its anterior aspect is enlarged at the expense of the posterior aspect. *Ranbp1* mutation also disrupts the formation of the trigeminal ganglion, mirroring (but significantly more severe than) anomalies we have observed in the *LgDel* mouse model of 22q11 DS. Heterozygous *Ranbp1* mutants display more subtle and variable forms of each of these phenotypes. Thus, *Ranbp1* appears to compromise palate formation, as well as disrupt the development of other craniofacial structures, possibly by disrupting the function of key craniofacial signals that pattern the cranial neural crest.

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Poster

555. Mechanisms of Developmental Disorders

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Topic: A.07. Developmental Disorders

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Title: MMP9/RAGE pathway overactivation underlies the inhibitory/excitatory imbalance induced by the feedforward loop of oxidative stress and neuroinflammation: A translation study in schizophrenia patients

Authors: *D. DWIR[1], B. GIANGRECO[1], L. XIN[2], L. TENENBAUM[3], J.-H. CABUNGCAL[1], P. STEULLET[1], A. GOUPIL[1], M. CLEUSIX[1,4], R. JENNI[1,4], P. BAUMANN[1,4], P. KLAUSER[1,4], P. CONUS[4], R. TIROUVANZIAM[5], M. CUENOD[1], K. Q. DO[1]
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Abstract: Besides oxidative stress (OxS), evidence indicates the implication of immune dysregulation in schizophrenia. As OxS is known to induce inflammation, we explored the mechanisms involved in their interaction using both a well-characterized cohort of early psychosis (EP) SZ patients that carry GAG trinucleotide-repeat polymorphisms in glutamate-cysteine ligase (GCL, the key synthesizing enzyme of the major antioxidant GSH) and a transgenic model of redox dysregulation, the Gclm knockout (*Gclm*-KO) mouse, which has a 70% reduction in brain GSH due to the lack of the GCL modulatory subunit (Gclm). OxS (8-oxoDG), microglia activation (Iba1, CD11b and CD68), parvalbumin interneurons (PVI) and perineuronal net (PNN), Receptor for Advanced Glycation End-product (RAGE) shedding, matrix-metalloproteinase 9 (MMP9), and NFkB activation (using an Adeno-Associated Virus) were investigated in the anterior cingulate cortex of *Gclm*-KO mice at both peripuberty (P40) and adulthood (P90), after an additional stress (dopamine uptake inhibitor GBR, P10-P20). At both P40 and P90, increased OxS and microglia activation were found in *Gclm*-KO, which peaked at P40, revealing a period of vulnerability during youth. In *Gclm*-KO at P40, RAGE shedding was increased in neurons and induced by MMP9, sensitive to OxS, as *In vivo* inhibition of MMP9 with a siRNA completely prevented RAGE shedding. Moreover, NFkB activation was increased in neurons of *Gclm*-KO, as well as pro-inflammatory cytokines. Then, in order to test the hypothesis that the following pathway: oxidative stress→MMP9 activation→RAGE shedding→NFKb activation→cytokines induction→microglia activation→reactive oxygen species production→OxS, could be causal to the long lasting PVI/PNN deficit observed in the 2 hits *Gclm*-KO model (±GBR, Cabungcal et al., 2013), the latter was treated with MMP9 inhibitor during puberty (P20-30). MMP9 inhibitor treatment, after the additional OxS, reversed PVI/PNN deficit, and reduced OxS as well as microglia activation in adulthood (P90). In EP patients with a genetic vulnerability to OxS, an increase in soluble RAGE was associated with low prefrontal GABA levels, potentially predicting a central inhibitory/excitatory imbalance, in line with our preclinical model in which OxS-induced MMP9 activation and increased RAGE shedding lead to PVI deficits. The circular pathway described above constitutes a positive feedforward process by which inflammation and OxS amplify each other, which is particularly damaging to PVI/PNN and might explain the persistence of the observed cellular damage. Therefore, MMP9 inhibitor holds promise for preventive treatment approaches.

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Poster

555. Mechanisms of Developmental Disorders

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Topic: A.07. Developmental Disorders

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Title: Somatic mutation in SLC35A2 leads to focal epilepsy

Authors: *A. PODURI[1], M. R. WINAWER[2], P. B. CRINO[3], E. L. HEINZEN[2]
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Abstract: Malformations of cortical development, including focal cortical dysplasia, have long been hypothesized to result from somatic, post-zygotic mutation. Recently, post-zygotic, or mosaic, variants in genes encoding mTOR-AKT-PI3K pathway proteins have been identified as a common cause of focal malformations through the study of brain tissue resected in the course of epilepsy surgery. We sought to identify novel causes of focal epileptic lesions through the study of brain tissue from patients with focal epilepsy with and without neuroimaging evidence of focal cortical dysplasia. We identified 18 patients without explanatory imaging findings (non-lesional) and 38 patients with focal malformations on imaging, all of whom were undergoing clinical evaluation and focal resection. We performed high-depth sequencing (gene panel and exome) on DNA from the resected brain tissue, as well as blood for comparison. We identified 5 distinct novel pathogenic variants in the same gene, *SLC35A2*, 3 from among the 18 who were non-lesional on imaging (2 of whom had neuropathological evidence of focal cortical dysplasia) and 2 from among the 18 with focal malformations on imaging, both of whom had radiological evidence of FCD. The variant allele frequency (VAF) ranged from 2-53%, with lower VAF for the non-lesional cases and higher VAF for the lesional cases. *SLC35A2* has been traditionally associated with epilepsy in the context of glycosylation defects. Our identification of post-zygotic variants in this gene—in cases ranging from non-lesional focal epilepsy to cases with neuroimaging or neuropathological evidence of abnormal cortical development—highlights the important role of somatic mutation in focal epilepsy. Further, we have identified a new role for glycosylation defects in epilepsy and in the pathogenesis of focal epileptic lesions.

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Poster

555. Mechanisms of Developmental Disorders

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Topic: A.07. Developmental Disorders

Support: NIH/NINDS Research Supplements to Promote Re-entry into Biomedical and Behavioral Research Careers - NS097305-01S1

Title: Cortical malformations in pediatric epilepsy

Authors: *L. SUBRAMANIAN, M. ANDREWS, A. BHADURI, M. PAREDES, A. R. KRIEGSTEIN
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Abstract: Medically intractable epilepsies are one of the most common neurological disorders that affect children. Focal Cortical Dysplasia (FCD) is a developmental malformation that is a major cause of surgically treated, medication-resistant pediatric epilepsy. FCD originates in the cerebral cortex of the embryo as a result of defects in proliferation, neuronal maturation, and/or neuronal migration. These developmental errors lead to focal regions of disorganization in the cerebral cortex of patients, characterized by disrupted lamination, misplaced neurons, dysplastic neurons and focal seizures. The molecular and cellular causes of these developmental errors are poorly understood. Recent studies suggest that somatic mutations in the genes regulating the mammalian Target of Rapamycin (mTOR) signaling pathway may be responsible for the condition. However, there is no clear understanding of the role played by the mTOR pathway in the development of the human cerebral cortex. It is also unclear if particular cell types are particularly vulnerable to these mutations during development and how the disrupted cellular identities contribute to the disease phenotype in patients with FCD. In order to gain a better insight into this condition, we examined the cellular composition of donated brain tissue from patients with focal cortical dysplasia. Our results suggest that the dysplasia may be the result of errors in the maturation of a specific group of progenitor cells. In order to understand how these errors in a progenitor cell type translate into the disease condition, it is necessary to build a detailed cellular profile of the disease focused on molecular and lineage relationships between cells. We are generating such a profile using advanced genomic technologies on donated human patient tissue samples to compare gene expression patterns between several thousand individual dysplastic and healthy neurons from multiple patients. In order to understand how FCD alters vulnerable cell types during development, we have also developed and validated slice culture models of human brain development. By pharmacologically manipulating mTOR signaling in this model, we can recapitulate key cellular characteristic of dysplastic cells in vitro. Together, these approaches will help unravel the developmental causes of FCD. In addition, they will

provide novel insights into the molecular and cellular events that shape the development of the human cerebral cortex, thus opening the door towards a broader understanding of other neurodevelopmental conditions.

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Poster

555. Mechanisms of Developmental Disorders

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Topic: A.07. Developmental Disorders

Support: 5R01MH107305-04
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Title: Defining links between an intellectual disability-associated RNA-binding protein and planar cell polarity in neurodevelopment

Authors: *E. B. CORGIAT, III[1], J. ROUNDS[1], P. CHEN[1], W. LEE[1], P. SHENG[1], A. CORBETT[2], K. MOBERG[1]
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Abstract: The human *ZC3H14* gene encodes a ubiquitously expressed zinc-finger polyadenosine RNA-binding protein. Mutations in *ZC3H14* that impair function of it encoded protein have been linked to an inherited form of non-syndromic intellectual disability (NS-ID). We developed a *Drosophila melanogaster* model of ZC3H14 NS-ID by deletion of *dNab2*, the fly ortholog of *ZC3H14*. These *dNab2*-deficient animals display defects in survival, locomotion, and memory which correlate at a cellular level with neurodevelopmental defects. Importantly, pan-neuronal expression of human ZC3H14 in *Drosophila* neurons can rescue the overt locomotor and survival phenotypes of *dNab2*-deficient flies, suggesting that dNab2 and ZC3H14 serve conserved roles in neurons. To probe this role, we used a dominant-modifier approach to identify alleles of genes that interact with *dNab2*. This approach has uncovered genetic interactions between *dNab2* and multiple components of the planar cell polarity (PCP) pathway, such as *Disheveled, Frizzled, and Van Gogh*. Additionally, we have characterized classic PCP-like defects in wing hair orientation and cochlea inner hair cell orientation in dNab2 null flies and ZC3H14 knockout mice, respectively. Furthermore, loss of function alleles of PCP components can rescue a portion of dNab2 null neuro-morphology defects observed in the mushroom bodies, twin neuropil structures analogous to the mammalian hippocampus. What underlies the rescue of this neurodevelopmental defect is of particular interest. Here we conduct a comparative proteomic analysis of control and dNab2 null brains at a critical timepoint in *Drosophila*

neurodevelopment. 4302 proteins were represented with significant changes in 144: 56 are increased and 88 are decreased in abundance by a factor of 1.6-fold or greater. Interestingly, a number of these are candidate PCP effectors or factors with PCP-like phenotypes by RNAi screens including: Treh, Akap200, and CG31738. Additionally, many actin and cytoskeletal-related proteins were identified including: Arc1, Lasp, Map205, and Polo. These data suggest that multiple pathways relevant to neurodevelopment are regulated by dNab2 but that PCP may be critical.

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Poster

555. Mechanisms of Developmental Disorders

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Title: The chromatin regulatory factor ASH1L regulates neuronal development by modulating the neurotrophin-signaling pathway

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Abstract: Autism spectrum disorders (ASD) are associated with defects in neuronal connectivity and are highly heritable. Genome wide association studies in large ASD family cohorts identified high risk variants associated with autism in genes that regulate histone modifications and remodel chromatin. These findings highlight the relevance of chromatin regulatory mechanisms in the pathology of ASD. Changes in Histone H3 methylation have been identified in a subset of neuronal genes in postmortem cerebral cortex of autism patients. ASH1L is a Histone H3-Methyltransferase that was previously identified in whole exome sequencing studies, as a gene strongly enriched for variants likely to increase ASD risk. ASH1L dimethylates Histone H3 on Lysine 36 (H3K36me2), this histone mark has been implicated in transcriptional activation. Therefore, ASH1L could modulate expression of genes that are essential for neuronal development. However, how mutations in ASH1L lead to deficits in neuronal connectivity associated with autism pathogenesis is largely unknown. We are using genome editing and shRNA knockdown approaches in stem cell derived human neurons to interrogate the function of

ASH1L. In particular we are defining how changes in chromatin structure and function elicited by loss of ASH1L could disrupt the structural development of early neuronal connectivity. Our preliminary data suggests that knockdown of ASH1L in human neurons impacts neurite outgrowth and that it might do so by modulating the expression of neurotrophic receptors. This is the first time that neurotrophic receptors gene expression have been shown to be regulated by the chromatin regulatory factor ASH1L, suggesting the relevance of ASH1L to human neuronal development.

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Poster

555. Mechanisms of Developmental Disorders

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Program #/Poster #: 555.27/D5

Topic: A.07. Developmental Disorders

Title: Altered gene expression in iPSC-derived cortical neurons predict risk for psychopathic violence

Authors: *M. KOSKUVI[1], J. TIIHONEN[2,3], I. HYÖTYLÄINEN[1], K. PUTTONEN[1], Y. GAO[1], O. VAURIO[2], I. OJANSUU[2], E. REPO-TIIHONEN[2], T. PAUNIO[4,6,8,9], M.-R. RAUTIAINEN[2,5,6], S. TYNI[10], S. LEHTONEN[1,7], J. KOISTINAHO[1,7]
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Abstract: Psychopathy is a disorder characterized by a loosely correlated set of interpersonal, affective, and behavioral features, including pronounced emotional deficits such as diminished sense of guilt and empathy. Psychopathy involves also an increased risk for antisocial behavior and poor impulse control. Although psychopaths represent less than 1% of the general population and 15-25% of prison populations, they perpetrate even 30-50% of all violent crimes. Thus, psychopathy is one of the strongest predictors of aggression and severe violence. This study aims to identify the neurobiological characteristics associated with psychopathic violence as markers, and targets for intervention and prevention of violent behavior by generating induced pluripotent stem cell (iPSC) lines from psychopathic violent substance abusers and healthy controls and substance abusers without psychiatric manifestations. The iPSC lines were differentiated to TUJ1+ and VGLUT1+ glutamatergic neurons via dual SMAD inhibition. The neuronal RNA

was sequenced with Illumina Hiseq sequencing system to compare the global gene expression profiles of the psychopaths and the two control groups. A total of 168 genes were up- or down-regulated (|FC| >3, p <0.05) in psychopathic violent substance abusers when compared to control groups. One particular gene showed strong and statistically significant upregulation among psychopathic prisoners (upregulated FC=4.4 p=0.044). Even though this gene has not been previously reported to be related to psychiatric disorders, its expression correlates positively with PCL-R scores of psychopathic violent substance abusers ($R^2 = 0.951$). Future proteomic studies will uncover interacting proteins for the gene and elucidate its contribution to psychopathic violence.

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Poster

555. Mechanisms of Developmental Disorders

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Program #/Poster #: 555.28/D6

Topic: A.07. Developmental Disorders

Support: NIMH 1R21MH113949-01

Title: Dysregulation of developmental and synaptic networks in a cellular model of intellectual disability

Authors: *B. J. WILKINSON[1,1], F. S. ALKURAYA[2], J. ICHIDA[1], M. P. COBA[1]
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Abstract: Advances in human genetics have identified a variety of candidate genes implicated in a number of developmental disorders such as intellectual disability (ID) and schizophrenia (SCZ). However, it is not known if mutations associated with candidate genes can be used to define alterations within developmental signaling networks. Here, we use the Traf2 and Nck Interacting Kinase (TNIK) as a model to explore changes in signaling pathways as a truncating mutation in the kinase domain (p.Arg180*) of TNIK has recently been shown to be causal for ID. TNIK plays essential roles in regulating synaptic function and interacts with multiple key postsynaptic density (PSD) proteins involved in complex brain disorders, including SHANK3 and SYNGAP1. We determined protein interaction networks of TNIK and potential substrates of the kinase domain in early neural development and adult PSD via mass spectrometry, which highlighted the involvement of TNIK in centrosomal dynamics and synaptic function. This

network was further expanded by immunoisolation of the centrosomal TNIK-interacting proteins AKAP9 and PDE4DIP. To investigate the function of TNIK within early stages of neural development, we generated multiple models using induced pluripotent stem cells (iPSCs). These include a TNIK kinase dead cell line and an iPSC line derived from a patient harboring the p.Arg180* mutation which was further corrected using CRISPR/Cas9 genome engineering. Functional analyses shows how TNIK regulates specific components within human neural progenitor cell (hNPC) developmental signaling networks such as beta-catenin and how they are dysregulated in mutant TNIK hNPCs. Furthermore, using mutant TNIK iPSC-derived glutamatergic neurons, we show the role of TNIK function in the regulation of synaptic activity through development and compare developmental and adult synaptic signaling networks in ID and SCZ.

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Poster

555. Mechanisms of Developmental Disorders

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Lookout Foundation

Title: Human neural progenitor cells harbor DSB clusters in genes linked to Autism

Authors: *M. WANG[1], P.-C. WEI[2], S. MARSHALL[1], I. S. GALLINA[1], C. K. LIM[1], F. W. ALT[2], F. H. GAGE[1]
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Abstract: Human neurons contain high level of somatic genomic variations that might derive from DNA double-strand break (DSB) intermediates. To study replication stress-induced DSB hotspots, we applied high throughput genome-wide translocation sequencing, identifying 36 replication-associated genomic fragile regions overlapping genes in neural progenitor cells (NPCs) derived from human pluripotent stem cells. Our analysis also reveals cell type-dependent gene fragility associated with transcription. Here we show that NPCs derived from autism

patients exhibit increased DNA damage and elevated DSBs in long genes associated with autism. Our results demonstrate that replication-associated genome instability may cause neurological dysfunction by disrupting long neural genes linked to neurodevelopmental diseases.

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Poster

555. Mechanisms of Developmental Disorders

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Title: Excitatory/Inhibitory imbalance in hiPSC derived cortical neurons from patients with autism associated with MEF2C haploinsufficiency

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Abstract: We and others have previously shown that transcription factor MEF2C is critical for neuronal differentiation, synapse formation and neuronal survival. Human MEF2C haploinsufficiency results in a syndrome with clinical features resembling autism spectrum disorder (ASD), intellectual disability, and epilepsy. However, molecular mechanisms underlying MEF2C haploinsufficiency syndrome (MHS) in patients remain poorly understood. Here we report that human induced pluripotent stem cell (hiPSC)-derived cerebrocortical neurons from MHS cultured on mouse astrocytes for 5-6 weeks exhibit excitatory to inhibitory (E/I) synaptic imbalance. By patch-clamp recording and fluo-4AM calcium imaging, we show greater spontaneous bursts of action potentials and increased frequency of calcium transients in MHS patient neurons when compared to controls including isogenic-correction. MHS patient neurons exhibit greater glutamate current density in response to 100 µM glutamate and smaller GABA current density in response to 100 µM GABA than control neurons. Sodium and potassium current density, cell size and resting membrane potential remain unchanged. MHS

patient neurons also display increased frequency and amplitude of miniature excitatory postsynaptic currents (mEPSCs), but decreased frequency of miniature inhibitory postsynaptic currents (mIPSCs). These results provide mechanistic insight into the abnormal neuronal electrical activity that leads to the observed functional deficits in patients with MHS. These aberrant electrical properties of MHS hiPSC-derived neurons will be useful for screening of putative novel therapeutic compounds in a patient-specific genetic context.

Disclosures: S. Ghatak: None. **D. Trudler:** None. **J. Parker:** None. **N. Dolatabadi:** None. **S. McKercher:** None. **R. Ambasudhan:** None. **M. Talantova:** None. **S.A. Lipton:** None.

Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 556.01/D9

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Title: The Unc93b1 mutation 3d attenuates neuropathic painthrough increasing M2 polarization of spinalmicroglia

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Abstract: Evolving evidence suggest that Tool-like receptors (TLRs) are associated with the maintenance of neuropathic pain. However, little is known about the precise mechanisms underlying the TLRs. UNC93B1 associated with TLR3, TLR7 and TLR9, mediating their translocation from the endoplasmic reticulum to the endolysosome, hence allowing proper activation of glia cells. We found that the triple deficient‘3d’mice, which lack functional UNC93B1, significantly attenuated the maintenance of tactile allodynia, activated microglial cellnumber. It was also noted that,either gene mutation of UNC93B1 or by neutralizing antibody, can significantly suppress the harmful cytokines (IFN-γ,IL-1β,TNF-α) expression and assist the beneficial cytokine (IL-10) expression. When applying the human recombinant HMGB1 into the MG6 microglial cell line, NF-κB and STAT1 activation was detected and significantly blocked by pretreating UNC93B1 neutralizing antibody. These observations suggest the crucial roles of TLR3, TLR7 and TLR9 in the development of neuropathic pain.

Disclosures: S. Li: None. **N. Hiroshi:** None. **D. shumin:** None.

Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 556.02/D10

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH R01 DA034748
the Medical Research Service of the Department of Veterans Affairs
NARSAD
We thank the National Neurological Specimens Bank, Los Angeles, NICHD Brain Bank and Netherlands Brain Bank for providing human brain tissue.

Title: Increased number of detected hypocretin (orexin) neurons in human heroin addicts

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Abstract: We found that human heroin addicts have, on average, a 54% increase in the number of detectable hypocretin neurons (N=5) relative to human controls (N=7, p=0.0009, t=8.89, df=10). Hypocretin cell size is reduced by 22% in the addicts (p= 0.01, t=2.78 df=10). In mice (C57BL/6) doses of 10 mg/kg or higher for 14 days produced a significantly elevated number of detected hypocretin neurons compared to saline. The increase in hypocretin cell number at 50 mg/kg was 38%. Doses above 50 mg/kg produced no further increase (10mg - p=0.009, t=-4.77 df=4; 25mg - p=0.019,t=-3.81, df=4; 50mg - p=0.002, t=-7.07, df=4; 75mg - p=0.01, t=-5.14, df=4; 100mg - p=0.01, t=-4.52 df=4). With daily dosing for a 60 day period changes in cell number were smaller than that after 14 days of administration. mRNA amounts of preprohypocretin, Narp and preprodynorphin were significantly elevated with morphine injection (Preprohypocretin p=0.03, t=2.99 df=5; Narp p=0.02, t=3.36, df=5; Prodynorphin p=0.01, t=3.65 df=5). The number of melanin concentrating hormone cells, a cell type intermixed with hypocretin cells in the hypothalamus, was not changed by morphine administration. BrdU labelling to identify new neurons showed no increase in the number of BrdU labelled cells in the hypothalamic hypocretin cell field after 14 days of morphine treatment in mice. Human narcoleptics with cataplexy given morphine over a long period were found to have a higher number of hypocretin cells than the available narcoleptic control case. Morphine administration

restored the population of detected hypocretin cells to the normal level in partially hypocretin depleted mice (orexin-tTA;TetO DTA mice), and eliminated or greatly decreased cataplexy in narcoleptic mice, suggesting that opiate agonists may have a role in the treatment of narcolepsy. Induction of specific long-term changes in peptide production may be useful in treating diseases characterized by neuronal loss. Our findings also indicate that some portion of the loss of specific cell types that have been reported in neurological diseases may be due to reduced production of the identifying label used for counting the neurons, rather than to neuronal death.

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Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 556.03/D11

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: RO1 NS094597
T32MH019934

Title: Protease systems in dense core secretory vesicles for neuropeptide biosynthesis and degradation analyzed via global proteomics, peptidomics, and multiplex substrate profiling-mass spectrometry (MSP-MS)

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Abstract: Dense core secretory vesicles (DCSVs) of neurons, glia, and neuroendocrine cells secrete peptide signal molecules to regulate physiological systems and facilitate cell-cell communication. These peptides perform crucial functions in biological processes that range from neurotransmission to hunger, analgesia, circadian rhythm, and cognition. Neuropeptide precursors, or proneuropeptides, are packaged in DCSVs along with a diverse array of proteases responsible for processing them into bioactive neuropeptides. This begins inside the DCSVs at pH ~5.5 and is followed by secretion into the extracellular environment of neutral pH 7.4. The acidic pH within DCSVs is thought to be an important factor for protease function. To gain understanding of the DCSV proteolytic systems, we utilized high-resolution liquid chromatography (LC)-mass spectrometry (MS) proteomics, peptidomics, and multiplex substrate

profiling (MSP)-MS of purified DCSVs from adrenal chromaffin cells (bovine) to determine 1) the identities of DCSV proteases and their endogenous inhibitors, 2) the primary cleavage properties of DCSV proteases at intravesicular and extracellular pH, and 3) which classes of DCSV proteases are responsible for the biosynthesis and degradation of neuropeptides such as Neuropeptide Y, Galanin, and Met-Enkephalin. Proteomics data identified approximately 65 proteases comprised of the cysteine, aspartyl, serine, and metallo protease sub-classes. The identification of this diverse group demonstrates the extensive spectrum of DCSV proteases. To characterize the specificity of proteolysis within the DCSVs at their internal pH of 5.5, MSP-MS was used to measure the relative abundances of cleavages of a comprehensive synthetic peptide library. Further, peptidomic analyses of endogenous peptides generated by DCSV proteases is being conducted to illustrate the protease sub-classes involved in neuropeptide production and degradation. Results will advance understanding of the vital DCSV proteolytic controls of neuropeptide precursor processing to generate active neuropeptides for intercellular communication.

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Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 556.04/D12

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: MYRG2014-00093-FHS
MYRG2016-00110-FHS
MYRG 2015-00036-FHS
FDCT 026/2014/A1
FDCT 025/2015/A1

Title: Desynchronized lower alpha rhythms were associated with functional ischemia in the prefrontal cortex in heroin patients after protracted abstinence: A concurrent EEG-fNIRS study

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Abstract: Opiate addiction involves cycles of lapse and relapse. Despite diverse treatment options nowadays, the relapse rate is still extremely high, and there is no biomarker to predict relapse. Prefrontal cortex (PFC) has been a target for drug addiction, in large part, because of its well-known executive functioning and its strong connection with limbic reward regions. However, the mechanism underlying the systems-level neuroadaptations during abstinence has

not been fully characterized across drug classes. It has been suggested that resting-state functional connectivity (rsFC) can serve as a systems-level biomarker to predict various neuropsychiatric trajectories. Is it possible to establish an intermediate level that explores a large population of cells and vessels within PFC network to better understand the adaptation in opiate addiction after prolonged cessation from the drug? The objectives of our study were to determine which neural oscillatory activity contributed to the chronic effect of opiate exposure on abstinence and whether the electrical activity could be coupled with neurovascular information in the PFC. The oscillatory activity was recorded through electroencephalography (EEG); whereas the hemodynamic activity was recorded through function near-infrared spectroscopy (fNIRS). Resting-state desynchronization in lower alpha rhythm, decreased functional connectivity and degree strength in PFC network among heroin-dependent patients. Through modern machine learning computation, asymmetric interhemispheric excitability evidenced by hemodynamic patterns in PFC was observed, suggesting as a potential biomarker for heroin protracted abstinence. Our findings have potentially important implications for future brain state-dependent electrotherapy applying wearable optical neuroimaging in clinical psychiatry to predict relapse.

Disclosures: H. Ieong: None. **Z. Yuan:** None.

Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 556.05/D13

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: ICMR 58/24-BMS-2011
Nalbuphine drug was gifted by RUSAN Pharma Ltd.

Title: Co-administration of mixed μ/ƙ-agonist attenuate the opioid dependence

Authors: *R. RAGHAV[1,2], R. JAIN[2], T. ROY[3], A. DHAWAN[2], P. KUMAR[3]
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Abstract: Background: The most actions of exogenous opioids, such as morphine, are mediated through μ-opioid receptors. By contrast, the activation of the κ-receptor antagonizes various μ-receptor mediated actions in the brain, including analgesia, tolerance, reward and memory processes. Therefore, the aim of present study was to provide more information about the possible action of acute and chronic co-administration of κ-agonist, nalbuphine on opioid dependence also it is not properly known whether the effect of acute and chronic doses of nalbuphine are similar in attenuating the opioid dependence.
Method: Male adult Wistar albino rats (n=160) were made physically dependent by

administrating increasing dose of morphine and withdrawals were precipitated with naloxone. Nalbuphine was co-administered acutely and chronically in variable doses (0.1, 0.3, 1.0, 3.0 mg/kg, i.p.) with morphine. Somatic signs of withdrawals were scored by using Gellert-Holtzman (GH) rating scale. Thereafter, brain was carefully dissected out for tyrosine hydroxylase, μ and κ expressions.
Results: Withdrawals from chronic morphine administration produces profound increase in GH-score whereas, TH levels were significantly decreased. Chronic co-administration of nalbuphine significantly suppressed the GH Score and μ-opioid receptor levels whereas, increase the TH and κ -opioid receptors levels. No change was observed with acute co-administration.
Conclusion: These findings suggest that withdrawal-induced reduction in TH levels could be responsible for somatic and as well as subjective symptoms of opiate withdrawal and anti-morphine action of the κ-receptor systems may lead to new drug design and therapeutic strategies for opioid addiction (Supported by ICMR, Govt. of India and nalbuphine gifted by RUSAN Pharma Ltd.).
Abbreviations: μ = mu; κ = Kappa; TH = Tyrosine Hydroxylase; n = Number

Disclosures: R. Raghav: 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.; Financially supported by Indian Council of Medical Research, Govt. of India. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Nalbuphine drug was gifted by RUSAN PHARMA Ltd. **R. Jain:** 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.; Financially supported by Indian Council of Medical Research, Govt. of India. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Nalbuphine drug was gifted by RUSAN PHARMA Ltd.. **T. Roy:** None. **A. Dhawan:** None. **P. Kumar:** None.

Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 556.06/D14

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH R01DK066604
NIH K12GM111725

Title: Characterizing reproductive function in POMC-deficient mice

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Abstract: The pro-opiomelanocortin (*Pomc*) gene encodes POMC, which is differentially processed to produce adrenocorticotrophin, beta-endorphin, and three melanocyte-stimulating hormones, among other peptides. POMC neurons are principally located in the arcuate nucleus (Arc) of the hypothalamus, where they are essential in the control of food intake, energy expenditure and body weight. Several different homozygous null mutations in the *POMC* gene have been shown to cause early-onset obesity and adrenal cortical insufficiency in a small number of humans. *Pomc* expression in Arc neurons is regulated by two distal enhancers. Mutations in these enhancers selectively reduce the amount of *Pomc* mRNA and POMC peptides in Arc neurons, but not pituitary cells. Furthermore, estrogen receptor alpha can bind to one of these enhancers *in vitro*, and about 25% of Arc POMC neurons express this receptor. Mice with combined deletions of both enhancers (FNΔ1Δ2) have less than one percent of Arc *Pomc* mRNA compared to wildtype mice. Like other mouse models of obesity, FNΔ1Δ2 mice are infertile, but it is unclear whether their reproductive disruption is due primarily to POMC-deficiency in the brain or is secondary to obesity. We are comparing aspects of reproductive function in wildtype and FNΔ1Δ2 female mice, including day of vaginal opening, day of first estrus, estrous cyclicity and fertility. In addition, we are using a related, conditional mutant mouse model (FNΔ2) in which *Pomc* gene expression can be restored by the action of a tamoxifen-inducible Cre-ERT2 transgene after the mice have developed obesity. Because humans with mutations in the *POMC* gene also experience disruptions in timing of puberty, or a cessation of pubertal development, understanding more about how hypothalamic POMC-deficiency impacts reproduction in mice may help to develop therapies for humans impacted by similar mutations.

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Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 556.07/D15

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH grant 5R01NS094597

Title: Human brain gene expression profiles of the cathepsin V and cathepsin L cysteine proteases, with the PC1/3 and PC2 serine proteases, involved in neuropeptide production

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Abstract: Proteases are required to generate active peptide neurotransmitters, known as neuropeptides, from pro-neuropeptides. Model animal systems have recently illustrated roles for

the cathepsin V (CTSV) and cathepsin L (CTSL) cysteine proteases, combined with the serine proteases PC1/3 (PCSK1) and PC2 (PCSK2), and exopeptidases in the production of neuropeptides. There is notable interest in the human-specific cathepsin V gene that is not present in rodent and other animal models used in prior studies of neuropeptide production. A gap in the field is of the human brain gene expression patterns of these neuropeptide-producing protease systems. Therefore, the goal of this study was to characterize the expression profiles of these pro-neuropeptide processing proteases in human brain. Quantitative gene expression microarray data for 169 human brain regions was obtained from the Allen Institute Human Brain Atlas resource, analyzed as $\log_2$ of gene expression intensity normalized to the mean of human genes (21,245 genes) expressed in human brain. These proteases had $\log_2$ values of 2-12, indicating expression levels above the average of all genes in the human brain, with varying expression levels among the 169 brain regions. CTSV and CTSL displayed moderate to high expression values of 1.9-8.6 and 7.1-10.6, respectively. Interestingly, CTSV and CTSL showed high expression in white matter composed of myelinated axons, consistent with the knowledge that neuropeptide production occurs within axons that transport neuropeptide secretory vesicles to nerve terminals. PCSK1 had a broad range of moderate to very high expression with $\log_2$ of 2-12. PCSK2 had somewhat lower expression levels than PCSK1. The exopeptidase genes RNPEP, CTSH, and CPE each showed fairly even levels of expression throughout the brain, with CPE displaying high expression. The prevalence of these processing proteases throughout human brain regions, including areas rich in neuropeptides such as hypothalamus, is consistent with their roles for neuropeptide production. Further, proenkephalin and NPY precursors, substrates of CTSV and CTSL shown in prior model animal studies, were co-expressed with CTSV and CTSL. These data demonstrate that the human brain expresses the neuropeptide-producing cysteine and serine proteases, with exopeptidases, throughout a multitude of brain regions.

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Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

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Program #/Poster #: 556.08/D16

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: Allen Institute Founders Paul G. Allen and Jody Allen
NIH R01NS092474
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Title: Cell-type-specific expression of neuropeptide precursor and receptor genes in mouse neocortical neurons

Authors: *S. J. SMITH, F. C. COLLMAN, L. ELABBADY, O. GLIKO, L. T. GRAYBUCK, M. KARLSSON, M. NAUGLE, J. SCHARDT, R. SERAFIN, S. SESHAMANI, B. TASIC, Z. YAO, H. ZENG
Allen Inst. for Brain Sci., Seattle, WA

Abstract: Function of the brain's synaptic networks depends profoundly upon adjustment of synaptic weights by spike activity and neuromodulatory chemical signaling. Among the numerous chemical signals known to modulate synaptic transmission, neuropeptides have long attracted attention due to discoveries of their potent effects in critical brain processes such as pain perception, mood and motivational state. Neuropeptides are produced as cleavage products of precursor proteins and stored in dense-cored secretory vesicles, which are released by regulated exocytotic secretion. In mouse, approximately 90 genes have been identified as encoding neuropeptide precursor proteins. In addition, similar numbers of protein species, predominantly G-Protein-Coupled Receptors (GPCRs), have been identified as neuropeptide receptors. The functional architecture of neuropeptide signaling systems has nonetheless remained enigmatic. The high ligand affinities of most neuropeptide receptors suggest the possibilities of humoral and volume transmission signaling, while the presence of neuropeptide secretory vesicles in many presynaptic boutons suggests more focal synaptic actions.
This poster presents new insights into neocortical neuropeptide signaling from deep single-cell RNA-seq transcriptomic analysis and clustering of approximately 20,000 neurons in VISp and ALM regions of mouse cortex (Tasic B, et al., *bioRxiv*. doi.org/10.1101/229542). This clustering identified 53 inhibitory neuron types and 58 excitatory neurons types in these two regions. Eighteen neuropeptide precursor genes with high and strongly cell-type-dependent expression patterns (Npy, Vip, Sst, Cck, Tac2, Penk, Crh, Tac1, Pdyn, Cort, Igf1, Nxph1, Pthlh, Pnoc, Cbln2, Cbln4, Adcyap1, Nucb2) were identified based on the RNA-Seq data. Strongly cell-type-specific expression of 20 GPCRs genes cognate to these 18 peptide precursors (Npy1r, Npy2r, Npy5r, Oprm1, Oprd1, OprK1, Oprl1, Ogfr, Sstr1, Sstr2, Sstr3, Sstr4, Vipr1, Vipr2, Cckb2, Tacr1, Tacr3, Hcrt1, Hcrt2) were similarly identified. The neuropeptide precursor mRNAs were found predominantly in the inhibitory neuron clusters, while the cognate receptor mRNAs genes were more commonly found in excitatory neuron clusters, suggesting a prevailing polarity of neuropeptidergic signaling from inhibitory to excitatory neurons. We are using array tomography to test for the presence of specific neuropeptide secretory vesicles in specific cell types as predicted by the transcriptomic analysis.
We thank Allen Institute for Brain Science founders, Paul G. Allen and Jody Allen, for their vision, encouragement and support.

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Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 556.09/D17

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: PHS Grant DA 024314

Title: Orphanin FQ/Nociceptin modulates energy homeostasis pleotropically by activating opioid receptor like-1 in a sex- and diet- dependent manner

Authors: *J. HERNANDEZ[1], C. FABELO[2], R. CHANG[1], E. J. WAGNER[2]
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Abstract: Orphanin FQ (aka nociception; OFQ/N) binds to its cognate opioid-receptor-like 1 (ORL1) in many different areas within the hypothalamus, including those that partake in the regulation of energy balance. It has been shown that ORL1 receptors are expressed in proopiomelanocortin (POMC) neurons within the arcuate nucleus (ARC), as well as in excitatory terminals impinging upon them, and that OFQ/N inhibits POMC neurons both pre- and post-synaptically[Conde, 2016]. Pre-synaptically, OFQ/N inhibits glutamatergic input onto POMC neurons, while post-synaptically, OFQ/N activates G-protein coupled inwardly-rectifying K^+ channels (GIRK) channels. Steroidogenic factor (SF) 1-expressing neurons in the dorsomedial ventromedial nucleus (VMN) of the hypothalamus, which are known to be glutamatergic, have been shown to synapse directly with ARC POMC neurons. Gonadal hormones regulate the hypothalamic energy balance circuitry in part by modulating $G_{i/o}$-coupled receptors and their linkage to GIRK channels. Thus, we tested hypothesis that OFQ/N inhibits neurotransmission via pleiotropic actions at VMN SF-1/ ARC POMC synapses. Electrophysiological recordings were done in slices from both intact male and female NR5A1-Cre mice and eGFP-POMC mice. In optogenetic recordings from POMC neurons in NR5A1-Cre mice, OFQ/N (1μM) significantly decreased the light-evoked excitatory postsynaptic current (leEPSC) more so males than in diestrus or proestrus females, and this inhibition was further accentuated in males fed a high- fat diet (HFD) for approximately 4-8 weeks. In recordings from POMC neurons in eGFP-POMC mice, OFQ/N induced a robust outward current and increase in conductance in voltage clamp, and a hyperpolarization and decrease in firing in current clamp. This effect was again greater in males than in diestrus, proestrus and estrus females. These pre- and postsynaptic actions were abolished upon application of the ORL-1 receptor antagonist BAN ORL-24 (10μM).These findings show that the OFQ/N-induced decrease in glutamate release and activation of GIRK channels at VMN SF-1/ ARC POMC synapses is greater in males than in females, and that diet-induced obesity caused by long term HFD exposure further potentiated OFQ/N-induced

inhibition of excitatory transmission at SF-1/POMC synapses. Overall, these findings demonstrate that OFQ/N regulates neurotransmission at SF-1/ POMC synapses in a sex- and diet-dependent manner.

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Poster

556. Opiates, Cytokines, and Other Neuropeptides

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Program #/Poster #: 556.10/D18

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: Cooper Medical School of Rowan University

Title: Colocalization of mor1 and gad67 in mouse nucleus accumbens

Authors: *C. HINKLE, T. N. FERRARO, E. I. DEDKOV, R. J. BUONO
Cooper Med. Sch. of Rowan Univ., Camden, NJ

Abstract: Current understanding of the rewarding and addictive effects of opioids involves mu-opioid receptor (MOR) binding within the nucleus accumbens (NAcc), a region of the basal forebrain. GABA neurons in the NAcc are thought to function to potentiate the rewarding response to opioids, and in fact, drugs that generally stimulate GABAergic activity are also addictive, a phenomenon mediated in part by endogenous opioid systems. It is still unclear how some individuals become susceptible to opioid addiction and thus, further understanding of the interaction between the MOR and other neurotransmitter systems in the reward pathway is needed. We report here evidence supporting the direct interaction between GABA and MOR within the mouse NAcc. Male and female FVB/NJ mice (12-16 months of age) were euthanized via carbon dioxide inhalation and brains processed for histology and immunohistochemistry (IHC). Coronal sections (10-12 um in thickness) were taken through the NAcc at the level of the anterior commissure. A mouse monoclonal antibody against GAD67, an enzyme catalyzing GABA production, was used in conjunction with an anti-mouse rhodamine red-X-labeled secondary antibody to identify GABA neurons. Alternating sections were stained for MOR using a rabbit polyclonal MOR1 antibody linked to the fluorophore FITC. The location of expression of GAD67 and MOR1 was identified using a DAPI nuclear stain. As expected, fluorescence microscopy results show that GAD67 staining is localized predominately in the cytoplasm. Unexpectedly, the MOR1-FITC stain tended to localize in the cytoplasm and cell membrane, but more prominently within the nucleus and nuclear membrane. In separate experiments, we used double-immunostaining to study the co-expression of MOR1 and GAD67 within the same NAcc neurons. A similar localization pattern for these proteins was detected. There are few published reports of GAD67 and MOR1 co-expression within neurons of the NAcc. Previous studies of

MOR expression show the receptor to be localized to the plasma membrane and, to a smaller degree, intracellularly. Here we found the MOR1 staining to be predominantly in the nucleus and nuclear membrane. Further studies are required to validate the nuclear expression of MOR in GABAergic NAcc neurons. We conclude that individual mouse NAcc neurons may express both MOR1 and GAD67, potentially providing a functional link between opioid and GABAergic systems in the reward pathway.

Disclosures: C. Hinkle: None. **T.N. Ferraro:** A. Employment/Salary (full or part-time):; Cooper Medical School of Rowan University. **E.I. Dedkov:** A. Employment/Salary (full or part-time):; Cooper Medical School of Rowan University. **R.J. Buono:** A. Employment/Salary (full or part-time):; Cooper Medical School of Rowan University.

Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 556.11/D19

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: DA034777

Title: Evaluation of novel dual-activity opioid-NPFF ligands for receptor affinity, antinociception and tolerance liabilities

Authors: *J. P. MCLAUGHLIN[1], K. L. MCPHERSON[2], M. MOTTINELLI[2], W. SHENG[3], S. O. EANS[3], V. B. JOURNIGAN[4,5], C. MESANGEAU[5], C. R. MCCURDY[2,5]
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Abstract: Tolerance limits the analgesic clinical value of mu-opioid receptor (MOR) agonists. Neuropeptide FF (NPFF) mediates hyperalgesia and opioid-induced tolerance through the activation of NPFF-1 and -2 receptors. We hypothesized that ligands with dual MOR agonist/NPFF receptor antagonist activity would produce antinociception with reduced tolerance. Accordingly, a series of ligands were designed with putative dual opioid and NPFF pharmacophoric elements. Nineteen of these novel ligands were synthesized and screened with competition radioligand binding assays *in vitro*, demonstrating a range of affinity for mu-, kappa-, and delta-opioid receptors (nM) as well as NPFF-1 and -2 receptors (μM). Subsequent *in vivo* screening of all compounds (30 nmol, i.c.v.) in mice with 55^{o}C and 48^{o}C warm-water tail-withdrawal assays identified three compounds with better analgesia and anti-hyperalgesia performance, VBJ-192, VBJ-215 and KGM01082. Following up with a more detailed assessment, all three compounds dose-dependently produced equipotent antinociception lasting

at least 50 min, with ED50 (and 95%CI) values of 6.9(4.7-9.5), 16(3.5-38.8) and 22.2(11.3-36.6) nmol, i.c.v., respectively that was antagonized by pretreatment with mu- or kappa-opioid receptors antagonists. All three compounds also dose-dependently attenuated NPFF-induced hyperalgesia. Unlike morphine, when tested in the acute antinociceptive tolerance test, repeated dosing of VBJ-215 showed no tolerance, while VBJ-192 and KGM01082 showed moderate tolerance commensurate with their magnitude of NPFF antagonism. In further examination of the three compounds, mice administrated with VBJ-192 or VBJ-215 showed neither respiratory depression nor elevated ambulation in the Comprehensive Lab Animal Monitoring System (CLAMS), and both VBJ-215 and a low dose of VBJ-192 did not impair coordinated locomotor activity on the rotorod (30 and 100 nmol, i.c.v.). Together, these results confirm the mediating effect of NPFF on opioid tolerance, and suggest the potential of dual-action opioid-NPFF ligands as analgesics with fewer liabilities of use.

Disclosures: J.P. McLaughlin: None. **K.L. McPherson:** None. **M. Mottinelli:** None. **W. Sheng:** None. **S.O. Eans:** None. **V.B. Journigan:** None. **C. Mesangeau:** None. **C.R. McCurdy:** None.

Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 556.12/D20

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH R01 Grant DE17794
NIH R01 Grant DE22743
NIH R01 Grant NS87988

Title: Different roles of PD-L1 and PD-L2 in regulating nociceptive synaptic transmission in spinal cord dorsal horn neurons

Authors: *C.-Y. JIANG, M. MATSUDA, Z. WANG, R.-R. JI
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Abstract: Programmed cell death ligand-1 (PD-L1) is typically produced by cancer cells and has been shown to suppress immunity through PD-1 receptor expressed on T cells. Emerging immune therapies such as anti-PD1 and anti-PD-L1 monoclonal antibodies have shown success in treating cancers such as melanoma, as well as lymphoma, lung cancer, ovarian cancer, and head and neck cancers. We recently demonstrated that PD-1 is also expressed by primary sensory neurons in dorsal root ganglion (DRG). PD-L1 inhibits acute and chronic pain by suppressing nociceptive neuron activity via PD-1 (Chen et al., Nat Neurosci, 2017). PD-L2 is another ligand of PD-1, but its role in nociception is unclear. We compared PD-L1 and PD-L2

expression using RNAscope. We found broad expression of PD-L1 in many DRG neurons but very limited expression of PD-L2 in mouse DRGs. We also tested the effects of PD-L1 and PD-L2 in spinal cord synaptic transmission using patch clamp recordings in isolated spinal cord slices. While PD-L1 significantly reduced sEPSC in lamina IIo neurons, PD-L2 had very mild effects on sEPSCs. Currently, we also comparing the antinoceptive effects of PD-L1 and PD-L2. Our findings suggest that PD-L1 has normal physiological function and may serve as an endogenous neuromodulator or neurotransmitter as well as an endogenous pain inhibitor.Thus, PD-L1 is not only an immune checkpoint inhibitor but may also act as a "neuro checkpoint inhibitor".

Disclosures: C. Jiang: None. **M. Matsuda:** None. **Z. Wang:** None. **R. Ji:** None.

Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 556.13/D21

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Title: Examining the chronic effects of indirect and direct cannabinoid receptor agonists on dopamine transmission in the nucleus accumbens of mice

Authors: K. M. HONEYWELL, T. FREELS, A. CHAFFIN, M. MCWAIN, H. NOLEN, H. J. SABLE, *D. B. LESTER
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Abstract: A major problem with current anxiolytic medications is abuse liability; thus, new pharmaceutical targets are being explored. The cannabinoid (CB) system is one potential target. Previous behavioral studies have shown that indirect agonists of the CB system may be more beneficial as anxiolytics than direct CB receptor agonists. Determining the effect of such CB agonists on dopamine release in the nucleus accumbens (NAc), a brain area well known for regulating the rewarding effects of drugs, is critical to assess potential abuse liabilities. The current study compared the effects of chronic administration (one injection per day for 7 days) of the indirect CB agonist arachidonoyl serotonin (AA-5-HT 2.5 mg/kg, i.p.), the direct CB receptor agonist arachidonyl-2-chloroethylamide (ACEA 1 mg/kg, i.p.), and vehicle (control solution with saline and 10% DMSO) on locomotor activity using open field tests and stimulation-evoked NAc dopamine release using in vivo fixed potential amperometry in anesthetized mice. AA-5-HT indirectly agonizes the CB system via inhibition of fatty acid amide hydrolase (FAAH) while also inhibiting transient vanilloid type 1 channels ($TRPV_1$), providing this drug with 2 anxiolytic mechanisms. Open field tests revealed that the 7^{th} injection of ACEA but not AA-5-HT decreased locomotor activity relative to pre-drug baseline (ACEA = 14.41% ± 6.51, AA-5-HT = 54.92% ± 11.85, and vehicle = 55.74% ± 13.2). Amperometric recordings revealed that mice

chronically treated with ACEA but not AA-5-HT had significantly decreased stimulation-evoked dopamine release (ACEA = 0.18 uM ± 0.03, AA-5-HT = 0.23 uM ± 0.03, and vehicle = 0.29 uM ± 0.03). Furthermore, mice chronically treated with ACEA but not AA-5-HT had an increased dopaminergic response to cocaine (10 mg/kg, i.p.) (ACEA = 289% ± 27, AA-5-HT = 197% ± 22, and vehicle = 223% ± 17). Overall, in regards to potential anxiolytic use, these findings suggest that indirect mechanisms of agonizing the CB system may be a better alternative than direct mechanisms if concerned with disrupting dopamine function and inducing abuse liability.

Disclosures: K.M. Honeywell: None. **T. Freels:** None. **A. Chaffin:** None. **M. McWain:** None. **H. Nolen:** None. **H.J. Sable:** None. **D.B. Lester:** None.

Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 556.14/D22

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: DA041336
DA00523

Title: 2-Aminoindan and its ring-substituted derivatives interact with plasma membrane monoamine transporters and α_2-adrenergic receptors

Authors: *A. L. HALBERSTADT[1], S. D. BRANDT[2], W. DONNA[3], M. H. BAUMANN[3]
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Abstract: Over the last decade many new controlled psychostimulant substance analogues have appeared on the recreational drug market and many of these substances are derivatives of amphetamine or cathinone. Another class of designer drugs are derived from the 2-aminoindan structural template. Several members of this class, including the parent compound 2-aminoindan (2-AI), have been available in Europe as designer drugs. Here we tested 2-AI and its ring-substituted derivatives 5-methoxy-2-aminoindan (5-MeO-AI), 5-methoxy-5-methyl-2-aminoindan (MMAI), and 5,6-methylenedioxy-2-aminoindan (MDAI) for their abilities to interact with plasma membrane monoamine transporters for dopamine (DAT), norepinephrine (NET) and serotonin (SERT). We also compared the binding affinities of the 2-aminoindans at 29 receptor and transporter binding sites. We found that 2-AI was a selective substrate for NET (EC_{50} = 86 nM) and DAT (EC_{50} = 439 nM). Ring substitution increased potency at SERT while reducing potency at DAT and NET. MDAI was moderately selective for SERT (EC_{50} = 114 nM) and NET (EC_{50} = 117 nM), with 10-fold weaker effects on DAT (EC_{50} = 1,334 nM). 5-MeO-AI exhibited some selectivity for SERT (EC_{50} = 134 nM), having 6-fold lower potency at NET

(EC_{50} = 861 nM) and 20-fold lower potency at DAT (EC_{50} = 2,646 nM). Conversely, MMAI was highly selective for SERT (EC_{50} = 31 nM), with 100-fold lower potency at NET (EC_{50} = 3,101 nM) and DAT (EC_{50} > 10,000 nM). In addition to their effects on monoamine release, the 2-aminoindans had relatively high affinity for α_2-adrenoceptor subtypes. 2-AI had particularly high affinity for α_{2C} receptors (K_i = 41 nM) and slightly lower affinity for the α_{2A} (K_i = 134 nM) and α_{2B} (K_i = 211 nM) subtypes. α_2-Adrenoceptor affinity was reduced by ring substitution but 5-MeO-AI, MMAI, and MDAI still bound with submicromolar or micromolar affinity. 5-MeO-AI and MMAI also had moderate affinity for the 5-HT_{2B} receptor (K_i values of 4,793 nM and 902 nM, respectively). Based on these results, 2-AI is predicted to have (+)-amphetamine-like effects and abuse potential whereas the ring-substituted derivatives may produce 3,4-methylenedioxymethamphetamine (MDMA)-like effects but with less abuse liability

Disclosures: A.L. Halberstadt: A. Employment/Salary (full or part-time):; UCSD, Veteran's Administration. 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.; NIDA. **S.D. Brandt:** A. Employment/Salary (full or part-time):; Liverpool John Moores University. **W. Donna:** A. Employment/Salary (full or part-time):; NIDA. **M.H. Baumann:** A. Employment/Salary (full or part-time):; NIDA.

Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 556.15/D23

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Title: Encoding of phasic nucleus accumbens dopamine release by ventral tegmental area neurons revealed through simultaneous single-unit recording and fast-scan cyclic voltammetry

Authors: *D. F. HILL[1], Z. OLSEN[1], M. L. HEIEN[2], S. L. COWEN[3,4]
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Abstract: Dopaminergic signaling is known for its role in reward valuation, reinforcement learning, and memory. Dysregulation of dopamine signaling is also implicated in neuropathological conditions such as depression, schizophrenia, movement disorders, and addiction. Dopamine is released from ventral tegmental area (VTA) neurons and acts on dopamine receptors in the nucleus accumbens (NAc) to modulate cortical input. Dopaminergic terminals in the NAc are thought to release dopamine in response to large population-level burst activity in the VTA. However, the exact relationship between VTA cell activity and NAc dopamine release has not been established due to technological limitations that have prevented

simultaneous measurement of both dopamine release and single-unit activity. To address this, we collected simultaneous measurements of cell firing in the VTA and dopamine release in the NAc using a novel measurement tool developed in our laboratory that integrates extracellular electrophysiological recording with fast-scan cyclic voltammetry. To induce phasic dopamine release, anesthetized Sprague Dawley rats (n = 10, 3 - 4 months old, 1 - 1.5 % isoflurane) were injected with dopamine transporter inhibitor GBR-12909 (17.5 mg/kg, i.p.) and D2 receptor antagonist eticlopride (0.75 mg/kg, i.p.). Although we predicted that a large portion of recorded dopamine neurons would fire before the onset of transient dopamine release events, we found instead that only ~ 8 % of VTA dopamine neurons exhibited reliable peri-event responses before a dopamine transient release event. Additionally, transient dopamine release events were associated with small (< 1 Hz) increases in dopamine neuron activity. Neurons that did exhibit reliable responses to transient dopamine release events responded long before the onset of transient dopamine release (980 ± 403 ms SEM; n = 5 neurons). We also observed that the firing rate of putative GABAergic neurons in the neighboring 'tail' of the VTA (tVTA), a region thought to be the 'master brake' of the VTA, decreased as cell firing of both dopaminergic and non-dopaminergic neurons in the VTA increased. Taken together these data suggest that NAc dopamine release is encoded by sparse signals from VTA dopamine neurons that are under tight control by inhibitory neurons of the tVTA.

Disclosures: D.F. Hill: None. **Z. Olsen:** None. **M.L. Heien:** None. **S.L. Cowen:** None.

Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 556.16/D24

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: Whitehall Foundation Research Grant #2017-08-43

Title: Electrochemical characterization of chemogenetically modulated dopamine transmission in the olfactory tubercle

Authors: *R. BHIMANI[1], C. E. BASS[2], J. PARK[1]
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Abstract: The selective targeting of specific neuronal subtypes using chemogenetic techniques, such as Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), has facilitated the understanding of the functional roles of complex brain circuits. While DREADDs are a powerful tool for transient and repeated manipulation of neurons, how activation of excitatory or inhibitory DREADDs affects neurotransmitter dynamics (release and clearance) is poorly understood. In this study, we used a combinatorial viral targeting system to restrict

DREADD expression to dopamine (DA) neurons in the ventral tegmental area (VTA)/substantia nigra (SN) of wild-type rats. We then employed in vivo fast-scan cyclic voltammetry (FSCV) to determine how systemic administration of Clozapine-N-oxide (CNO), a biologically inert ligand for DREADDs, modulates DA transmission in the olfactory tubercle (OT), an important limbic structure located in the ventral-most part of the ventral striatum that is implicated in mediating the rewarding effects of drugs. Through immunohistochemical and electrochemical evidence, we demonstrated selective viral targeting of DA neurons and determined that CNO dose-dependently (0.3 - 6.0 mg/kg, i.p) activates DREADDs, leading to excitation and/or inhibition of DA release evoked by electrical stimulation of the VTA/SN in urethane-anesthetized rats. These results will facilitate the understanding of DA neurons in essential brain functions, as well as establish guidelines for the use of DREADDs in behavioral studies.

Disclosures: R. Bhimani: None. **C.E. Bass:** None. **J. Park:** None.

Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 556.17/D25

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Title: Robust expression of 5HT2A and 5HT2B in glia cells: A comparative immunohistochemical study of non-principal cells

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Abstract: Serotonin action on principal excitatory cells is implicated in mood regulation and thought to be mechanistic for part of the dysfunction in many psychiatric disorders. While pharmacological treatments targeting serotonin signaling can be highly effective their exact mechanism is not clearly understood. Released serotonin may bind to any of seven 5HT receptor subtypes, with the 5HT2 family having a critical role in mood disorder pathology. Recent findings in cultured cells and expression systems have demonstrated serotonin receptor expression and function in non-principal cells, yet a comprehensive and comparative localization of these receptors in intact tissue has yet to be completed. In the present study, we examined 5HT2A and 5HT2B receptor expression in parvalbumin-positive inhibitory interneurons, GFAP-positive astrocytes, and Iba1-positive microglia in the mouse cortex and hippocampal CA1 region. Using immunohistochemistry and confocal microscopy, we characterize differential 5HT2A and 5HT2B receptor expression that varies both by cell type and brain region. We detected robust expression levels of 5HT2A in microglia cells, which are not conventionally thought to participate in serotonin signaling. These findings elucidate the potential contributions

of specific 5HT2 receptor subtypes to normal brain function via non-principal cells and may have implications for the mechanisms of action of drugs that target these receptors.

Disclosures: A. Contreras: None. **R.M. Hines:** None. **D.J. Hines:** None.

Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 556.18/D26

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: Davidson Research Initiative
NIH grant DA045714
NIH grant DA031725

Title: *Caenorhabditis elegans* as a molecular model organism for drug addiction

Authors: *M. HAY, M. SMITH, R. EL BEJJANI
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Abstract: Drugs of abuse cause addiction through neuronal modulations, though the molecular mechanisms of addiction are poorly understood and targeted treatment options are scarce. The immense social and financial costs of drug addiction necessitate further investigation. The rewarding effects of these drugs act through many of the same monoamine proteins in humans as in invertebrates. Since the human nervous system is quite complex, this research utilizes a simple, reliable model for investigation of the molecular mechanisms of action underlying cocaine and MDMA using the invertebrate nematode *Caenorhabditis elegans*. Previous research confirms high molecular conservation of the dopamine and serotonin reward systems between humans and *C. elegans*. We set out to investigate the effects of cocaine and MDMA on *C. elegans* egg laying as a marker for activation of the serotonergic system. Cocaine inhibits the dopamine, serotonin and norepinephrine transporters in mammals, whereas MDMA interferes in vesicle packaging of neurotransmitters and is a known serotonergic agonist. Our results show a dose-dependent increase in egg-laying in response to cocaine (mean eggs laid/animal/hour = 6.56 at 70 mM and 2.82 at 35 mM, $p < 0.0001$ for both concentrations as compared to an osmotic and a negative control) and a smaller increase in response to MDMA (mean eggs laid/animal/hour = 1.1 at 35 mM, $p = 0.0048$ compared to osmotic control and $p = 0.0019$ compared to negative control). Significantly, we observed a curled posture of worms subjected to MDMA (88.00% curled). Reduction of function mutations in the ortholog of the vesicular proton ATPase required for neurotransmitter transport into vesicles show an identical posture in the same assay (*unc-32;him-5* control strain, 79.49% curled, Fisher's exact test MDMA treated wild type *vs* untreated *unc-32* mutants $p = 0.3802$). To further investigate this finding, we will test for resistance to

MDMA when *unc-32* is overexpressed. Finally, we are also adapting a Conditioned Place Preference experiment to be applied on relevant mutant strains. We aim to use this system and our posture and egg laying assays to measure addictive behavior to MDMA and cocaine and elucidate the molecular mechanisms involved.

Disclosures: M. Hay: None. **M. Smith:** None. **R. El Bejjani:** None.

Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 556.19/D27

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: R01 MH100292

Title: Role of dopaminergic modulation of thalamo-prefrontal connectivity in social behavior

Authors: *J. IAFRATI[1], S. INCONTRO[2], C. C. BAVLEY[3], A. M. RAJADHYAKSHA[4], J. L. WHISTLER[5], V. S. SOHAL[6]
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Abstract: The medial prefrontal cortex (mPFC) plays a key role in cognitive and emotional behaviors affected in many neuropsychiatric disorders. Dopamine is a major modulator of layer 5 (L5) pyramidal neurons, which are a main output from mPFC. L5 neurons can be divided into subpopulations based on their expression of various dopamine receptors. Previous studies from our laboratory showed that subcortically projecting L5 pyramidal neurons exhibit a specific modulation of intrinsic excitability after dopamine type 2 receptor (D2R) activation. However, the effects of dopaminergic modulation on specific circuits and behavior remain unclear. To test whether dopamine modulates behavior through defined circuits, we have used a combination of genetic models, behavioral assays, optogenetic manipulations, pharmacology, patch-clamp recordings and *in vivo* calcium imaging. We have found that D2R activation can enhance responses of mPFC neurons to inputs from the mediodorsal thalamus (MD) but not other sources. This mechanism is cell-type specific and depends on specific voltage gated ion channels. D2R deletion in the mPFC disrupts normal social behavior. Furthermore, optogenetic inhibition of projections from the MD thalamus to mPFC can decrease social interactions. Together, these observations suggest a role for this mechanism in social behaviors. Interestingly, we found that the D2R-mediated potentiation of MD-mPFC synapses can be abolished under

pathological conditions: cocaine administration, DISC1 dominant negative and chronic social defeat stress. In these cases, the D2R-mediated potentiation can be rescued by preventing D2R internalization and degradation, suggesting that under some conditions, the downregulation of surface D2Rs may lead to the loss of this modulation, potentially driving behavioral effects.

Disclosures: J. Iafrati: None. **S. Incontro:** None. **C.C. Bavley:** None. **A.M. Rajadhyaksha:** None. **J.L. Whistler:** None. **V.S. Sohal:** None.

Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

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Program #/Poster #: 556.20/D28

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NSF GRFP
UCLA Cota Robles
UCLA Depression Grand Challenge
NSF IOS-1455869

Title: A novel serotonergic microcircuit in the *Drosophila* visual system

Authors: *M. M. SAMPSON[1], K. MYERS GSCHWENG[2], M. FRYE[3], D. KRANTZ[2]
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Abstract: Serotonergic projections densely innervate the visual system of the fruit fly *Drosophila melanogaster*, yet the role of serotonin signaling in the optic lobe remains unknown. Here, we characterize cells expressing serotonin receptors and identify a microcircuit modulated by serotonin. *Drosophila* have five serotonin receptors that are homologous to mammalian 5-HT1A, 5-HT1B, 5-HT2A, 5-HT2B and 5-HT7. We found that 5-HT1A, 5-HT1B and 5-HT7 are expressed in serotonin-immunoreactive projections, presumably functioning as autoreceptors. We also identified several neurons that house serotonin heteroreceptors, including lamina monopolar cell 2 (‘L2’), expressing excitatory 5-HT2B and 5-HT7 receptors, and ‘T1’, expressing inhibitory 5-HT1A and 5-HT1B receptors. Serotonin neurons were not found to synapse onto L2 or T1 neurons, indicating signaling via volume transmission. However, L2 and T1 neurons both synapse onto serotonergic projections, in contrast to several other serotonin receptor-expressing cells we examined. Intriguingly, there are reciprocal synaptic connections between L2 and T1 neurons themselves. Thus, activation of serotonin receptors independently modulates each visual neuron, while the L2/T1 synaptic connection acts as a potential integration site. L2 and T1 both form reciprocal synapses with lamina monopolar cell one (‘L1’), together encompassing the light-OFF and -ON visual pathways. This is the first description of serotonin signaling to visual processing neurons that function as inputs to fly visuomotor behaviors. The

Drosophila visual system's well-characterized circuitry provides an ideal model system to inform our understanding of long range serotonergic signaling and reveal basic principles of modulatory network function.

Disclosures: M.M. Sampson: None. **K. Myers Gschweng:** None. **M. Frye:** None. **D. Krantz:** None.

Poster

556. Opiates, Cytokines, and Other Neuropeptides

Location: SDCC Halls B-H

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Program #/Poster #: 556.21/D29

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIMH R21MH108867
NINDS F99NS105208

Title: CTR1 in normal striatum, substantia nigra, and cortex

Authors: *K. E. SCHOONOVER[1], C. NGUYEN[2], C. FARMER[3], R. C. ROBERTS[4]
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Abstract: Copper is crucial for several cellular functions including proper monoamine metabolism, mitochondrial activity, and myelination. Abnormal copper levels are implicated in several brain disorders such as Menke's disease, Wilson's disease, and probably schizophrenia (SZ). In fact, copper-decreasing experimental manipulations (such as a diet containing the copper chelator cuprizone) produce demyelination, increased dopamine, decreased expression of oligodendrocytic (OLI) proteins and SZ-like behavioral impairments. The copper transporter CTR1 transports copper across the blood brain barrier, but has rarely been studied in human postmortem brain. In this study we used immunohistochemistry to localize CTR1 in the striatum, substantia nigra (SN), and prefrontal cortex of twelve normal controls, with four cases examined for each brain area (5F&7M; overall mean PMI and age were 11.83hr and 46yrs). Rabbit anti-CTR1 (dilution 1:2000, Novus Biologicals NB100-402) was used. Preadsorbtion with CTR1 blocking peptide neutralized all staining, confirming antibody specificity. Cellular staining was infrequent throughout most of the striatum, while endothelial cells, labeled punctate structures, and beady fibers were observed. There was dramatic staining of fibers and ependymal cells at the ventricular border of the caudate. Very few, if any, stained astrocytes (AST) and OLI were observed in the striatum; however, AST were occasionally labeled in the external and internal capsule. Diffusely labeled puncta were observed in the white, but not grey, matter of the striatum. The SN contained a large number of stained endothelial cells within dopaminergic and

nondopaminergic regions; prominently labeled endothelial cells were also common in white matter. Most dopaminergic neurons appeared to be labeled. Very few, if any, labeled AST and OLI were observed. However, densely labeled neuropil was observed throughout the SN. Labeled beady fibers were also present. Labeling was present in neuropil, neurons and glia of the cortex. Labeled neurons, including pyramidal neurons, were scattered throughout grey matter. Blood vessels lined with stained endothelial cells were prominent in both grey and white matter. Subcortical white matter was heavily stained with fibers, punctate structures, glial cells and endothelial cells. CTR1 labeling differed between regions. The rich staining of CTR1 in the SN, cortex and hippocampus (previous work) parallels the known concentrations of copper in the brain. This study yields novel information about cell-specific CTR1 copper transport in postmortem human cortex, SN and striatum, which could elucidate disease state etiology.

Disclosures: K.E. Schoonover: None. **C. Nguyen:** None. **C. Farmer:** None. **R.C. Roberts:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 557.01/D30

Topic: B.02. Ligand-Gated Ion Channels

Title: Dual actions of Psalmotoxin at ASIC1a and ASIC2a heteromeric channels (ASIC1a/2a)

Authors: R. H. HAGAN, J. SCHOELLERMAN, *Y. LIU
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Abstract: Acid-Sensing Ion Channels (ASICs) are gated by extracellular protons and play important roles in physiological and pathological states, such as pain and stroke. ASIC1a and ASIC2a, two of the most highly expressed subunits in the brain, form functional homo- and hetero-meric (ASIC1a/2a) channels. The function of ASIC1a has been widely studied using psalmotoxin (PcTx1), a venom-derived peptide, as an ASIC1a-selective antagonist. Here, using whole-cell patch clamp, we show that PcTx1 has dual actions at rodent ASIC1a/2a. It can either inhibit or potentiate the heteromeric channel, depending on the conditioning and stimulating pHs. Potent inhibition occurs only at conditioning pHs that begin to desensitize the channel (IC_{50} = 2.9 nM at pH7.0, a threshold pH for desensitization of ASIC1a/2a). By contrast, potent potentiation of the channel can occur at the physiological pH in both CHO cells (EC_{50} = 56.1 nM) and cortical neurons (threshold concentration < 10 nM). PcTx1 potentiates ASIC1a/2a by increasing the apparent affinity of channel activation for protons. As such, potentiation is the strongest at moderate pHs, diminishing with increasing proton concentrations. Our findings identify PcTx1 as a valuable tool for studying ASIC1a/2a function and expand the diverse and complex pharmacology of PcTx1.

Disclosures: **R.H. Hagan:** A. Employment/Salary (full or part-time):; Janssen R&D. **J. Schoellerman:** A. Employment/Salary (full or part-time):; Janssen R&D. **Y. Liu:** A. Employment/Salary (full or part-time):; Janssen R&D.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 557.02/D31

Topic: B.02. Ligand-Gated Ion Channels

Support: DFG VI586
DFG SO328
Graduate School of Life Sciences (GSLS)
Scientia-career development program

Title: New insights into the pathology of glycine receptor autoantibodies in stiff person syndrome

Authors: V. ROEMER[1], N. VON WARDENBURG[1], N. SCHAEFER[1], E. TÜZÜN[2], C. L. SOMMER[3], *C. VILLMANN[1]
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Abstract: The rare neurological disease stiff person syndrome (SPS) is triggered by autoantibodies against various neuronal structures including intracellular proteins like the GABA-synthesizing enzyme glutamate decarboxylase (GAD) and the vesicle-associated protein amphiphysin or membrane-bound proteins like NMDA receptors and glycine receptors (GlyR). Typical symptoms of SPS-patients are stiffness and painful spasms in axial and proximal limb muscles sometimes accompanied by anxiety and sudden falls. So far, immunotherapy is the most effective treatment but relapses often occur. We focus on SPS-patients carrying GlyR autoantibodies. The first identification of GlyR autoantibodies in a patient was in 2008. This patient showed symptoms similar to the hereditary neurological disorder hyperekplexia, rigidity, brainstem signs and CSF lymphocytosis. Enhanced GlyR internalization induced as a consequence of autoantibody-binding was suggested as the underlying pathomechanism. Furthermore, internalized GlyRs colocalized with the late endosomal marker LAMP2. We use live staining experiments of transfected HEK293 cells to identify GlyR autoantibody-positive sera from SPS-patients. Live stainings of cultured motoneurons resulted in a similar staining pattern. Competition analyses were used for evaluation of the GlyR autoantibody epitope. Therefore, monoclonal anti-GlyR alpha1-antibody mAb2b and sera positive for GlyR autoantibodies were co-incubated in different dilution ratios, thus revealing a shared epitope of both antibodies. Chimeric construct of human and zebrafish GlyR alpha1 could further restrict

the epitope to the N-terminus. Autoantibody-binding to GlyRs had no effect on the ligand affinity of glycine. HEK293 cells transfected with GlyRs as well as spinal cord motoneurons labeled by GlyR-autoantibodies were investigated for further functional GlyR analysis. Using electrophysiological recordings, GlyR efficacy and potency were determined following autoantibody binding in a time window that excluded GlyR internalization as the primary mechanism. Hence, our data widen the knowledge of the pathomechanism of GlyR autoantibodies in SPS.

Disclosures: V. Roemer: None. **N. von Wardenburg:** None. **N. Schaefer:** None. **E. Tüzün:** None. **C.L. Sommer:** None. **C. Villmann:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

Location: SDCC Halls B-H

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Topic: B.02. Ligand-Gated Ion Channels

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NIH R01AA025718 (LA)

Title: Pka-mediated phosporylation diminished single channel conductance of the glycine receptor alpha 3 subunit

Authors: *G. MORAGA[1], V. SAN MARTIN[2], C. LARA[2], B. MUÑOZ[2], A. MARILEO[2], L. AGUAYO[2], J. FUENTEALBA[2], P. CASTRO[2], C. BURGOS[2], C. MUÑOZ-MONTESINO[2], J. GUZMAN[2], G. YEVENES[2]
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Abstract: Glycine receptors (GlyRs) are anion-permeable ligand-gated ion channels of the Cys-loop superfamily. In the mammalian CNS, the enhancement of the chloride conductance through the activation of GlyRs results in a transient hyperpolarization of the membrane potential, which is critical to control the neuronal excitability. The relevance of glycinergic inhibition is underlined by the presence of malfunctional GlyRs in many pathophysiological states, including chronic pain. Previous studies have shown that the dysfunction of GlyRs containing the alpha 3 subunit is a pivotal mechanism of pain hypersensitivity. This pathway involves the activation of prostaglandin receptors and the subsequent PKA-dependent phosphorylation of alpha 3 GlyRs

within the intracellular domain (ICD), which decrease the GlyR-associated currents and in turn enhance the neuronal excitability. Despite the importance of this pain sensitization pathway associated with the dysfunctional alpha 3 GlyRs, our current understanding of the molecular events involved is very limited. Here we report that PKA-mediated phosphorylation of alpha 3 GlyR decreases the ion channel conductance. We show in addition that the substitution of the PKA-targeted serine with a negatively charged residue within the ICD of alpha 3 GlyRs and of chimeric GLIC-GlyR receptors was necessary and sufficient to generate receptors with impaired conductance. Furthermore, we show that a recently characterized GlyR modulators showing in vivo analgesic activity normalized the impaired conductance of phospho-mimetic alpha 3 GlyRs. Our findings thus propose a molecular framework for a pain sensitization mechanism involving neuronal dis-inhibition and suggest that the allosteric modulation of alpha 3 GlyR alleviates chronic pain at least in part through the restoration of phosphorylated ion channels with impaired chloride conductance.

Disclosures: G. Moraga: None. **V. San Martin:** None. **C. Lara:** None. **B. Muñoz:** None. **A. Marileo:** None. **L. Aguayo:** None. **J. Fuentealba:** None. **P. Castro:** None. **C. Burgos:** None. **C. Muñoz-Montesino:** None. **J. Guzman:** None. **G. Yevenes:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

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Program #/Poster #: 557.04/D33

Topic: B.02. Ligand-Gated Ion Channels

Support: NHMRC 1045608
NHMRC 1058542

Title: Modulating ivermectin sensitivity at glutamate-gated chloride channels (GluCls) of haemonchus contortus (Hco) and biophysical properties of inhibitory postsynaptic currents mediated by alpha and alphabeta HcoGluCl receptors

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Abstract: Background: Glutamate-gated chloride channel receptors (GluClRs) are expressed by invertebrates where they mediate neuronal and muscle inhibition. GluClRs are important therapeutic targets for controlling parasitic pest species in agriculture, veterinary practice and human health. The most widely used drug to control pest species is ivermectin (IVM). This drug acts by binding to and potentiating the activity of GluClRs with high potency. However, the continuous and long-term use of IVM has led to the emergence of resistance in many pest

species.
Aim: Our aim was to explore possible mechanisms of IVM resistance in pest species by measuring the glutamate and IVM sensitivity of different homomeric and heteromeric isoforms of GluClRs. We used different methods for this purpose a) two-electrode voltage clamp electrophysiology (TEVC) and oocyte expression b) heterosynapses to measure the inhibitory postsynaptic currents (IPSCs) of GluClRs subunits from the ruminant animal parasite, *Haemonchus contortus* (HcoGluClR).
Results: Glutamate dose-response experiments demonstrated that homomeric HcoGluClRs comprising α subunits and heteromeric HcoGluClRs made of α and β subunits of have similar EC_{50}s, being between 20-30 μM. In contrast, homomeric HcoGluClRs containing β subunits exhibit an increased EC_{50} of 300 μM. The most IVM-sensitive receptors were the α HcoGluClRs with an EC_{50} value of 20 nM. An intermediate IVM sensitivity was exhibited by heteromeric αβ HCoGluClRs with EC_{50} of 130-200 nM. Homomeric receptors of HCoGluClRs comprising β subunits were insensitive to IVM (EC_{50} > 10 μM).
We also studied IPSCs mediated by the two isoforms in a cortical neuron-HEK 293 co-culture assay. The IPSC decay time constant was faster for the heteromeric receptors (αβ:15 ms) than for α homomeric receptors (α:40 ms). IVM application prolonged the decay times for both the isoforms wherein increasing the decay time of the α homomer by 2.5 fold to 100 ms and that of αβ heteromer to 70 ms.
Conclusion: Our data from TEVC and IPSCs suggests that a significant determinant of IVM sensitivity at GluClRs is the subunit composition. This implies that an organism can increase resistance to IVM without losing glutamate sensitivity by upregulating the expression of an IVM-insensitive subunit to produce heteromeric receptors.

Disclosures: J. Smith: None. **A. Keramidas:** None. **J. Lynch:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

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Topic: B.02. Ligand-Gated Ion Channels

Support: FONDECYT 1170252 (to G.E.Y)
FONDECYT 1160851 (to G.M)
FONDECYT Postdoctoral Fellowship 3170108 (to C.F.B)

Title: Modulation of inhibitory receptors by gelsemine, a gelsemium plant alkaloid

Authors: *C. O. LARA[1], A. M. MARILEO[1], V. P. SAN MARTÍN[1], B. MUÑOZ[2], C. F. BURGOS[1], A. SAZO[1], L. G. AGUAYO[1], J. L. GUZMÁN[1], J. FUENTEALBA[1], G. MORAGA-CID[1], G. E. YÉVENES[1]

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Abstract: $GABA_A$ and glycine receptors ($GABA_A$Rs and GlyRs) are anion-selective ligand-gated ion channels that mediate the inhibitory neurotransmission in the central nervous system. Activation of $GABA_A$Rs and GlyRs controls relevant biological processes, including pain processing, sleep and anxiety. Recent reports coincidentally have shown that natural alkaloids from the *Gelsemium* genus plants, such as gelsemine and koumine, displayed analgesic and anxiolytic activity in behavioral models. Interestingly, these effects were dependent on the activity of inhibitory receptors. However, it is currently unknown whether *Gelsemium* alkaloids can directly modulate the function of GlyRs and $GABA_A$Rs. Here, we examined the functional effects of gelsemine, one of the principal alkaloids produced by the *Gelsemium* genus of plants, on recombinant and native $GABA_A$Rs and GlyRs by using electrophysiological techniques. Using whole-cell recordings of inhibitory receptors expressed in HEK293 cells, we determined that gelsemine exerted conformation-specific and subunit-selective effects on GlyRs. On the other hand, recombinant benzodiazepine-sensitive $GABA_A$Rs were inhibited by gelsemine. The gelsemine modulation of GlyRs was associated with differential changes in the apparent affinity for glycine and in the open probability of the ion channel. Additional electrophysiological studies with chimeric and mutated GlyRs indicated that specific residues within the extracellular domain of GlyRs were essential for the gelsemine effects. Molecular modeling and docking calculations suggest that gelsemine binds to the orthostheric site of GlyR. Similar studies performed on $GABA_A$Rs also suggest that gelsemine binds to the GABA binding site at the interphase between α and β subunits. Further studies performed on cultured neurons showed that gelsemine significantly diminished the frequency of glycinergic, GABAergic and glutamatergic miniature post-synaptic events without altering the average amplitude. Our results show that gelsemine can directly modulate the activity of recombinant and neuronal GlyRs and $GABA_A$Rs. At the molecular level, our data also suggest that gelsemine binds to the orthostheric site of GlyRs and $GABA_A$Rs. In addition, our results showed that gelsemine negatively modulate both inhibitory and excitatory neurotransmission. Future studies may contribute to shed light on the mechanisms underlying the beneficial effects of the *Gelsemium* alkaloids in the control of pathological pain and anxiety through the modulation of inhibitory receptors.

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Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 557.06/D35

Topic: B.02. Ligand-Gated Ion Channels

Support: DFG VI586
Scientia-career development program

Title: The GLRB mouse mutant spastic - A model system to study agoraphobic behavior

Authors: *N. SCHAEFER, C. VILLMANN
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Abstract: The adult glycine receptor is a pentamer of three α and two β subunits that enables fast inhibitory neurotransmission in the central nervous system. The β subunit is encoded by the *GLRB* gene. Mutations in the *GLRB* gene have been assigned as the third most common cause of the neuromotor disorder startle disease. Patients with this rare neurological disorder suffer from exaggerated startle responses following unexpected noise or tactile stimuli. Moreover, a genome-wide association study provided evidence for a link between *GLRB* gene polymorphisms of agoraphobic (AG) patients with an anxiety phenotype based on an Agoraphobic Cognitions Questionnaire (ACQ) in healthy German volunteers. To further investigate the issue of a panic phenotype, we used the mouse model *spastic*. Homozygous *spastic* mice show a neuromotor phenotype due to a splice defect within the *Glrb* gene. The splice defect generates aberrant splice products that lack exon 6 or a combination of exons 5 and 6. As a consequence, reduced amounts of full-length GlyR β protein have been observed. Since homozygous animals die at the age of three weeks after birth, the homozygous animals do not represent a good model to study panic behavior. Heterozygous *Glrb* mutants lack about 50% of full-length GlyRβ in brain regions including cortex, cerebellum, thalamus, striatum, hippocampus, brain stem and spinal cord. Heterozygous animals have no motor phenotype, which is in line with the human volunteers carrying *GLRB* gene polymorphism that most probably do not result in large changes of glycine receptor β expression. Thus, heterozygous *spastic* mice were analyzed at the age of 8-10 weeks for an anxiety phenotype. Heterozygous animals show an avoidance of a novel open space, a behavior in line with the agoraphobic fear in human volunteers with *GLRB* polymorphisms. In addition, the distance the animals walked as well as the entries into the open field did not change between heterozygous animals and controls. Furthermore, mice were analyzed in the elevated plus maze, the dark/light field, for the startle reaction, and in the Morris water maze. The last was used as a control for lack of a motor phenotype and a control for learning and memory in heterozygous *spastic* animals. In summary, our findings show that the *Glrb* gene does not only contribute to the neurological disorder hyperekplexia, but also represent a model system useful to investigate agoraphobic behavior associated with differences in the expression level of the glycine receptor β subunit.

Disclosures: N. Schaefer: None. **C. Villmann:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 557.07/D36

Topic: B.02. Ligand-Gated Ion Channels

Support: MRCMR/L021676

Title: The role of phenylalanine residues in the extracellular domain of the glycine receptor

Authors: *S. C. LUMMIS, B. TANG
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Abstract: The extracellular domains (ECDs) of Cys-loop receptors characteristically contain many aromatic amino acids, but only those in the binding pocket have been extensively studied. Here we show that many Phe residues in the ECD which are not located in the binding pocket are also involved in the function of the glycine receptor, a typical Cys-loop receptor. The Phe residues were explored by creating a range of mutant receptors, characterising them using two electrode voltage clamp in *Xenopus oocytes*, and interpreting changes in receptor parameters using currently available structural information on the open and closed states of the receptor. The data reveal that substitution of most of the Phes in the ECD with Ala alters the function of the receptor; of the 14 Phe residues 2 Ala substitutions ablate function, 3 cause >100 fold changes in EC_{50}, 3 cause changes in EC_{50} 10-100 fold, and 2 change EC_{50} 2-10 fold. Only 4 of these mutants resulted in EC_{50}s similar to WT. Substitution with other amino acids, combined with examination of nearby residues that could potentially interact with these Phes, suggests interactions that could be important for the correct functioning of glycine receptors, and possibly also for other members of the Cys-loop receptor family. Overall the data suggest many regions of the ECD are important for receptor function, and they also indicate potential novel regions that could be targeted in the design of novel therapeutic agents.

Disclosures: S.C. Lummis: None. **B. Tang:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

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Topic: B.02. Ligand-Gated Ion Channels

Support: NRF-2017R1D1A1B03034551

Title: Gastrokinetic agent, mosapride inhibits 5-hydroxytryptamine 3 receptor currents in NCB-20 cells

Authors: *K.-W. SUNG[1], S. JEUN[2], Y. PARK[3]
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Abstract: Mosapride accelerates gastric emptying through acting on a 5-hydroxytryptamine (5-HT) 4 receptor and is used for the treatment of gastritis, gastroesophageal reflux diseases, and irritable bowel syndrome. But its mechanism is still unclear, therefore we tested the effect of mosapride on 5-HT_3 receptor currents, because the 5-HT_3 receptors are known to be expressed in the gastrointestinal system and have an important role for the regulation of bowel movement. Using whole cell voltage clamp method, we compared the currents of 5-HT_3 receptor when 5-HT was applied alone and co-applied with mosapride in cultured NCB-20 cells known to express the 5-HT_3 receptors. The 5-HT_3 receptor current amplitudes were inhibited by mosapride in a concentration dependent manner. Mosapride blocked the peak currents in a competitive manner, because the EC50 was shifted to the right without the change of maximal effect evoked by the 5-HT application. The 5-HT_3 receptor current rise slopes were decreased by the mosapride. It accelerated the desensitization of 5-HT_3 receptor, but did not affect the receptor deactivation. There were no voltage-, and use-dependency in its blocking effects. Mosapride also did not change the recovery process from the receptor desensitization. These results suggest that mosapride inhibits the 5-HT_3 receptor through a competitive blocking mechanism. From this study, we could expand our understanding the pharmacological and therapeutic mechanisms of mosapride to improve gastrointestinal motility and to treat several gastrointestinal disorders.

Disclosures: K. Sung: None. **S. Jeun:** None. **Y. Park:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

Location: SDCC Halls B-H

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Program #/Poster #: 557.09/D38

Topic: B.02. Ligand-Gated Ion Channels

Title: Effects of acid-sensing ion channels on modulation of locomotor activity by amphetamine

Authors: Q. JIANG[1], N. GOVALLA[1], *X. CHU[2]
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Abstract: Drug addiction is a persistent mental illness and there is no effective therapy for patients. The precise mechanisms underlying addictive responses have not been completely deciphered. New evidence has been shown that ion channels in the brain reward circuits are believed to play a vital role in drug addiction. Acid-sensing ion channels (ASICs) are highly expressed in brain with ASIC1a and ASIC2 channels being the predominant subtypes. These channels are enriched at synaptic sites and are central for the regulation of normal synaptic transmission. Moreover, increasing evidence is linking ASICs to the pathogenesis of various neurological and neuropsychiatric disorders. We and others have shown that ASICs are involved in cocaine addiction. Here, we hypothesized that amphetamine, a psychostimuland similar to cocaine, may also impact the function of ASICs. Adult wild-type (WT) C57BL/6J, ASIC1 and ASIC2 knock-out (KO) mice were placed in individual test chambers to allow accommodation to novel environment for 60 minutes. They then received a single intraperitoneal (i.p) injection of amphetamine at 3.0 mg/kg, and their locomotor activities were recorded for 150 minutes. The experiment was repeated daily for a total of 5 days. After a 2-week withdrawal period, the mice were brought back to the behavioral chamber followed by a final challenge i.p injection of amphetamine at 1.5 mg/kg. Locomotor activity to this challenge dose was measured for 150 min. Acute amphetamine injection induced a typical dose-dependent increase in locomotor activities in WT, ASIC1 and ASIC2 KO mice (both male and female mice). However, the increase in locomotor activities were attenuated in ASIC1 and ASIC2 KO mice as compared to WT mice. Both WT, ASIC1 and ASIC2 KO mice showed sensitization to amphetamine. However, ASIC1 KO mice showed more, while ASIC2 KO mice showed less behavioral sensitization to amphetamine. Our data provides new understanding of the complex genetic and molecular mechanisms of ASICs in response to amphetamine exposure.

Disclosures: Q. Jiang: None. **N. Govalla:** None. **X. Chu:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

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Program #/Poster #: 557.10/D39

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH/NIA T32AG020494

Title: Development of $GABA_A$receptor positive modulators with low abuse potential

Authors: *M. CLAUDIO[1], R. HUANG[2], G. H. DILLON[1], K. A. EMMITTE[3], N. MISHRA[3]
[2]Pharmacol. and Neurosci., [3]Pharmaceut. Sci., [1]UNT Hlth. Sci. Ctr., Fort Worth, TX

Abstract: $GABA_A$ receptors have long been considered as targets for treatment of acute pain and neuropathic pain conditions. Our previous studies have shown that carisoprodol (CSP), a widely

prescribed muscle relaxant to treat low back pain, differentially potentiates $GABA_A$ receptor subtypes with strongest potentiation on α1- containing $GABA_A$ receptors. Furthermore, it has been reported that α1-containing $GABA_A$ receptors are associated with abuse-related effects while α2/α3-containing receptors are linked with anti-nociceptive and muscle relaxant effects. The purpose of the present study is to investigate the structure and function relationship of CSP in an effort to seek a compound with lower α1 efficacy and putative α2/α3 selectivity and thus maximize CSP's clinical utility with lower abuse liability. Focusing on particular positions of our parent compound, CSP, a series of novel compounds were synthesized with various side-chain modifications. The subtype selectivity profile was examined on HEK cells expressing various recombinant $GABA_A$ subtypes with whole-cell patch clamp. Animal behavioral assays were performed to assess the effects of drugs on motor function, nociception and abuse potential. We show that modifications made to CSP's structure were able to shift its original subunit selectivity profile. Some of the synthesized CSP analogs have shown relatively high efficacy on α2-containing receptors. Our preliminary results provide a future direction for the development of subtype-selective GABAergic drugs for the treatment of chronic pain and other neuropathic pain conditions.

Disclosures: M. Claudio: None. **R. Huang:** None. **G.H. Dillon:** None. **K.A. Emmitte:** None. **N. Mishra:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

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Program #/Poster #: 557.11/D40

Topic: B.02. Ligand-Gated Ion Channels

Support: Albany Medical College Bridge Grants

Title: Function and pharmacology of the $GABA_A$θsubunit

Authors: *J. NUWER, M. W. FLECK
Albany Med. Col., Albany, NY

Abstract: $GABA_A$ receptors are chloride channels that are the primary mediators of inhibitory neuronal transmission. These channels are pentamers composed from 19 possible subunits (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, θ, π, and ρ_{1-3}). The canonical $GABA_A$ receptor contains 2 α subunits, 2 β subunits, and 1 other subunit - typically γ. However, non-canonical subunit combinations can also create functional receptors with unique pharmacology. Non-canonical θ-containing combinations have been shown to create plasma membrane-expressed receptors that are not activated by GABA or known $GABA_A$ ligands. θ is expressed primarily in aminergic midbrain and brainstem nuclei, the hypothalamus, and the hippocampus. This is a discrete expression pattern, unlike that of the

subunits that make up the canonical receptor. Therefore, we believe the θ subunit could prove to be a novel, druggable target for aminergic dysfunction without widespread side effects. To date, there are only two publications exploring the role of θ in $GABA_A$ pharmacology, however they report contradictory findings. Our lab has observed that θ does not assemble into pentamers when expressed in recombinant HEK293 or COS7 cells. Whole-cell voltage-clamp comparisons of $\alpha_3\beta_3$, $\alpha_3\theta$, and α_3-only transfections reveal that $\alpha_3\theta$ is not different than α_3-only with regard to current amplitude (both ~90 pA), EC_{50} (both ~30 uM), and histamine potentiation (both ~300% potentiation). Non-permeabilized staining of $^{HA}\theta$ in combinations that were previously shown by another lab to be present at the plasma membrane was nonexistent in our hands; however, $^{HA}\theta$ expression was confirmed intracellularly and the protein was not degraded, as shown by permeabilized staining and Western blotting. The absence of assembly could be due to a lack of an accessory protein, the presence of a protein that inhibits assembly, or cell type and species differences. To circumvent the problem with recombinant systems, we have identified cell lines that natively express the θ subunit. If θ is assembled into the $GABA_A$ receptors in these cell lines, the results of this study are expected to reveal any interacting proteins as well as expand our knowledge about the pharmacological contributions of θ to $GABA_A$ function.

Disclosures: J. Nuwer: None. **M.W. Fleck:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 557.12/D41

Topic: B.02. Ligand-Gated Ion Channels

Support: Russian Science Foundation grant No. 16-14-00201

Title: GABA accumulation during theta-rhythms is responsible for second order oscillations

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Abstract: Hippocampal theta-rhythm appears in oscillation epochs (episodes of synchronized activity followed by silent intervals) that contribute to encoding of spatial and behavioral information. Here we suggest a mechanism responsible for appearance of these second-order oscillations. We recorded hippocampal theta-rhythms with a wireless system in freely moving mice. Rhythmic modulation of the theta power was studied using wavelet analysis. 1 μM

picrotoxin was injected through a chronically implanted cannula to selectively block tonic $GABA_A$ conductance. The injection significantly increased the length of the theta-rhythm epochs and decreased the inter-epoch intervals. Larger concentrations of picrotoxin (10 μM and 100 μM), which also block synaptic $GABA_A$ mediated signalling, shortened the theta-rhythm epochs and prolonged the inter-epoch intervals. This finding is consistent with previous reports that synaptic GABAergic signalling is required for synchronization of neuronal networks. Then we facilitated activity-dependent tonic $GABA_A$ conductance increase by blocking GABA uptake. Both a GAT-3 inhibitor SNAP-5114 (100 μM) and a GAT-1 inhibitor NNC-711 (10 μM) significantly shortened the epochs and increased the inter-epoch intervals. 100 nM allopregnanolone, highly potent positive allosteric modulator of extrasynaptic $GABA_A$ receptors, shortened the epochs and increased the inter-epoch intervals likewise. In summary, we suggest that accumulation of extracellular GABA during synchronized neuronal activity inhibits neurons and stops their firing, hence rhythmic activity. When the neuronal activity is reduced, the ambient GABA concentration also decreases. Such fluctuations in the ambient GABA are responsible for the rhythmic modulation of the theta oscillations. GABA uptake or enhancement of tonic $GABA_A$ current with allopregnanolone modulate the epochs of the theta rhythms. Our findings also shed light upon possible mechanisms by which endogenously produced allopregnanolone exerts its physiological effects.

Disclosures: A.V. Semyanov: None. **A. Pushkarev:** None. **E. Ivlev:** None. **I. Novozhilov:** None. **A. Mironov:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 557.13/D42

Topic: B.02. Ligand-Gated Ion Channels

Support: U54NS079202
T32GM099608

Title: The GABA-A receptor antagonists TETS and RDX bind in the pore of the GABA-A channel

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Abstract: The rodenticide tetramethylenedisulfotetramine (TETS) is a potent convulsant (lethal dose in humans 7-10 mg) that is listed as a possible threat agent by the United States Department of Homeland Security. TETS has previously been studied *in vivo* for toxicity and *in vitro* in

binding assays, with the latter demonstrating it to be a non-competitive antagonist on $GABA_A$ receptors. However, since it was unknown whether TETS exhibits subtype selectivity for a particular $GABA_A$ receptor combination, we used whole-cell patch-clamp to determine the potency of TETS on the major synaptic and extrasynaptic $GABA_A$ receptors associated with convulsant activity. We found that TETS exhibited the highest potency towards blocking α2β3γ2 (IC_{50} 480 nM, 95% CI: 320-640 nM) and α6β3γ2 (IC_{50} 400 nM, 95% CI: 290-510 nM). We next decided to map the TETS binding site on the α2β3γ2 receptor by using a combination of electrophysiology, site-directed mutagenesis and molecular modeling with the Rosetta membrane method to help identify critical residues. In parallel, we are also investigating the structurally related compound RDX (royal demolition explosive; 1,3,5-trinitroperhydro-1,3,5-triazine), a high energy explosive, which is widely used for military and civilian purposes and regulated as an environmental contaminant. RDX induces seizures in humans following accidental exposure during manufacture or in soldiers following inadvertent or intentional ingestion as a perceived illicit drug. We first constructed a homology model of the α2β3γ2 receptor using the published structures of the β3 homopentamer and the β3α5-TMD chimera as templates. Both TETS and RDX were docked in the homology model using Rosetta ligand docking with the membrane function. Rosetta identified three possible interaction sites for both compounds in the pore region of the channel: Site one at the 2' position previously hypothesized as a site of TETS action; site two at T6', which is the traditional non-competitive antagonist site for picrotoxin, TBPS and EBOB; and site three located much higher up in the pore with favorable interactions at the R19' position. We are currently experimentally testing these predictions but hypothesize that both the TETS and the RDX binding sites overlap with, but are not identical to, the picrotoxin binding site.

Disclosures: B. Pressly: None. **I. Pessah:** None. **H. Wulff:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 557.14/D43

Topic: B.02. Ligand-Gated Ion Channels

Title: Adapting the proteostasis network to restore function of epilepsy-associated GABAA receptors

Authors: *T. MU, X. DI
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Abstract: Proteostasis deficiency in ion channels leads to a variety of ion channel diseases called channelopathies, which are often caused by excessive endoplasmic reticulum-associated degradation (ERAD) and inefficient membrane trafficking of mutant ion channels. We focus on

proteostasis maintenance of gamma-aminobutyric acid type A (GABAA) receptors, the primary inhibitory ion channels in the mammalian central nervous system. Numerous epilepsy-associated missense mutations in the receptor subunits predispose them to rapid ERAD, reduce their cell surface expression, and cause loss of their function. We aimed to use small molecules to adapt the proteostasis network in the ER to restore the surface trafficking and function of such mutant receptors. Our screening assay from a structurally diverse FDA-approved drug library identified lead compounds that enhanced the surface expression of a number of trafficking-deficient mutant receptors. Furthermore, patch clamping electrophysiology showed that these lead compounds restored their function on the plasma membrane. Mechanistic studies revealed that they reduced the degradation by attenuating the ERAD pathway. In addition, they enhanced the folding of the mutant subunits by enhancing their interactions with major GABAA receptors-interacting chaperones. Both ERAD inhibition and folding enhancement contributed to the improved ER-to-Golgi trafficking efficiency of the mutant receptors. These compounds hold the promise to be further developed to ameliorate idiopathic epilepsy resulting from excessive GABAA receptor degradation.

Disclosures: T. Mu: None. **X. Di:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 557.15/D44

Topic: B.02. Ligand-Gated Ion Channels

Title: Impaired GABAergic signaling at the axon initial segment alters bursting activity and sleep architecture

Authors: *A. J. BOREN, D. J. HINES, R. M. HINES
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Abstract: Interneurons act on principal neurons to pattern excitatory output allowing for neuronal synchronization and maintenance of homeostatic rhythms. Changes in excitatory patterning due to disruptions in inhibitory signaling are thought to be mechanistic in neuropsychiatric and neurodevelopmental disorders including developmental epilepsy, autism spectrum disorder, and schizophrenia. In support of a critical role of inhibitory regulation, neuropsychiatric and neurodevelopmental disorders share common symptomology including spontaneous recurrent seizure, abnormal sleep architecture, and altered circadian rhythmicity. Providing phasic inhibition necessary for patterning excitatory output, γ-aminobutyric acid type A receptors ($GABA_ARs$) are heteropentameric chloride channels constructed from a diverse set of subunits resulting in 26 known human isoforms with several subtypes having unique signaling characteristics. $GABA_ARs$ containing the α2 subunit are trafficked to the axon initial segment

(AIS) postsynaptic to chandler cells, a synaptic site central to neuronal synchronization. Despite the high abundance of α2 at the AIS, little is known about its contribution to common symptomology seen in neuropsychiatric and neurodevelopmental disorders. Behavioral and electroencephalographic techniques were used to examine cortical bursting activity, sleep-state architecture, and circadian rhythmicity in transgenic mice with a knock-in mutation that reduces α2 trafficking onto the AIS (Gabra2-1). Our results indicate reduced α2 signaling at the AIS plays a pivotal role in the breakdown of rhythmic homeostatic functions and abnormal cortical activity common to neuropsychiatric and neurodevelopmental disorders. Increased understanding of the contribution of α2 signaling on the AIS to common symptomology seen in these disorders will allow for the development of novel, targeted, therapeutics
as well as further our understanding of the mechanisms underlying excitatory patterning.

Disclosures: A.J. Boren: None. **D.J. HInes:** None. **R.M. Hines:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 557.16/D45

Topic: B.02. Ligand-Gated Ion Channels

Support: NSERC
CIHR

Title: Effects of neurosteroids and phosphorylation on $GABA_A$receptor currents and piriform cortex circuit in epilepsy

Authors: *J. JEONG, M. O. POULTER
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Abstract: Neurosteroids such as tetrahydrodeoxycorticosterone (THDOC) are well known modulators of gamma-aminobutyric acid (GABA) receptor activities, and thus, they have great influence on circuit activities. THDOC is a potent positive allosteric modulator of $GABA_A$ receptor, and it has been shown to be involved in epilepsy. THDOC potentiates $GABA_A$ receptor by prolonging the decay of inhibitory postsynaptic currents (IPSCs). The modification of IPSC kinetics by neurosteroid can also be influenced by phosphorylation by kinases. However, the interaction of neurosteroids and kinases have not yet been thoroughly investigated and the available information is conflicting.
Previous studies have shown widely varying effects of neurosteroids on GABAergic currents. We hypothesized that the variability is due to varying phosphorylation states of $GABA_A$ receptors. In this study, we have investigated the activity of neurosteroids THDOC on GABAergic miniature IPSCs (mIPSCs) and tonic currents after activation of various kinases, and

their activities in brain circuit. Tonic currents and IPSCs were measured from Sprague-Dawley rat pyramidal neurons (>14 days) in primary culture. Decay of IPSCs were in two phases, fast decay lasting ~10 ms (τ_1) followed by slow decay lasting ~50 ms (τ_2).
THDOC by itself caused shortening of τ_1 and prolongation of τ_2. It also greatly increased the negative charge transfer. Activation of protein kinase C (PKC) using the phorbol ester PMA, or activation of tyrosine receptor kinase B (TrkB) by 7,8-dihydroxyflavone (DHF), did not significantly alter the effects of THDOC. Treatment with THDOC after activation of protein kinase A (PKA) by forskolin resulted in two distinct populations of response. One population showed increased mIPSC amplitude. The other showed prolonged τ_2 compared to the first population. For tonic currents, THDOC caused a large inward shift in holding current, and thus increase in tonic inhibition. All three kinases suppressed the change in tonic current by THDOC. These data show that kinases differentially modulate the effects of neurosteroids on phasic and tonic GABA currents.
Next, we utilized voltage sensitive dye imaging (VSDI) technique to visualize circuit activity of the piriform cortex (PCtx) in brain slice, and how these kinases and THDOC function to modulate circuit activity. The PCtx is one of the most seizure-susceptible region of the brain. Application of THDOC decreased the circuit activity by 40%. However, our preliminary data show that when THDOC is applied in the presence of PMA, THDOC failed to suppress circuit activity. Our preliminary finding suggests that PKC activation blocks the effect of THDOC in the PCtx.

Disclosures: J. Jeong: None. **M.O. Poulter:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 557.17/D46

Topic: B.02. Ligand-Gated Ion Channels

Support: SNSF, Project 31003A_153276
SNSF, Project 31003A_176321
Roche Postdoctoral Fellowship

Title: Nonlinear α5-$GABA_A$Rs effectively regulate NMDAR recruitment in CA1 pyramidal neuron dendrites

Authors: *J. M. SCHULZ[1], F. KNOFLACH[2], M.-C. HERNANDEZ[2], J. BISCHOFBERGER[1]
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Abstract: Dendrite-targeting GABAergic interneurons powerfully control dendritic electrogenesis, synaptic plasticity and learning. However, the underlying mechanisms are not well understood. In the current study, we show that dendritic $GABA_A$ receptors ($GABA_ARs$) in CA1 pyramidal neurons exhibit pronounced outward rectification. Optogenetically activated IPSCs from somatostatin (SOM)- and NOS-positive interneurons linearly increased with holding potential above -50 mV. By contrast, at more negative potentials the synaptic peak conductance was about 3-fold smaller, showing a pronounced non-linear voltage-dependence. On the other hand, fast peri-somatic IPSCs evoked in parvalbumin (PV)-ChR2 mice showed a classical, much more linear behavior. Pharmacological experiments showed that α5-subunit containing $GABA_ARs$ substantially contributed to the non-linear IPSCs generated by SOM and NOS interneurons, while somatic IPSCs evoked by PV interneurons were independent of a5-$GABA_ARs$. Next we tested the impact of synaptic α5-$GABA_ARs$ on the activation of NMDARs. Application of an α5-NAM (RO4938581) increased the amplitude of subthreshold burst PSPs to about 140% of control. This increase was strongly dependent on NMDARs, as it was fully reversible by AP5-application. By contrast, low concentration of gabazine (100 nM) increased burst EPSP to a similar extent, however, in a largely NMDAR-independent manner. Computational modeling in NEURON demonstrated that the slow time course and the nonlinear voltage-dependence of the IPSCs evoked by SOM and NOS interneurons are essential for its powerful control of voltage-dependent NMDAR recruitment. Taken together, nonlinear rectifying α5-$GABA_ARs$ with slow kinetics match functional NMDAR properties and thereby mediate powerful control of dendritic postsynaptic integration by dendrite-targeting interneurons**.**

Disclosures: J.M. Schulz: None. **F. Knoflach:** None. **M. Hernandez:** None. **J. Bischofberger:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 557.18/D47

Topic: B.02. Ligand-Gated Ion Channels

Support: UAB Research Acceleration Funds

Title: Elevated O-GlcNAcylation depresses inhibitory transmission recorded from granule cells in the rat dentate gyrus

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Abstract: O-GlcNAcylation is crucial for protein function, and involves the addition and removal of an O-linked N-acetylglucosamine (O-GlcNAc) to serine or threonine residues by the enzymes O-GlcNAc transferase (OGT) and OGlcNAcase (OGA), both of which are highly expressed in the hippocampus. We have previously shown that acutely increasing O-GlcNAcylation using the substrate glucosamine or the OGA inhibitor, thiamet-G (TMG), induces long-term depression (LTD) at CA3-CA1 hippocampal synapses that requires O-GlcNAc modification of GluA2 AMPA type glutamate receptor (AMPAR) subunits. Additionally, we found that increasing O-GlcNAcylation dampens epileptiform activity at these same synapses. Because function of $GABA_A$ receptors ($GABA_A$Rs) is modulated by serine phosphorylation, we have begun to ask whether the strength of $GABA_A$R mediated synaptic inhibition is modulated by O-GlcNAcylation. We have found that pharmacologically increasing O-GlcNAc levels in acute slices decreases the amplitude and frequency of spontaneous IPSCs (sIPSCs) but only the amplitude of miniature IPSCs recorded from CA1 pyramidal cells. This indicates that much like phosphorylation, O-GlcNAcylation affects postsynaptic $GABA_A$R function. To assess if this is general mechanism by which inhibition is modulated in the brain, we used whole-cell voltage-clamp recordings from dentate granule cells (DGCs) to investigate whether increasing O-GlcNAcylation modulates the frequency and/or amplitude of spontaneous IPSCs (sIPSCs) onto DGCs. Preliminary experiments found a reduction in both sIPSC amplitude and frequency following pharmacologically increasing O-GlcNAcylation. Collectively, these data will help us parse the effects of O-GlcNAcylation on the input and output stages of hippocampal processing.

Disclosures: K. Abiraman: None. **L.T. Stewart:** None. **L.L. McMahon:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 557.19/D48

Topic: B.02. Ligand-Gated Ion Channels

Support: University of Connecticut Research Excellence Program (grant 4466280)

Title: Cell-specific transgenic manipulation of $GABA_A$ receptor synaptic clustering in hippocampal neurons

Authors: *S. GEORGE, A. L. DE BLAS
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Abstract: Synaptic inhibition in the brain is essential to regulate neuronal excitability and is primarily achieved through gamma-aminobutyric acid (GABA) acting on fast-acting GABAA receptors. Collybistin (CB) is critical in the postsynaptic localization of gephyrin and GABAA

receptors at these synapses. The constitutively active isoform of collybistin, CBSH3-, has been shown to be particularly adept at recruiting gephyrin and GABAA receptors to post-synaptic clusters. This effect, which we have shown both in culture and in vivo, is coupled with an increase in synaptic strength, suggesting that CBSH3- is a good target candidate for modulating the strength of inhibitory neurotransmission. For this purpose, we have created an adeno-associated virus (AAV) with a double-floxed cassette that bicistronically expresses the constitutively active isoform of collybistin, CB2SH3-, and mCitrine (as a transduction marker), in a cre-dependent manner. Through unilateral stereotactic injection of our AAV construct into the hippocampus of two mouse lines, VGLUT1-ires2-cre or VGAT-ires-cre, we were able to investigate changes in GABAergic synaptic clusters specifically in hippocampal glutamatergic pyramidal neurons and GABAergic interneurons, respectively. Interneurons transduced with this construct display higher densities and larger sized gephyrin clusters compared to contralateral interneurons or transduced interneurons of VGAT-cre mice injected with a control eGFP-AAV construct. Preliminary data in VGLUT1-cre mice indicate a similar increase in GABAergic postsynaptic proteins in transduced glutamatergic neurons. We therefore conclude that this approach can be used for the controlled manipulation of the strength of GABAergic synapses in specific brain regions and in specific cell-types. We are exploring the possibility of using this AAV in a mouse epilepsy model to reduce seizure seizures and provide neuroprotective effects against excitotoxicity.

Disclosures: S. George: None. **A.L. de Blas:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 557.20/D49

Topic: B.02. Ligand-Gated Ion Channels

Support: Milton lev memorial fund

Title: Neuroimmune interactions of interleukin 10 (IL-10) with $GABA_AR$

Authors: *S. DECKER, A. SURYANARAYANAN, E. ALTOWAIRQI
Univ. of the Sci., Philadelphia, PA

Abstract: We have previously shown that Interleukin-10 (IL-10), an anti-inflammatory cytokine, is upregulated following a single intoxicating exposure to ethanol (EtOh) in rats (*Neuropharmacology, 2016*). In addition, we have also shown that IL-10 inhibits GABAergic transmission in the hippocampus, causing hyperexcitability via both pre- and postsynaptic mechanisms. However, the molecular mechanisms of the interaction of IL-10 with $GABA_AR$ are not known. Understanding these mechanisms are important in elucidating neuroimmune

regulation of $GABA_AR$ and other ion channels. To further explore these neuroimmune interactions, we are studying the effects of IL-10 exposure on cell-surface expression and phosphorylation of $GABA_AR$ in human neuroblastoma and glial cells. Our results suggest that IL-10 exposure increases the phosphorylation of the β_3 subunit of the $GABA_AR$. To explore a direct interaction of IL-10 with $GABA_AR$, we have also employed two-electrode voltage clamp (TEVC) studies in *xenopus laevis* oocytes expressing human $\alpha_1\beta_2$ and $\alpha_1\beta_2\gamma_2$ $GABA_AR$. The TEVC experiments are looking at the effect of dose-dependent IL-10 exposure on $GABA_AR$ mediated currents. Based on results obtained from *in vitro* cell lines and TEVC experiments, we will perform *ex vivo* studies on rat hippocampal slices to elucidate the mechanisms of interaction of IL-10 with $GABA_AR$.

Disclosures: A. Suryanarayanan: None. **E. Altowairqi:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

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Topic: B.02. Ligand-Gated Ion Channels

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National Health Research Institutes, Taiwan (NHRI-EX107-10733NI
National Institutes of Health, USA (R01 NS076517)
National Institutes of Health, USA (R01 MH096463)

Title: Implications of trigeminal $\alpha6GABA_A$ receptors in migraine and orofacial pain

Authors: *H.-R. TZENG[1], S. S. BALLON ROMERO[2], Y.-H. CHEN[2], W. SIEGHART[3], D. E. KNUTSON[4], J. COOK[4], L.-C. CHIOU[1,5,2]
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Abstract: We found that the α6-subunit-containing $GABA_A$ receptors ($\alpha6GABA_ARs$) in trigeminal ganglia (TG) are functional since $\alpha6GABA_AR$ positive allosteric modulators (PAMs) attenuated the activation of the trigeminovascular (TGVS) system in a migraine model induced by intracisternal injection of capsaicin (Lee et al., SFN2018 poster). Here, we further evaluate

the potential of α6GABA$_A$R PAMs in migraine treatment in a clinical presentation align animal model, the repetitive nitroglycerin (NTG) -induced migraine model in mice since NTG can trigger migraine in human migrainer. NTG (10 mg/kg, *i.p.*) was injected into mice (6-8 weeks, male ICR) every two days for 5 sessions. The mouse grimace scale (MGS) was used to score the severity of migraine. All five parameters of MGS in NTG-treated mice were significantly greater than in saline-treated mice. In the 5th session, Compound 6 (3 and 10 mg/kg, *i.p.*), an α6GABA$_A$R-selective PAM, injected 20 min after NTG administration significantly attenuated the elevated grimace score in the NTG group to the level as in the saline-treated group. Importantly, this effect of Compound 6 was antagonized by *i.p.* injection of furosemide (20 mg/kg), an α6 GABA$_A$ R-selective antagonist.
There are three trigeminal nerve branches from TG. In addition to the dural ophthalmic branch (V1) that is involved in migraine pathogenesis, the maxillary (V2), and mandibular (V3) branches are important for transmitting orofacial pain. We therefore also assessed the effect of Compound 6 in an orofacial pain model. Type III dental pulp injury (DPI) in mice (8 weeks, male ICR), which resembles irreversible pulpitis in humans and causes severe orofacial pain, was induced by drilling their left maxillary first molars. The DPI-induced orofacial pain in mice was accessed by the reduction of their burrowing behaviors, which are indicators of well-being of mice. DPI significantly decreased the burrowing activity in mice on Day 1, 3, 7, but not Day 14. Ibuprofen (30 mg/kg, *p.o.*) and Compound 6 (3 mg/kg, *i.p.*) restored the burrowing activity on Days 1, 3 and 7 to the level as in sham-operated mice. These results support the effectiveness of an α6GABA$_A$R PAM in animal models with clinical representations of migraine and orofacial pain.

Disclosures: H. Tzeng: None. **S.S. Ballon Romero:** None. **Y. Chen:** None. **W. Sieghart:** None. **D.E. Knutson:** None. **J. Cook:** None. **L. Chiou:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

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National Institutes of Health, USA (R01 MH096463)
Psychoactive Drug Screening Program, National Institute of Mental Health, USA

Title: α6GABA$_A$R-selective positive allosteric modulators: A novel pharmacotherapy for neuropsychiatric disorders

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Abstract: The α6 subunit-containing GABA$_A$ receptors (α6GABA$_A$Rs) are abundant in cerebellar granule cells while their functions remained unclear due to a lack of selective ligands. Recently, we identified several pyrazoloquinolinones to be positive allosteric modulators (PAMs) selective to α6GABA$_A$Rs.[1,2] Using the prototypical Compound 6 (PZ-II-029), we have revealed that positively modulating cerebellar α6GABA$_A$Rs, via attenuating granule cell activity, can rescue disrupted prepulse inhibition (PPI), which reflects sensorimotor gating deficits manifested in several neuropsychiatric disorders.[3] Compound 6 (3 and 10 mg/kg, *i.p.*) significantly rescued methamphetamine-induced PPI disruption in mice. Importantly, this effect was prevented by intra-cerebellar (*i.cb.*) microinjection of furosemide, an α6GABA$_A$R antagonist, and mimicked by Ro15-4513 and loreclezole, (two α6GABA$_A$R PAMs), but not by diazepam (an α6GABA$_A$R-inactive benzodiazepine). In the same animal model, we further examined effects of Compound 6 and three other pyrazoloquinolinones, Compound 11 (PZ-II-028), LAU159 and LAU463, as well as their methoxy-deuterated derivatives. All compounds, applied at 10 mg/kg (*i.p.*) rescued methamphetamine-induced PPI disruption in mice with an efficacy similar to Compound 6. These results indicate that methoxy-deuterated derivatives of pyrazoloquinolinones, which are less susceptible to metabolic O-demethylation,[1] retain the *in vivo* efficacy in the PPI-disrupted animal model. This suggests that deuterated pyrazoloquinolinones with appropriate half-lives (9-13 hr)[1] are first-in-class drugable candidates for treating sensorimotor gating deficits in neuropsychiatric disorders, including but not limited to schizophrenia.
[1]Knutson et al. (2018) Design and synthesis of novel deuterated GABA$_A$R α6 subtype functionally selective ligands with improved metabolic stability and enhanced bioavailability. J. Med. Chem. 61:2422-2446.
[2]Treven et al. (2018) Towards functional selectivity for α6β3γ2 GABA$_A$ receptors: a series of novel pyrazoloquinolinones. Br. J. Pharmacol. 175:419-428.
[3]Chiou et al. (2018) Cerebellar α6 subunit-containing GABA$_A$ receptors: A novel therapeutic target for disrupted prepulse inhibition in neuropsychiatric disorders. Br. J. Pharmacol. DOI: 10.1111/bph.14198.

Disclosures: L. Chiou: None. **H. Lee:** None. **D.E. Knutson:** None. **C. Witzigmann:** None. **L. Wimmer:** None. **M.D. Mihovilovic:** None. **M. Ernst:** None. **J. Cook:** None. **W. Sieghart:** None.

Poster

557. Glycine Receptors and Other Ligand Gated Ion Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 557.23/E1

Topic: B.02. Ligand-Gated Ion Channels

Support: Ministry of Science and Technology, Taiwan (MOST 105-2314-B-002-150)
Ministry of Science and Technology, Taiwan (MOST 104-2923-B-002-006-MY3)
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Ministry of Science and Technology, Taiwan (106-2811-B-002-124-)
National Taiwan University Hospital (NTUH 105-S3057)
National Health Research Institutes, Taiwan (NHRI-EX107-10733NI)

Title: The α6-subunit-containing $GABA_A$ receptor is a novel drug target for migraine: Capsaicin-induced migraine model in rats

Authors: *M.-T. LEE[1], P.-C. FAN[3], W. SIEGHART[4], D. E. KNUTSON[5], J. M. COOK[6], L.-C. CHIOU[1,2,7]
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Abstract: Migraine remains an unmet medical need. Its pathogenesis is attributed to activation of the trigeminal vascular system (TGVS). The α6-subunit-containing $GABA_A$ receptors (α6$GABA_A$Rs) are expressed in trigeminal ganglia (TG), the hub of the TGVS. Here we revealed an unprecedented role of α6$GABA_A$Rs in TGVS activation using several pharmacological approaches in a migraine model induced by intra-cisternal (*i.c.*) instillation of capsaicin. Capsaicin (*i.c.*) induced both central and peripheral TGVS responses in rats. Centrally, it activated the trigeminal cervical complex (TCC), measured by the increased number of c-Fos-immunoreactive (c-Fos-ir) TCC neurons. Peripherally, it elevated calcitonin gene-related peptide immunoreactivity (CGRP-ir) in TG and caused CGRP release from sensory nerve fibers, which was measured by the reduced length of CGRP-ir fibers, in the dura mater. Pharmacological approaches included a recently identified α6$GABA_A$R-selective positive allosteric modulator (PAM), the pyrazoloquinolinone Compound 6[1] and its 4'-deuterated derivative (4'-OCD_3-Compound 6)[2], two α6$GABA_A$R-active PAMs (Ro15-4513 and loreclezole), an α6$GABA_A$R-inactive benzodiazepine (diazepam), an α6$GABA_A$R-selective antagonist (furosemide), and a

clinically effective antimigraine agent (topiramate). Compound 6 (3-10 mg/kg, *i.p.*) and 4'-OCD_3-Compound 6 (3-10 mg/kg, *i.p.*) significantly attenuated the TCC neuronal activation and TG CGRP-ir elevation, and dural CGRP depletion induced by *i.c.* capsaicin. All effects of Compound 6 were mimicked by Ro15-4513, loreclezole and topiramate, but not diazepam. The brain-impermeable furosemide antagonized the peripheral, but not central, effects of Compound 6 and 4'-OCD_3-Compound 6. These results suggest that the α6$GABA_AR$ in TG is a novel drug target for migraine and the potential of α6$GABA_AR$-selective PAMs as novel anti-migraine agents.

[1]Chiou et al. (2018) Cerebellar α6 subunit-containing $GABA_A$ receptors: A novel therapeutic target for disrupted prepulse inhibition in neuropsychiatric disorders. Br. J. Pharmacol. DOI: 10.1111/bph.14198.

[2]Knutson et al. (2018) Design and synthesis of novel deuterated $GABA_AR$ α6 subtype functionally selective ligands with improved metabolic stability and enhanced bioavailability. J. Med. Chem. 61:2422-2446.

Disclosures: M. Lee: None. **P. Fan:** None. **W. Sieghart:** None. **D.E. Knutson:** None. **J.M. Cook:** None. **L. Chiou:** None.

Poster

558. Potassium Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 558.01/E2

Topic: B.04. Ion Channels

Title: Aromatic-dependent interactions control Kv1.1 voltage-gated potassium channel conformational equilibria

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Abstract: We found in a young proband who displayed continuous myokymia, ataxic gait episodes, motor incoordination and spastic skeletal muscle contractions, a novel heterozygous mutation in the *KCNA1* gene that encodes the delayed-rectifier potassium channel Kv1.1. The missense mutation causing debilitating symptoms involved a highly evolutionary conserved aromatic non-polar phenylalanine at position 303. Using site-directed mutagenesis mutant and wild-type *KCNA1* constructs were heterologously expressed in *Xenopus laevis* oocytes, after which electrophysiological characterization followed. The mutation resulted in decreased Kv1.1 current amplitude, significant positive shifts of voltage-dependence, altered kinetics of activation, deactivation and slow inactivation, and reduced window currents. We constructed a model using rat Kv1.2 coordinates that shows the open channel structure may be stabilized by

hydrophobic interactions. Substitution of the aromatic phenylalanine with the smaller aliphatic valine in the model revealed altered neighboring interactions important in keeping the channel open. These findings suggest the bulky hydrophobic phenylalanine occupies a sterically confined area that allows essential hydrophobic interactions and restricts conformational movements towards channel closure. We propose the rigid phenylalanine at position 303 as an open-state conformation stabilizing residue and aromatic-dependent interactions as a mechanism for the fine-tuning of conformational equilibria in the Kv1.1 channel.

Disclosures: S.M. Hasan: None. **T. Hunter:** None. **G. Hunter:** None. **M. Pessia:** None. **M.C. D'Adamo:** None.

Poster

558. Potassium Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 558.02/E3

Topic: B.04. Ion Channels

Title: Regulation of Eag1 K^+ channel biosynthesis by a RING E3 ubiquitin ligase

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Abstract: The Eag1 K^+ channel is a member of the e*ther-à-go-go* (Eag) potassium (K^+) channel that belongs to the superfamily of voltage-gated K^+ channel. In mammals, the expression of Eag1 is largely restricted to the brain. Mutations in the gene encoding human Eag1 (KCNH1) K^+ channel have been associated with the congenital neurodevelopmental diseases Temple-Baraitser syndrome and Zimmermann-Laband syndrome. Some of the disease-associated Eag1 mutants may manifest enhanced protein degradation. A dynamic equilibrium between synthesis and degradation contributes to the proteostatic regulation of K^+ channels. However, little is known about the molecules mediating protein synthesis and degradation of Eag1 channels. To better understand the physiological function of Eag1 K^+ channel, we study the molecular machinery responsible for the ubiquitin-dependent regulation of Eag1 K^+ channels. By performing yeast two-hybrid screening of a rat brain cDNA library, we identified a specific RING E3 ubiquitin ligase, MKRN1 (also known as RNF61), as a potential binding partner of Eag1 proteins. We have performed the co-immunoprecipitation, GST pull-down assay, and immunofluorescence staining to confirm the interactions between Eag1 and MKRN1. The expression level of Eag1 proteins was significantly increased in HEK293T cells when using siRNA to knockdown the expression of MKRN1. Immunoblotting analyses of Eag1 *per se* revealed two protein bands that correspond to full- and core-glycosylated channel proteins. Interestingly, when co-expressed with MKRN1, Eag1 displayed a third low molecular-weight band that was also be detected when

treated with the proteasomal inhibitor MG132. Deglycosylation treatment showed that the third band had the molecular weight similar to the deglycosylated form of Eag1. Co-immunoprecipitation also indicated that MKRN1 interacted mostly with the immature Eag1 at the endoplasmic reticulum (ER). Moreover, MKRN1 overexpression enhanced Eag1 ubiquitination and prevented protein maturation. Taken together, our data suggest that MKRN1 may contribute to the ER quality control of Eag1 channels.

Disclosures: Y. Fang: None. **Y. Gan:** None. **C. Tang:** None. **C. Jeng:** None.

Poster

558. Potassium Channels

Location: SDCC Halls B-H

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Program #/Poster #: 558.03/E4

Topic: B.04. Ion Channels

Support: NIH grant DC 01919 (L.K.K)

Title: Kv3.3 channels regulate the formation of multivesicular bodies

Authors: *Y. ZHANG[1], L. VARELA[2], K. SZIGETI-BUCK[3], T. L. HORVATH[4], L. K. KACZMAREK[1]
[1]Yale Univ. Sch. Med., New Haven, CT; [2]Comparative Med., [3]Yale Univ., New Haven, CT; [4]Comparative Med., Yale Med. Sch., New Haven, CT

Abstract: The voltage-dependent potassium channel Kv3.3 is encoded by the KCNC3 gene. Human mutations in this gene result in Spinocerebellar ataxia type 13 (SCA 13), a condition associated with cerebellar degeneration. We have found by electron-immunostaining that Kv3.3 channels are highly expressed in Purkinje cells of the cerebellum at locations where the plasma membrane comes into close apposition with underlying mitochondria. We have also found that depolarization of Kv3.3 channels directly activates Tank Binding Kinase 1 (TBK1), an enzyme that plays a key role in the formation of multivesicular bodies, autophagy and mitophagy. A disease-causing mutation, G592R Kv3.3, produces enhanced TBK1 activation both in cell lines and in the cerebellum of knock-in mice bearing this mutation. By electron microscopy, we find that the enhanced activation of TBK1 in G592R Kv3.3 knock-in mice is associated with increased numbers of intracellular multivesicular bodies, and increased levels of CD63, a molecular marker for these structures. Mitochondrial function may also be impacted by the mutation because levels of mitofusin-1 and mitofusin-2, proteins that are required for fusion and maintenance of mitochondrial structure, are altered in the mutant mice. Using cell lines expressing the mutant channel, we have shown that the G592R Kv3.3-induced multivesicular bodies contain Hax-1, a protein essential for the survival of cerebellar neurons. Inhibition of TBK1 in the cell lines prevents in increase in CD63 levels produced by the Kv3.3 mutation. Our

findings suggest that Kv3.3 channels are directly coupled to pathways that regulate the trafficking of proteins into multivesicular bodies. Moreover, disease-causing mutations of these channels may promote the formation of autophagosomes and potentially trigger mitophagy of mitochondria that are co-localized with the channels at the plasma membrane.

Disclosures: Y. Zhang: None. **L. Varela:** None. **K. Szigeti-Buck:** None. **T.L. Horvath:** None. **L.K. Kaczmarek:** None.

Poster

558. Potassium Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 558.04/E5

Topic: B.04. Ion Channels

Support: USAF contract number FA8650-14-D-6519
AFOSR LRIR Number 18RHCOR039

Title: 1869 nm infrared laser pulses inhibit action potential firing and hyperpolarize the membrane potential in postnatal hippocampal neurons and neuroblastoma X glioma NG108-15 cells by modulating thermo-sensitive potassium channels

Authors: *A. V. SEDELNIKOVA, G. P. TOLSTYKH[1,2], A. J. WALSH[3], A. S. TIJERINA[1,4], A. D. SHINGLEDECKER[5], C. M. VALDEZ[1,6], H. T. BEIER[1]
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Abstract: Infrared laser pulses (IRLPs) modulate the activity of excitable cells, making it a potentially valuable tool for a variety of clinical applications. We have shown that 1869 nm IRLPs can reversibly inhibit action potential (AP) firing in hippocampal neurons. One explanation for this observation is that the IRLPs influence the activity of the ion channels contributing to the APs. By applying the patch-clamp technique, we investigated the effect of IRLPs on membrane potential and channel activity in hippocampal neurons. We found that while IRLPs induce a depolarization of the plasma membrane of up to 18 mV and above the AP firing threshold, the majority of hippocampal neurons failed to fire synchronized APs. Moreover, when APs were stimulated by voltage-step protocol, we observed complete inhibition of APs by 3 and 3.5 ms IRLPs applied at the beginning of the voltage step. By reducing IRLPs duration to ≤2.5 ms some AP recovery was observed, but with shorter duration or/and smaller magnitude. The membrane depolarization induced by IRLPs was followed by a longer-lasting hyperpolarization. The hyperpolarizing current was voltage-dependent and blocked in neurons sensitive to low

concentrations of tetraethyl ammonium (TEA, 1mM), indicating the involvement of Kv3.1 channels, a thermo-sensitive potassium channel subtype. When K+ channels where blocked in TEA-sensitive neurons, 3.5 ms IRLPs induced only a 3% reduction in magnitude of the voltage-step-stimulated depolarization waveform, suggesting a relatively small effect on Nav+ channels from IRLPs. Involvement of Kv3.1 was confirmed in NG108 cells, which primarily express Kv3.1 channels, where IRLPs caused an instant potentiation of current when channels were opened by a voltage (Vh) stimulus. The amplitude of the response depended on holding voltage and was significantly decreased by 20 mM TEA.

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Poster

558. Potassium Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 558.05/E6

Topic: B.04. Ion Channels

Support: NIH

Title: Cooperative synaptic and intrinsic plasticity onto NAc D1MSNs drive depressive-like behavior induced by aversive learning

Authors: *M. PIGNATELLI[1], H. TEJEDA[2], L. BONTEMPI[2], A. LOPEZ[3], D. BARKER[2], R. MARINO[2], S. PALMA RIBEIRO[2], J. WU[2], Z.-L. CAI[4], M. XUE[4], M. MORALES[2], A. BONCI[2]
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Abstract: Anhedonia and behavioral despair represent core symptoms of depression. While these features are in part mediated by maladaptive neuroadaptation within the brain reward circuitry, a comprehensive framework of how information flows through these nodes in depression is lacking. Here, we show that aversive learning induces anhedonia and behavioral despair. These phenotypes can be reversed independently by depotentiating the observed increased synaptic strength of ventral hippocampus (VH) excitatory synapses onto D1 medium spiny neurons (D1R-MSNs) in the nucleus accumbens shell (NAc), or by restoring depression-induced decreased potassium channel function in hyperexcitable D1R-MSNs. Moreover, mimicking the observed decreased potassium channel function observed after aversive learning in D1R-MSNs in naïve animals is sufficient to drive depressive behavior. Finally, utilizing a novel disconnection procedure, we demonstrate that strengthening of VH synapses and excitability changes in D1-MSNs induced by potassium channel dysfunction are serial processes that promote anhedonia and behavioral despair after aversive learning. These results provide a

novel, cellular mechanism for decreased motivation and increased behavioral despair after an aversive experience. They highlight a previously unappreciated role for D1R-MSNs in driving negative affective states, thus elucidating innovative targets for treatment of depression and other psychiatric disorders characterized by negative affective states.

Disclosures: M. Pignatelli: None. **H. Tejeda:** None. **L. Bontempi:** None. **A. Lopez:** None. **D. Barker:** None. **R. Marino:** None. **S. Palma Ribeiro:** None. **J. Wu:** None. **Z. Cai:** None. **M. Xue:** None. **M. Morales:** None. **A. Bonci:** None.

Poster

558. Potassium Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 558.06/E7

Topic: B.04. Ion Channels

Title: SUMOylation of the mouse voltage-gated potassium channel Kv4.2 at two distinct sites independently regulates surface expression and the biophysical properties of the A-type potassium current (I_A)

Authors: *M. A. WELCH[1], L. A. FORSTER[2], S. I. ATLAS[1], D. J. BARO[1]
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Abstract: Small ubiquitin-like modifier (SUMO) is a ~100 amino acid peptide post-translationally added to lysine (K) residues on target proteins to alter their protein-protein interactions. SUMOylation can occur in an activity-dependent manner, and ion channel SUMOylation can regulate surface expression and biophysical properties. These data suggest that dynamic SUMOylation could mediate activity-dependent regulation of ionic currents. The voltage-gated potassium channel Kv4.2 mediates the A-type potassium current, I_A. We are examining the role of Kv4.2 channel SUMOylation. Immunoprecipitation followed by western blot experiments showed that Kv4.2 channels were SUMOylated in the rodent CNS and in a human embryonic kidney (Hek) cell line stably expressing a GFP-tagged Kv4.2 channel (Hek-Kv4.2g). Transiently cotransfecting Hek-Kv4.2g cells with plasmids encoding SUMO and the SUMO conjugating enzyme, Ubc9, produced a significant 35% increase in Kv4.2 SUMOylation ($p<0.05$ t-test) and a significant 27% reduction in I_A maximal conductance (G_{max}) as measured with whole cell patch clamp recordings (34.75±2.65 vs 25.45±2.24µS $p<0.05$ MWU). Surprisingly, the conductance decrease was accompanied by a significant 78% increase in Kv4.2 channel surface expression as determined with biotinylation experiments (0.80±0.09 vs1.42±0.28 $p<0.05$ MWU). Prediction software identified two high-probability SUMOylation sites on the Kv4.2 channel, K437 and K579, and we surmised that they had opposing functions in Hek cells. We tested this by mutating one or both sites: K437R and K579R. Preliminary data showed that mutating both sites abolished the 35% increase in Kv4.2 SUMOylation elicited by transient

overexpression of SUMO and Ubc9. When one site was mutated, the 35% increase was reduced to ~16%. These data suggest that both predicted sites can be SUMOylated. Consistent with our hypothesis, SUMOylation at each site produced a distinct effect. Transient overexpression of SUMO+Ubc9 in Hek-Kv4.2g K579R cells still produced the increase in Kv4.2 surface expression (1.0±0.19 vs 1.9±0.37 $p<0.05$ t-test) but not the decrease in I_A G_{max}. In fact, increased SUMOylation now elicited a mean increase in I_A G_{max} consistent with augmented surface expression (27.6 vs 36.2µS $p=0.17$). We are currently examining the properties of Hek-Kv4.2g K437R. Preliminary data suggest that the mutation prevented the increase in surface expression elicited by transient overexpression of SUMO+Ubc9 (1.00 vs 1.027 n=1), but we have not yet examined I_A G_{max}. In sum, Kv4.2 channel biophysical properties and surface expression can be independently regulated by SUMOylation of the channel at two distinct sites.

Disclosures: M.A. Welch: None. **L.A. Forster:** None. **S.I. Atlas:** None. **D.J. Baro:** None.

Poster

558. Potassium Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 558.07/E8

Topic: B.04. Ion Channels

Title: Resistance to chronic stress by GABAergic regulation via TREK-1 channel

Authors: *J. CHOI[1,2], H. JUNG[1], A. KIM[1], S.-C. KIM[1], Y.-E. KIM[1], E. HWANG[1]
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Abstract: Exposure to chronic stress is responsible for many psychiatric diseases, such as depression, anxiety, and reduction of social ability. TREK-1 has been believed to play an important functional role in mood regulation, as already known that TREK-1 Knockout (Kcnk2 -/-) mice showed a depression-resistant phenotype. TREK-1 localized with GABAergic interneuron in mouse hippocampus. Particularly, in the temporal DG(Dentate gyrus), a sub region related to stress response and emotion. However, TREK-1's involvement in GABAergic regulation under chronic stress has not been studied. we made a depression model in TREK-1 Knockout (KO) and Wild type (WT) littermate control mice, and depression validation was performed through a forced swim test (FST), a tail suspension test (TST) and social interaction behavior After 21 days of stress, social interaction was reduced in WT stressed mice, whereas the TREK-1 KO stressed mice showed increased social interaction significantly. These findings suggests that TREK-1 may be involved in the inhibitory regulation of social behavior circuits as well as the depression resistant phenotype.

Disclosures: J. Choi: None. **H. Jung:** None. **A. Kim:** None. **S. Kim:** None. **Y. Kim:** None. **E. Hwang:** None.

Poster

558. Potassium Channels

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Program #/Poster #: 558.08/E9

Topic: B.04. Ion Channels

Support: MINECO-FEDER/BFU2014-56164-P
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Fundación Tatiana Perez de Guzmán el Bueno
UCLM Plan Propio Program

Title: Role of G protein gated inwardly rectifying potassium (Kir3/GirK) channels in mouse dorsal hippocampus

Authors: *L. JIMENEZ-DIAZ[1], S. TEMPRANO-CARAZO[1], I. SÁNCHEZ-RODRÍGUEZ[1], S. DJEBARI[1], A. NAJERA[1], M. O. NAVA-MESA[2], A. MÚNERA[3], J. YAJEYA[4], A. GRUART[5], J. DELGADO-GARCIA[5], J. D. NAVARRO-LOPEZ[1]
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Abstract: The hippocampus is an essential brain structure for learning and memory processes. Its correct performance relies on the balance between excitatory/inhibitory synaptic transmission. G protein-activated inwardly rectifying potassium (GirK) channels regulate neuronal excitability by mediating inhibitory effects of different G protein-coupled receptors. Activation of GirK channels induces neurons to hyperpolarize, compensating neuronal excitation excess. In addition, they are constitutively active contributing to resting conductances. Recent evidence shows that GirK-dependent signal is altered in pathologies related to excitatory/inhibitory neuronal activity imbalances, such as Down syndrome, epilepsy, and Alzheimer's disease. Here, we have examined the role of GirK-dependent signalling in the mouse dorsal hippocampus at different levels of complexity (synaptic, network and behavioral) and its relevance in the maintenance of normal cognitive functions. To reach that objective, GirK-dependent signal was pharmacologically modulated by specific drugs, the GirK opener ML297, and the blocker Tertiapin-Q. *In vitro,* we studied in dorsal hippocampal slices, the effect of GirK-dependent signalling modulation on LTP induced in CA1 by Schaffer collaterals stimulation. *In vivo*, we performed acute intracerebroventricular injections of GirK specific drugs and studied: 1) at the synaptic level, I/O and PPF protocols in CA3-CA1 synapse, 2) at the circuit and network levels, oscillatory properties of CA1 region and LTP induction in CA3-CA1 synapse and 3) at the

behavioral level, learning and memory capabilities during open field and object recognition tests, both dependent on CA3-CA1 synapse. Our data shows that an imbalance of GirK-dependent signalling, whether caused by increased or decreased activity of the channel, results in abnormalities on neuronal excitability, LTP and oscillatory rhythms recorded from CA3-CA1 hippocampal synapse. These effects are accompanied by learning and memory impairments in behavioral tasks. Taken together, our results suggest that GirK channels are necessary for normal hippocampal activity at synaptic, neural network and behavioral levels and an accurate control of its activity must take place in the hippocampus to sustain cognitive faculties.
STC, ISR, SD contributed equally. ISR, MONM, AM held fellowships from UCLM Plan Propio Program.

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Poster

558. Potassium Channels

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Topic: B.04. Ion Channels

Support: NSFC Grant 31571063

Title: Characterization of Kir channels in neurovascular pericytes in the brain

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Abstract: Pericytes are one of the most important component of neurovascular units and are essential in angiogenesis, blood-brain barrier formation, and blood flow regulation. Pericytes can be identified by expressing different molecular markers, such as PDGF$_{\beta}$R, α-SMA, CD13, desmin and nerve-glia antigen 2 (NG2 / CSPG4) [1]. Interestingly, NG2-glia as the fourth type of macroglia which are broadly distributed in the white and grey matter in the central nervous system (CNS), also express the pericytic specific marker NG2. Our previous study has shown that NG2-glia exhibit very hyperpolarized resting membrane potentials (RMPs) around -90 mV which is close to the K^+ equilibrium potential (E_K) and this hyperpolarized RMP is largely due to the high expression of inwardly rectifying K^+ channels subtype Kir4.1. However, compared with NG2-glia, our recent data has found that the neurovascular pericytes have much depolarized RMPs which is around -60 mV. It's well known that Kir4.1 channels express in glial cells and

play an important role in the maintenance of RMP and extracellular K^+ uptake in the CNS. In addition, Kir channels are also reported to be expressed in smooth muscle cells (SMCs) as well as in pericytes [2,3]. However, the functions of Kir channels in pericytes are largely unknown. To explore what is the major subtype of K^+ channel contributing to a depolarized RMP in pericytes and what is the physiological relevance underlying, we will combine RNA-Seq transcriptional analysis with electrophysiological patch recordings technique obtained from NG2DsRed transgenic mice. In this poster, we will present the characteration and function of K^+ channels especially Kir channels in neurovascular pericytes. This study will reveal a potential significance of how pericytes expressing Kir channels integrate into the maintenance of the BBB homeostasis in the brain.

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Disclosures: X. Zhang: None. **X. Hong:** None. **X. Tong:** None.

Poster

558. Potassium Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 558.10/E11

Topic: B.04. Ion Channels

Support: NIH intramural research program

Title: Seizure induces Kv4.2 phosphorylation by p38 MAPK

Authors: *J.-H. HU[1], D. A. HOFFMAN[2]
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Abstract: Kv4.2 is the main A-type voltage-gated potassium channel in hippocampal CA1 pyramidal neuron dendrites. Their currents play an important role in regulating the back-propagating action potentials and limiting the propagation of local dendritic spikes. Kv4.2 control of dendritic excitability impacts neuronal plasticity and contributes to learning and memory. Thus, it is important to know how Kv4.2 is regulated. Previous findings showed that Kv4.2 can be phosphorylated at T602, T607 and S616 by ERK in vitro. However, how phosphorylation is regulated and the identity of the proline-directed kinase us unknown. Here, we report that Kv4.2 is dynamically phosphorylated at T607 in mouse brain. We first

characterized specific antibodies against phosphor-T602 and phosphor-T607 using Kv4.2 mutations expressed in HEK293T cells. Kv4.2 phosphorylation at T602 and T607 were increase when co-expressed Erk1 suggesting that Erk1 can phosphorylate the two sites. However, the increase was small, which leads us to consider other kinases that could phosphorylate these two sites. We co-transfected Kv4.2 with other proline-directed kinases such as CDK5, GSK3B and P38 in HEK293 cells and found that P38 had the largest effect. Interestingly, a point mutation of P38 which abolished P38 kinase activity largely blocked Kv4.2 phosphorylation suggesting P38 is the main kinase responsible for T602 and T607 phosphorylation in HEK293T cells. We found that P38 binds to Kv4.2, supporting this idea. Furthermore, we found that both P38 activity and T607 phosphorylation of Kv4.2 was induced by seizure that is triggered by pentylenetetrazol (PTZ) administration, while T602 phosphorylation remained unchanged in mouse brain. Interestingly, P38 inhibitor SB203580 administration blocked PTZ-induced Kv4.2 phosphorylation at T607 site in mouse hippocampi. Taken together, these data show that P38 phosphorylates Kv4.2 at T607 in mouse hippocampi after seizure induction suggesting that Kv4.2 phosphorylation may play an important role in its pathophysiology.

Disclosures: J. Hu: None. **D.A. Hoffman:** None.

Poster

558. Potassium Channels

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 558.11/E12

Topic: B.04. Ion Channels

Title: The regulation of Kir4.1 in the development of epilepsy

Authors: *J. BONI[1], A. RANDOLPH[2], M. L. OLSEN[3]
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Abstract: Kir4.1, a glial-specific inwardly rectifying potassium channel, is essential for astrocytic maintenance of K+ homeostasis. Underscoring the role of Kir4.1 in CNS functioning, genetic mutations in Kcnj10, the gene which encodes the Kir4.1 protein have been linked to seizures, ataxia and developmental disability. Furthermore, numerous studies consistently demonstrate reduced levels of Kir4.1 protein and mRNA expression in multiple injury paradigms and disease models, typically accompanied by reactive gliosis. While reduced Kir4.1 protein and mRNA expression is a common observance in CNS insult, it is unclear what molecular mechanism/s govern this process. Utilizing a pilocarpine model of status epilepticus in adult rats we demonstrate Kir4.1 protein is significantly reduced as early as 24 hours post-status. This downregulation persists though 30 days, the last time point examined. A concomitant loss of mRNA was observed at all time points examined, suggesting a transcriptional mechanism of

regulation. Using a different injury paradigm, a fifth cervical (C5) vertebral hemi-contusion model of spinal cord injury, we observed similar results. Previous work by our group revealed the DNA methylation status of the Kcnj10 gene functions to regulate developmental levels. To analyze the role of DNA methylation in injury, studies were completed in the hippocampus and in isolated astrocytes after SE. Here we demonstrate hypermethylation of 7 CpG sites in CpG Island 2 in both the SE and SCI models. In contrast, our previous work indicates during development when Kir4.1 expression increases this region demonstrates significant hypomethylation. Our results suggest that bidirectional modulation of methylation may function to modulate Kcnj10 gene transcription. DNA methylation may represent a candidate mechanism to rescue astroglial Kir4.1 expression following CNS insult providing therapeutic benefit.

Disclosures: J. Boni: None. **A. Randolph:** None. **M.L. Olsen:** None.

Poster

558. Potassium Channels

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Topic: B.04. Ion Channels

Support: NIH Grant NS083402
Epilepsy Foundation Grant C4107

Title: Missense epilepsy mutations in neuronal KCNQ/Kv7 channels occur at hotspots within highly conserved functional domains of Kv7.2 and Kv7.3

Authors: *J. ZHANG[1], C. CHEN[1], E. KIM[1], E. PROCKO[2,3], J. PATEL[1], R. CHOI[1], M. HONG[1], D. JOSHI[1], G. LEE[1], A. GZAPLICKI[1], E. C. COOPER[4], E. BOLTON[1], H. CHUNG[1,3]
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Abstract: Neuronal KCNQ/K_v7 channels composed of KCNQ2/K_v7.2 and KCNQ3/K_v7.3 subunits are voltage-gated potassium channels that potently inhibit neuronal excitability. Close to 200 mutations in KCNQ2 and KCNQ3 genes are associated with neonatal epilepsy in humans including Benign Familial Neonatal Epilepsy (BFNE) and Epileptic Encephalopathy (EE) (rikee.org). In particular, EE patients show refractory seizures and severe behavioral comorbidities including developmental delay, psychomotor retardation, and autism. Currently, it remains unknown whether these mutations are randomly distributed throughout the coding region of K_v7.2 and K_v7.3. In this study, we analyzed 177 KCNQ2 and 14 KCNQ3 missense epilepsy mutations using nonrandom mutation clustering (NMC) and resampling statistical methods. Nonpathogenic missense mutations and silent mutations were used as negative

controls. These analyses revealed that KCNQ2 mutations are more likely to locate in the voltage-sensing S4 transmembrane domain, the pore loop and S6 that control ion permeability, helix B in the intracellular C-terminal tail that mediates calmodulin (CaM) binding, and helix B-C linker. When mutations are analyzed based on the severity of outcomes, EE mutations are more concentrated in the same hotspots whereas BFNE mutations do not exhibit any enrichment. Mutations in KCNQ3 are enriched in pore loop and helix A. For characterization of mutation hotspots, we selected EE mutations including L203P in S4, L268F in the pore, K524T and R525L in helix B. K524T and R525L decrease apoCaM and Ca^{2+}-CaM binding. All 4 mutations decrease channel expression at the membrane surface. The pore mutant L268F nearly abolishes the enrichment of the channel at the axon initial segment (AIS). Furthermore, we demonstrated that L203P induces right-shift voltage dependence, while K524T and R525L disrupt PIP_2 dependence with electrophysiolgy. Altogether, our results reveal the hotspots of pathogenic epileptic mutations in KCNQ2 and KCNQ3. Current efforts include systematic analyses of mutations within the hotspots to predict the functional and clinical outcome of the mutations.

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Poster

558. Potassium Channels

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Program #/Poster #: 558.13/E14

Topic: B.04. Ion Channels

Support: Grants NASU # II-1-12 to PB
Grants NASU # 67/15 to PB

Title: Neurocalcin delta translocation is not dependent on its dimerization

Authors: N. I. KONONENKO[1], A. DOVGAN[1], J. VIVIANO[2], A. DROMARETSKY[1], J. ZHANG[2], V. VENKATARAMAN[2], *P. V. BELAN[1,3]
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Abstract: Similar neuronal Ca^{2+} sensor (NCS) proteins, Neurocalcin δ (NCALD) and Hippocalcin (HPCA), control many neuronal functions via their differential Ca^{2+}-dependent translocation from the cytosol to the plasma membrane. Our preliminary results indicate that in solutions Ca^{2+}-bound HPCA is mainly present as a monomer while Ca^{2+}-bound NCALD exists as a homodimer. Besides, three AA that are critical to the formation of the dimer interface of NCALD were identified in biochemical experiments. However, the functional consequences of

NCALD dimerization have never been studied in cellular systems. We suggested that disruption of NCALD dimerization would result in its translocation to the plasma membrane that is similar to one for HPCA. To test this hypothesis, we developed a set of NCALD mutants having single, double, and triple point mutations disrupting dimerization interface of NCALD, tagged them by fluorescent proteins and co-expressed paired wise NCALD and HPCA or one of the NCALD mutants in cultured rat hippocampal neurons. Fast depolarization-induced $[Ca^{2+}]_i$ transients led to the very different time courses and amplitudes of HPCA and NCALD translocation to the plasma membrane. Surprisingly, all NCALD mutants did not reveal a significant difference in Ca^{2+}-dependent translocation compared the wild type NCALD. Even a triple NCALD mutant, which revealed HPCA-like monomeric behavior in the biochemical experiments, demonstrated no signs resembling HPCA translocation in the cellular system. We conclude that in the cellular system Ca^{2+}-bound NCALD promptly translocates to the plasma membrane before it can be dimerized in the cytosol. Thus, dimerization of NCALD revealed by its crystal structure is not important for this protein translocation in native cellular systems.

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Poster

558. Potassium Channels

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Topic: B.04. Ion Channels

Support: BBSRC CASE PhD studentship

Title: Localisation of Kv3 subunits within the spinal micturition reflex and the effect of novel modulation on bladder output

Authors: *P. MULLEN[1], N. PILATI[2], C. H. LARGE[3], J. DEUCHARS[1], S. DEUCHARS[1]
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Abstract: Kv3 channels are voltage-gated potassium ion channels that are highly expressed in the brain and important in neuronal firing and synaptic transmission (Rudy et al., 1999). However, in some brain regions, Kv3 channel expression decreases with age with functional consequences (Zettel et al., 2007). Kv3 channels are also expressed in the spinal cord (Deuchars et al., 2001) but little is known about their role in spinal circuitry and whether age-related changes are also observed here. To investigate this, we studied the expression of Kv3 subunits in spinal circuitry that underlies bladder function, and the effect of AUT1, a novel modulator selective for Kv3 channels (Alvaro et al. 2011), on bladder function. Parasympathetic

preganglionic (PGN), sympathetic preganglionic (SPN) and dorso-lateral nucleus (DLN) motoneurones were retrogradely traced with fluorogold (1%, i.p). 3 month (n=3) and 28 month (n=3) C57bl6 mice were anaesthetised intraperitoneally (i.p.) with 60mg/kg pentobarbitone, perfused transcardially and fixed with 4% paraformaldehyde. Lumbosacral spinal levels (L1, L6 and S1) were dissected, sectioned to 20 µm and processed for double labelling immunohistochemistry of Kv3 subunits, Kv3.1b and Kv3.3, with inhibitory synaptic markers, VGAT and GlyT2, and excitatory synaptic marker, VGluT2. 3 and 20 month mice were given vehicle or AUT1 (30 mg/kg, 60 mg/kg, i.p.) and their behaviour (micturition and locomotor activity) was recorded in metabolic cages over a 3 hour period. Kv3 puncta around bladder motoneurones in the lumbo-sacral spinal cord co-localised with both excitatory and inhibitory synaptic immunoreactivity. In a comparison of young and aged mice, the number of Kv3 puncta around motoneurones was significantly reduced (DLN, Kv3.3; Ind. Equ. T-test, young, 57.67 ± 16.5 vs aged, 49.26 ± 14.3, $p<0.01$, PGN, Kv3.3; Ind. Equ. T-test, young, 67.18± 14.8 vs aged, 43.8 ± 15.47, $p<0.001$, SPN, Kv3.1b; Ind. Unequ. T-test, young, 124.4 ± 27.0 vs aged, 87.7 ± 36.5, $p<0.001$. Data as mean ± SEM). AUT1 treatment produced an acute dose dependent reduction in bladder output (60mg/kg, 100%, $p<0.05$; 30mg/kg, 42.3%, $p<0.05$) and reduced activity (recorded by a sedation rating scale and video tracking software). We hypothesise that the reduced Kv3 expression observed in aged mice may have a functional significance within bladder circuitry and on the bladder reflex. Furthermore, the reduction in bladder output produced by AUT1 may have therapeutic relevance to age-related conditions such as nocturia.

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Poster

558. Potassium Channels

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Program #/Poster #: 558.15/E16

Topic: B.04. Ion Channels

Support: Marquette committee on research
Marquette department of biological sciences

Title: Excitability increases while BK channel contribution decreases in neonatal hippocampal neurons

Authors: *M. HUNSBERGER, A. MONICAL, M. MYNLIEFF
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Abstract: There are fundamental differences between the immature and the mature brain resulting in a greatly increased risk of seizures in infancy and early childhood. We used heterogeneous cultures of rat CA1 hippocampal neurons to investigate the maturation of the action potential and excitability as well as the contribution of Ca^{2+}-activated K^+ channels to these biophysical characteristics. Whole-cell patch clamp recording in the current clamp configuration was used to study action potentials and excitability. Single action potentials (AP) were evoked by a 0.1 ms, 8 nA current and AP trains were evoked by 100 ms depolarizing pulses. The mean action potential duration, represented by spike width at half-amplitude, decreased from 3.43±0.12 ms (22 animals, 133 cells) in cells from 0-5 day old pups to 2.65±0.10 ms (23 animals, 144 cells) in cells from 6-10 day old pups ($p<0.001$). Excitability of neurons, measured by the maximum number of APs that could be evoked by a 100 ms depolarizing pulse, increased between these age ranges from 2.00±0.10 (22 animals, 157 cells) to 2.86±0.13 (23 animals, 150 cells; $p<0.001$). These data may represent a maturation of inhibitory circuitry that can better coordinate hippocampal outputs and reduce seizure risk. As BK currents have been implicated in seizure susceptibility, we analyzed APs in cells treated with the BK antagonist Iberiotoxin (IbTx). Recordings were taken before, during, and after 100 nM IbTx perfusion and the drug effect was calculated by comparing the values during drug treatment to the average of the values before and after treatment to account for rundown effects. IbTx decreased action potential duration by 0.74±0.15 ms in cells from 3-5 day old pups (5 animals, 38 cells) but only by 0.22±0.06 ms in cells from 6-8 day old pups (5 animals, 41 cells; $p<0.002$). The Ca^{2+} activated K^+ channels SK and KCNQ also significantly contributed to AP kinetics but did not exhibit significant age-related changes in their effects so the difference in BK effect is likely not due to unrelated changes in AP kinetics. Preliminary data from voltage clamp recordings suggest that the BK component of the total K^+ current halves from day 5 to day 8. Our data demonstrate that action potential durations shorten and excitability increases in a heterogenous population of hippocampal neurons in the early postnatal period. We also demonstrate that contributions of BK channels to total K^+ currents and the action potential waveform decreases during this period. These results suggest that reduced excitability of inhibitory neuron populations and increased BK currents may underlie seizure susceptibility in infants.

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Poster

558. Potassium Channels

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Topic: B.04. Ion Channels

Title: Mechanisms of suppression by the amyloid peptide fragments 1-42 and 25-35 on Kv1.1 channel activity

Authors: *K. DEBOEUF, M. ISLAM, N. THELEN, J. FARLEY
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Abstract: The beta amyloid peptide (Aβ) has long been a hallmark of Alzheimer's Disease (AD) pathology. Past studies have linked its involvement in the disruption of Ca^{2+} homeostasis, synaptic communication, and long-term potentiation (LTP), but the underlying mechanism(s) is still largely unclear. Because Kv1.1 and related channels are activated during an action potential, regulate depolarization Ca^{2+} influx, and inhibition of Kv1 channels can be neurotoxic, we speculate that Aβ-suppression of Kv1 channels may be early targets in AD pathogenesis. Using murine Kv1.1 channels expressed in *Xenopus* oocytes, we have observed the effects of both Aβ(1-42) and the "core" peptide (25-35) on both macro- and micro-scopic currents. Both the bath application of Aβ(1-42) and Aβ(25-35) produced 40-50% suppression of macroscopic Kv1.1 current within 30 m. The suppression of Kv1.1 by Aβ(1-42) was partially dependent on intracellular Ca^{2+} and PP2B, being reduced by ~50% when cells were loaded with BAPTA-AM or exposed to the PP2B-inhibitor cyclosporine A (CsA). Patch-clamp results suggest that Aβ-suppression of Kv1.1 involves both PP2B-dephosphorylation and direct protein-protein interaction of Aβ with Kv1.1 channel subunits. Exposure of inside-out single Kv1.1 in ripped-off oocyte patches to application of purified, catalytically-active PP2B produced gradual reductions in *p*(open), followed by the abrupt disappearance of Kv1.1 activity. Application of Aβ to the intracellular face of Kv1.1 channels also produced dramatic reductions in *p*(open). To better study the direct interaction between Aβ and Kv1.1, we have made use of artificial membranes which have more stable preparations and easier access to both intra- and extra-cellular faces of the channel compared to oocyte patch clamping. Using "tip-dip" methods, Aβ(25-35) exposure eliminated Kv1.1 channel activity when applied to the intracellular face. Experiments are currently underway looking at the effects of Aβ on Kv1.1 channels incorporated in a Black Lipid Membrane (BLM) apparatus. Suppression of Kv1.1 and related K^+ channels presynaptically could lead to larger and longer action potentials, allowing more influx of Ca^{2+}, increased release of glutamate, and possibly the beginning of a disruption of Ca^{2+} homeostasis. Postsynaptically, the increased glutamate release, through activation of AMPA and NMDA receptors, may contribute to excitotoxicity.

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Poster

558. Potassium Channels

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Topic: B.04. Ion Channels

Support: NIH T32 HL007446
NIH R01 NS094461

Title: Novel enhancement of KCNQ M-type K+ channels and TRPC cation channels after muscarinic receptor activity in hippocampus controlling neuromodulation and excitability

Authors: *C. CARVER, M. S. SHAPIRO
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Abstract: KCNQ2/3 (M-channel) current maintains homeostatic control over neuronal firing and excitability. Classically, M-channels have been characterized by sensitivity to suppression by Gq-coupled muscarinic acetylcholine receptor stimulation due to intracellular membrane-PIP2 hydrolysis. The muscarinic signaling plays important functional roles in modulation of neuronal excitability. We investigated the neuromodulatory effects of muscarinic receptor stimulation on hippocampal M-currents in-depth.
Patch-clamp electrophysiology recordings were aquired from mouse dentate gyrus granule cells (DGGCs) and CA1 pyramidal neurons in brain slice. Stimulation of M1Rs with M1-selective allosteric agonist 77LH281 resulted in surprising and significant enhancement of M-current in DGGCs, however CA1 neurons exhibited muscarinic-induced suppression of M-current. We also observed significant increase to GIRK current acting as a a PIP2 biosensor, supporting our hypothesis that in DG, net intracellular PIP2 was increased after Gq-coupled muscarinic stimulation. The PIP5 kinase inhibitor UNC3230 was included to block PIP2 synthesis, and M1R stimulation then suppressed M-current in DGGCs. KCNQ2 knockdown in DG ablated the muscarinic receptor-dependent enhancement of M-current. Furthermore, the net effect of muscarinic stimulation was an increase in action potential firing frequency, despite enhancement of M-current. We found intracellular Ca2+ signals were robustly increased in DGGCs after muscarinic stimulation, as detected with a calcium sensor dye. Increased Ca2+ was dependent on TRPC channel activation of neuronal excitability, as TRPC blockers ablated excitability and Ca2+ increase. Interestingly, activation of TrkB receptors also enhanced M-current via PLCgamma activity in DGGCs.
We describe novel muscarinic receptor-induced effects on neuronal excitability in the DG involving a heretofore undiscovered, cell-type specific role of M-channel neuromodulation. Muscarinic receptors may serve a wider and more complex role in governing hippocampal excitability through cholinergic signals. These novel findings in DG suggest an entirely different relationship between muscarinic receptors, PIP2 availability, and M-channels than found in peripheral sensory neurons or other glutamatergic CNS neurons.

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Poster

558. Potassium Channels

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Topic: B.04. Ion Channels

Support: NIH Grant NS073981
NIH Grant HL137094
NIH GRANT NS101596

Title: Deletion of KCNQ2/3 potassium channels from PV+ interneurons leads to homeostatic potentiation of excitatory transmission

Authors: *H. SOH[1], S. PARK[2], K. SPRINGER[3], K. RYAN[4], A. MAHESHWARI[2], A. TZINGOUNIS[3]
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Abstract: Potassium channels play important roles in a range of cellular physiological processes in normal brain function. KCNQ2/3 channels, in particular, have arisen as critical regulators of neonatal brain excitability. Highlighting the importance of these channels, a growing number of loss-of-function (LOF) and gain-of-function (GOF) variants in*KCNQ2* and *KCNQ3* have been reported in patients with severe neonatal and infantile epileptic encephalopathy. The main features of KCNQ2/3-associated epileptic encephalopathy are progressive cognitive deterioration and early onset of a severe seizure disorder that does not respond to most anticonvulsant drugs. In contrast to the wealth of knowledge on KCNQ2/3 channels functioning in excitatory neurons, the roles of these channels in interneurons is still unclear. This is a major gap in our knowledge as interneurons play a critical function in shaping the activity of neuronal populations and promoting the development of excitatory synaptic circuits. KCNQ2/3 channels have been associated primarily with neurons that undergo pronounced spike frequency adaptation, a feature not traditionally associated with interneurons. However, using microscopy and more recently single cell exome sequencing, KCNQ2/3 channels have been detected in cortical interneurons such as parvalbumin (PV)- and somatostatin (SST)-positive interneurons. Here, by using *ex vivo* and *in vivo* electrophysiology show that deletion of *Kcnq2/3* channels from parvalbumin, but not somatostatin, interneurons increased their excitability, leading to elevated inhibitory transmission and homeostatic excitatory drive potentiation in CA1 pyramidal neurons. Additionally, *PV-Kcnq2* null-mice showed increased seizure susceptibility, suggesting that decreases in KCNQ2/3 activity in interneurons remodels excitatory networks, providing a previously unrecognized function of KCNQ2/3 channels in the brain.

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Poster

558. Potassium Channels

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Program #/Poster #: 558.19/E20

Topic: B.04. Ion Channels

Support: BRFSF_2015-05
NIH P20 GM113132

Title: The action potential as a modulator of synaptic transmission

Authors: *L. PANZERA[1], M. CHIN[2], M. B. HOPPA[2]
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Abstract: Individual presynaptic terminals often behave differently to electrical stimuli in terms of vesicle fusion probability. The probability of vesicle fusion or synaptic strength of a terminal can be modulated by many factors including vesicle pool dynamics and fusion machinery, as well as the properties of voltage gated calcium channels. It is often assumed that the electrical inputs are digital in nature and that individual synapses along an axon receive uniform action potential waveforms. However, recent experiments have called this observation into question for many central neurons. Action potential broadening is one of the most classically observed phenomena during various stimulation protocols, yet it is unclear how this broadening alters vesicle fusion and release of neurotransmitter. This is primarily due to the small size of *en passant* central neuron synapses which are not easily accessed by classic electrophysiology. Here we provide the first simultaneous quantitative measurements of presynaptic action potentials, calcium influx and exocytosis in *en passant* synapses using genetically encoded fluorescent indicators in cultured hippocampal neurons. We combine these measurements with genetic and pharmacological manipulations to understand how changes in action potential waveform shape alter presynaptic transmission. Inhibition of Kv1s, one of the predominant voltage gated potassium channels found in these synapses, results in a predictable increase in action potential amplitude and full width at half maximum. Interestingly, we found only excitatory cells rely on Kv1 channels to modulate the presynaptic action potential waveform. Further, action potential broadening results in an uncoupling of the classically defined relationship between vesicle fusion probability and net calcium influx, with an enhancement of neurotransmission over what is predicted. To determine the mechanism behind this phenotype we restricted the size of presynaptic calcium microdomains using the calcium chelator EGTA and found no effect on the percent change of vesicle fusion, indicating the radius of microdomains are not responsible for

the increase in fusion with action potential broadening. Our results suggest that modulation of the action potential waveform is a powerful modulator of synaptic strength.

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Poster

558. Potassium Channels

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Topic: B.04. Ion Channels

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Title: A *Drosophila* model of essential tremor

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Abstract: Essential Tremor (ET) is one of the most common neurological diseases, with an estimated 7 million affected individuals in the US; the pathophysiology of the disorder is poorly understood. Recently, we identified a mutation (*KCNS2* (*Kv9.2*), c.1137 T>A, p.(D379E) in an electrically silent voltage-gated K^+ channel α-subunit,*Kv9.2*, in a family with ET, that modulates the activity of Kv2 channels. We have produced transgenic *Drosophila* lines that express either the human wild type Kv9.2 (hKv9.2) or the ET causing mutant Kv9.2 (hKv9.2-D379E) subunit in all neurons. We show that thehKv9.2 subunit modulates activity of endogenous *Drosophila* K^+ channel Shab. The mutant hKv9.2-D379E subunit showed significantly higher levels of Shab inactivation and a higher frequency of spontaneous firing rate consistent with neuronal hyperexcitibility. We also observed behavioral manifestations of nervous system dysfunction including effects on night time activity and sleep. This functional data further supports the pathogenicity of the *KCNS2* (p.D379E) mutation, consistent with our prior observations including co-segregation with ET in a family, a likely pathogenic change in the channel pore domain and absence from population databases. The *Drosophila* hKv9.2 transgenic model recapitulates several features of ET and may be employed to advance our understanding of ET disease pathogenesis.

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Poster

558. Potassium Channels

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 558.21/E22

Topic: B.04. Ion Channels

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NIH U01 NS090581
NIH T32 GM0007377

Title: Organization of Ca^{2+} and lipid signaling domains by the Kv2 family of voltage-gated potassium channels

Authors: *M. KIRMIZ, N. C. VIERRA, J. S. TRIMMER
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Abstract: Voltage-gated potassium (Kv) channels play diverse roles in regulating neuronal excitability. Of these, Kv2.1 and Kv2.2 are robustly expressed as micron-sized plasma membrane (PM) clusters on the soma, proximal dendrites, and axon initial segment of most brain neurons. These Kv2 paralogs are distinct in their cellular expression patterns, extent of multisite phosphorylation, and responses to stimuli that trigger phosphorylation-dependent changes in the localization and voltage activation of Kv2.1, but not Kv2.2. The clusters of Kv2.1 and Kv2.2 are found at endoplasmic reticulum (ER)-PM junctions (EPJs), and we have recently demonstrated that organization of EPJs is a conserved and *bona fide* non-conducting function of both members of the Kv2 family (Kirmiz et al., 2018. bioRxviv 10.1101/296731). As such, Kv2 ion channels are the first family of PM proteins whose expression is sufficient to govern organization of these membrane contact sites. EPJs in diverse cell types are known to participate in Ca^{2+} signaling [*e.g.*, excitation-contraction coupling in striated muscle *via* the association of PM L-type Ca^{2+} channels and ER ryanodine receptors (intracellular Ca^{2+} release channels)] and/or regulation of lipid trafficking/metabolism (*e.g.*, regulation of phosphatidylinositol-4-phosphate levels by Sac1). Kv2-containing EPJs are also defined by the presence of vesicle associated membrane protein-associated proteins (VAPs). VAPs are known to participate in regulation of lipid metabolism in cultured mammalian cells, and Kv2 clusters often colocalize with RyRs in cultured hippocampal neurons, raising the question as to whether EPJs mediated by the Kv2 family have the capacity to function in dual capacities as distinct sites of lipid metabolism/trafficking and Ca^{2+} signaling. Here, using total internal reflection fluorescence and conventional fluorescence microscopy of cultured rat hippocampal neurons and heterologous

cells, we investigated the potential role(s) of Kv2-associated EPJs as sites of lipid metabolism/trafficking and Ca^{2+} signaling. We found that expression of Kv2 channels is sufficient to reorganize/recruit L-type Ca^{2+} channels, as well as proteins involved in lipid metabolism/trafficking including Sac1 and PITPNM1/Nir2, to EPJs organized by Kv2 channels. We also found that L-type Ca^{2+} channel-mediated Ca^{2+} responses are enhanced in cells coexpressing either conducting or nonconducting isoforms of Kv2.1. Our findings suggest that these abundant neuronal Kv2 channels can influence lipid trafficking/metabolism and Ca^{2+} signaling in brain neurons via a non-conducting function that is dependent on their ability to organize EPJs.

Disclosures: M. Kirmiz: None. **N.C. Vierra:** None. **J.S. Trimmer:** None.

Poster

558. Potassium Channels

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Topic: B.04. Ion Channels

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NIH T32 GM0007377

Title: Neuronal Kv2 channel-associated endoplasmic reticulum-plasma membrane junctions are sites of localized spontaneous Ca(2+) entry

Authors: *N. VIERRA, M. KIRMIZ, J. S. TRIMMER
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Abstract: Endoplasmic reticulum-plasma membrane junctions (EPJs) have long been assumed to serve critical roles in modulating neuronal Ca^{2+} handling, as sites with enhanced expression of Ca^{2+} channels and pumps. The voltage gated K^+ channels Kv2.1 and Kv2.2 are prominent constituents of neuronal EPJs, facilitating the spatial concentration of L-type Ca^{2+} channels (LTCCs), ryanodine receptors (RyRs), and Ca^{2+} store-regulated PM Orai1 channels (Kirmiz et al., 2018. bioRxviv 10.1101/296731). Although these observations suggest a role for Kv2 channel-associated EPJs in neuronal Ca^{2+} handling, their participation in Ca^{2+} signaling events has not been demonstrated. This is an important question, as autosomal dominant *de novo* mutations in Kv2.1 that disrupt Kv2.1 clustering at EPJs are associated with severe neurological disorders. Here, we have visualized distinct Ca^{2+} signaling events at neuronal Kv2 channel-mediated EPJs. We used genetically encoded Ca^{2+} indicators (GECIs) fused to conducting and nonconducting Kv2 channel isoforms or to the Kv2 auxiliary subunit AMIGO1 to image Ca^{2+} at these sites in cultured rat hippocampal neurons. We found that Kv2 channel-mediated EPJs are

sites of Ca^{2+} entry during global Ca^{2+} influx events. We found that in addition to these global events, Ca^{2+} transients consisting of rapid and often stochastic spikes occurred at a subset of individual Kv2-containing EPJs. These transients at individual Kv2-containing EPJs occurred independent of one another, even at junctions located <1 µm away from one another, such that the spatial extent of the Ca^{2+} transients was confined within individual Kv2 channel clusters. The frequency and amplitude of these local Ca^{2+} transients were sensitive to membrane potential depolarization, nimodipine, caffeine, and thapsigargin, implicating the involvement of LTCC and RyR-mediated Ca^{2+}-induced Ca^{2+} release. Moreover, following global Ca^{2+} entry, Ca^{2+} was cleared more quickly within a Kv2 channel cluster than in regions outside of clusters. Importantly, we determined that Kv2.1 channel conductance was not necessary for the occurrence of Kv2 channel cluster-associated spontaneous Ca^{2+} transients, as neither their frequency nor their amplitude differed between neurons expressing the "wild-type" Kv2.1-GECI or a non-K^+-conducting Kv2.1 pore mutant (P404W)-GECI. Together these findings demonstrate that Kv2-channel mediated EPJs are sites of distinct and compartmentalized neuronal Ca^{2+} signaling events with unique Ca^{2+} handling properties. Our findings further support a critical role for EPJs in neuronal Ca^{2+} handling and demonstrate an important function for Kv2 channels in this process.

Disclosures: N. Vierra: None. **M. Kirmiz:** None. **J.S. Trimmer:** None.

Poster

558. Potassium Channels

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Topic: B.04. Ion Channels

Support: NIH Grant DC01919
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Title: Activation of Slack potassium channels (KCNT1) triggers an increase in mRNA translation

Authors: *T. J. MALONE[1], P. LICZNERSKI[2], E. A. JONAS[2], L. K. KACZMAREK[3]
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Abstract: The Slack ion channel is a member of a family of large conductance sodium-activated potassium channels. It is expressed predominantly in neurons of the central nervous system where it regulates neuronal excitability. FMRP, an important regulator of mRNA translation, binds both Slack mRNA and the Slack protein. The association of Slack with FMRP stimulates

channel activity, raising the possibility that activation of Slack channels may also regulate the function of FMRP. Our laboratory has previously identified Slack as required for a protein translation-dependent recovery from an extended period of inhibition in Aplysia neurons following stimulation, further suggesting that Slack may play a role in the regulation of mRNA translation. Here we provide the initial evidence for such a role. We transfected cells with a fluorescent reporter for mRNA translation, which contains the 5' and 3' sequences of the mRNA for β-actin, but with the coding region replaced with that for the irreversibly-photoconvertible fluorescent protein dendra2. We were able to visualize real-time translation in HEK cell cultures and in mouse cortical neurons. Based on the observed translation levels in a stable Slack-expressing HEK cell line along with pharmacological manipulation and silencing RNA knockdowns, we propose a mechanism whereby Slack activation causes an increase in translation that is enhanced in the absence of FMRP. This increase in translation persists in the presence of the Slack channel blocker quinidine, indicating that it does not require ion flux through the channel. Experiments on cultured neurons from wild-type, Slack knockout, and FMRP knockout mice show that Slack-stimulated translation also occurs in native neurons. Additional experiments in HEK cell culture suggest that the Slack binding partners CYFIP1, another FMRP binding protein; and Phactr-1, a PP1 binding protein, may also modulate this channel-dependent translation. This mechanism of Slack-dependent translation potentially represents the first instance of the direct modulation of mRNA translation by activation of an ion channel.

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Poster

558. Potassium Channels

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Title: Interactions of Slack (KCNT1) channels with Phactr1 are altered by a human disease-causing mutation

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Abstract: The Slack gene encodes sodium-activated potassium channels that are abundantly expressed in the central nervous system. Human mutations alter the function of Slack channels, resulting in epilepsy and intellectual disability. Most of the disease-causing mutations are located in the extended cytoplasmic C-terminus of Slack channels and most result in increased Slack current. Previous experiment using a yeast two-hybrid system indicate that the C-terminus of Slack channels binds a number of cytoplasmic signaling proteins. One of these is Phactr1, a protein that is believed to target protein phosphatase 1 (PP1) to its phosphoprotein substrates. Phactr1 is also known to be an actin-binding protein. We have now found by co-immunoprecipitation that all three components, Phactr1, PP1 and actin exist in a complex with Slack channels. We then investigated the role of disease-causing Slack mutations in the modulation of the Slack-Phactr1 complex and found, using FRET and coimmunoprecipitation experiments that residue R1085 modulates the formation of Slack-Phactr1 complex and that the disease-associated mutation R1085A increases the affinity of the channel for Phactr1. In patch clamp experiments, however, the amplitude of both wild type and $Slack^{R1085A}$ currents was suppressed by co-expression of Phactr1. It has been proposed that activation of Slack channels could stimulate mRNA translation and preliminary experiments suggest that the $Slack^{R1085A}$ mutation may alter this aspect of channel function. Our data support the hypothesis that, in addition to regulating electrical excitability directly, Slack channels participate in intracellular signaling pathways via PP1 or actin. In this regard, targeting Slack-Phactr1 interactions may be helpful in developing novel therapies for brain disorders associated with the malfunction of Slack channels.

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Poster

558. Potassium Channels

Location: SDCC Halls B-H

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Program #/Poster #: 558.25/E26

Topic: B.04. Ion Channels

Support: HIH DC01919
NIDCD RO1DC00273

Title: Role of kv1.3 potassium channels in the auditory function

Authors: *L. EL-HASSAR[1], L. SONG[2], V. R. GAZULA[1], D. NAVARATNAM[3], J. SANTOS-SACCHI[2], L. K. KACZMAREK[1]
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Abstract: Kv1.3 is a low threshold voltage-dependent potassium channel expressed in both excitable and non-excitable cells. In non-excitable cells, Kv1.3 channels are involved in various functions including cell volume regulation, proliferation, and insulin signaling. Within the central nervous system, deletion of Kv1.3 gene from mitral cells of the olfactory bulb dramatically increased the sensitivity of the olfactory system. Our group has previously shown that Kv1.3 channels are also present in the presynaptic terminals of the medial nucleus trapezoid body (MNTB) within the auditory brainstem (also called Calyx of Held). More specifically, they were found to be expressed along the plasma membrane and in internal vesicular structures of the calyx of Held. Whether these Kv1.3 channels with distinct localization in the presynaptic terminals can regulate the synaptic transmission and contribute to the auditory function is unknown. To examine the functional role of Kv1.3 channels in the auditory system, we used *in-vitro* and *in-vivo* approaches in Kv1.3 knockout (KO) and Bl6 Wildtype (WT) mice. Our preliminary results from *in-vitro* whole cell patch-clamp recordings in young mice (P13-17) show that lack of Kv1.3 channels significantly increases the spike firing frequency of the presynaptic calyx in response to square pulses of injected currents. In contrast, no significant changes were detected in the postsynaptic MNTB neurons. We have also found aberrant evoked postsynaptic activity in MNTB neurons in response to presynaptic fibers stimulation in Kv1.3 KO mice, suggesting that kv1.3 channels regulate synaptic transmission between Calyx of Held and MNTB neurons. To further investigate their role in auditory function, we carried out *in-vivo* recordings of the Auditory Brainstem Responses (ABR) of Kv1.3 KO and found that the thresholds of ABR are elevated in young (P13-17) and old (2-4 months) Kv1.3 KO mice over those in WT mice. Latencies of peaks I, II and IV are prolonged in Kv1.3 KO mice. In addition, mice lacking Kv1.3 potassium channels show a desynchronization of ABR waves suggesting an alteration of synaptic transmission and changes in spike fidelity within auditory pathways. Altogether, our data suggest that loss of Kv1.3 potassium channels primarily influences the properties of presynaptic terminals, alters synaptic transmission between Calyx of Held and MNTB neurons, and impairs auditory function. Ongoing experiments are now characterizing the dynamic of Kv1.3 channels distribution in response to presynaptic fibers stimulation.

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Poster

558. Potassium Channels

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NIH 1R21NS101182

Title: A mutant SK channel that is hypersensitive to Ca^{2+}

Authors: *A. VIEGAS[1], Y.-W. NAM[1], S. BASKOYLU[3], R. O. ORFALI, 92866[2], A. HART[3], M. ZHANG[1]
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Abstract: Small-conductance Ca^{2+}-activated K^+ (SK) channels mediate medium afterhyperpolarization in the neurons which limits the firing frequency of action potentials and, thus, play a key role in the regulation of neuronal excitability. Given their importance in neurons, SK channels are potential drug targets for movement disorders, including ataxia and Amyotrophic Lateral Sclerosis (ALS). The SK channels are activated exclusively by the Ca^{2+}-bound calmodulin. Previously, we identified an intrinsically disordered fragment that is essential for the mechanical coupling between Ca^{2+}/calmodulin binding and the channel opening. Here, we report that substitution of one amino acid residue in the intrinsically disordered fragment caused a ~6 fold increase in the Ca^{2+} sensitivity of SK2-a channels. Subsequent tests with equivalent substitutions in SK1 and SK3 channels also exhibited Ca^{2+} hypersensitivity. Additionally, an equivalent phenylalanine substitution in the *Caenorhabditis elegans* (*C. elegans*) SK2 ortholog *kcnl-2* partially rescued locomotion defects in an existing *C. elegans* ALS model, in which human SOD1G85R is expressed at high levels in neurons. This supports the idea that the phenylalanine substitution impacts SK channel function *in vivo*. This work confirms that the intrinsically disordered fragment plays a crucial role in SK channel regulation and - for the first time - provides a critical reagent for future studies: an SK channel that is hypersensitive to Ca^{2+} concentrations with increased activity *in vivo*.

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Poster

558. Potassium Channels

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Topic: B.04. Ion Channels

Support: NIH P20 GM113132
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Title: Distinct subsets of presynaptic K+ channels modulate frequency-dependent synaptic transmission in excitatory and inhibitory hippocampal neurons independent of net calcium influx

Authors: *I. CHO, S. ALPIZAR, M. HOPPA
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Abstract: Presynaptic terminals are fundamental computational units in the brain, and their dysfunction is associated with several neurological diseases. They mediate the transduction of incoming electrical signals (action potentials) into chemical signals (neurotransmitter release), and the efficiency of conversion determines the strength of circuits underlying memory and behavior. Many synapses in the hippocampus display frequency-dependent changes in transduction efficiency. The shape of the presynaptic action potential is of fundamental importance in determining the timing and magnitude of neurotransmitter release. However, the plasticity of action potential waveform shape during frequency dependent stimulation and a role in transduction efficiency is unknown. This is largely due to the fact that the *en passant* synapses that are prevalent in the hippocampus are difficult to measure with classic electrophysiology owing to their small size. To overcome these limitations in our current study we combine a genetically encoded far-red voltage indicator named QuasAr with quantitative measurements of presynaptic calcium and vesicle fusion by GCaMP and vGlut-pHluorin imaging, respectively. We found significant frequency-dependent changes in presynaptic action potential shape even from paired pulse stimulation in primary cultured rat hippocampal neurons. Namely, a significant broadening of action potentials at excitatory synapses and narrowing at inhibitory synapses were shown. We determined that these changes were due to unique molecular identities and functional role of K^+ channels that can modulate the electrogenic properties of the presynaptic membrane at excitatory and inhibitory terminals. Our results indicate that voltage-dependent inactivation of $K_v1.1/1.2$ channels underlies the broadening, while calcium-gated potassium channels underlie narrowing of the action potential for excitatory and inhibitory neurons respectively. Furthermore, while the changes in AP shape are strongly correlated with vesicle fusion probability, however they are independent of net calcium influx as classically predicted, suggesting a role for calcium-microdomain signaling. Taken together, these results suggest that variability in presynaptic K^+ channels may play a fundamental role in controlling frequency-dependent changes in synaptic strength.

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Poster

559. Presynaptic Organization

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Topic: B.05. Neurotransmitter Release

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Title: Proteomic and functional analysis of presynaptic actin regulation during synaptic transmission

Authors: *S. DUBE[1], T. BRADSHAW[1], A. UEZU[2], E. SODERBLOM[3], B. RÁCZ[4], S. H. SODERLING[1,2]
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Abstract: Proper nervous system function requires dynamic remodeling of the actin cytoskeleton, which is highly enriched at both presynaptic terminals and postsynaptic spines. Although actin dynamics and regulation have been well-characterized at postsynapses, studies on actin in mature presynapses have been limited by difficulties in visualizing presynaptic terminals and purifying them by biochemical fractionation. Here, we show the Arp2/3 complex, a nucleator of branched actin filaments, is present at presynaptic terminals *in vivo*. We also use electron microscopy, electrophysiology, and optical methods to probe the effects of its presynaptic disruption on synaptic transmission. Finally, we used *in vivo* BioID to identify 47 additional actin regulatory proteins that reside in presynaptic terminals in the mouse brain. We are currently using stimulated emission depletion (STED) microscopy to verify the localization of these proteins and CRISPR/Cas9-based approaches paired with electrophysiology to determine their functions during synaptic transmission. We expect to uncover several lines of genetic evidence for the functions and regulation of presynaptic actin, creating a framework for how presynaptic terminals may be structurally altered during synaptic plasticity. Additionally, since defects in actin regulation are associated with many neurological disorders (including autism spectrum disorders, intellectual disability, and schizophrenia), our findings may help inform potential presynaptic pathologies in these diseases.

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Poster

559. Presynaptic Organization

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Topic: B.05. Neurotransmitter Release

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Title: Endocytic scaffold Intersectin 1 regulates vesicle reclustering in the reserve pool of the giant vertebrate synapse

Authors: *O. SHUPLIAKOV[1,2], K. FREDRICH[1], A. PECHSTEIN[1], F. GERTH[4], O. VORONTSOVA[1], E. SOPOVA[3,1], O. KORENKOVA[3], V. HAUCKE[4,5], C. FREUND[5]
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Abstract: Synaptic vesicles (SVs) are accumulated at active zones in clusters, which are comprised of ready-releasable and reserve pools. Phosphoprotein synapsin I plays critical role in organizing SVs in the reserve pool in many central synapses. SVs in the reserve pool replenish the ready-releasable pool to sustain neurotransmission during high-rate activity and are rapidly reclustered after stimulation. How these SV trafficking events are regulated is largely unknown. Using the giant reticulospinal model synapse in lamprey we show that the scaffolding protein intersectin 1 (ITSN1) regulates the synapsin 1 function during synaptic activity by forming a dynamic complex with synapsin. Like in mammalian synapses ITSN1 is a component of an extravesicular matrix of the reserve pool of SVs in giant lamprey synapses. The complex formation with synapsin 1 is mediated by SH3A (Src-homology 3 A) domain of ITSN1, which binds to the D domain of synapsin I. An intramolecular switch within ITSN1 regulates the interaction between the proteins. Microinjection of antibodies against SH3A domain into giant synapses at rest does not perturb SV organization, while during stimulation it disrupts the vesicle clustering in the reserve pool thus supporting that ITSN1 and synapsin 1 come into interaction during synaptic activity. Our data suggest that the SH3A domain of ITSN1 serves to sequester synapsin 1 within the reserve pool when it dissociates from SV during stimulation and promotes efficient reclustering when stimulation ceased by releasing dephosphorylated synapsin within the reserve pool. Thus, our experiments uncover the molecular mechanism regulating vesicle reclustering within the reserve pool of SVs.

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Poster

559. Presynaptic Organization

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Topic: B.05. Neurotransmitter Release

Support: MOE2016-T2-1-097
NMRC/CBRG/0094/2015

Title: MAP kinase phosphorylation gates regulation of SV trafficking and neurotransmitter release by J domain of synapsin III

Authors: *S.-H. SONG, G. J. AUGUSTINE
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Abstract: Among the 3 mammalian synapsin genes, synapsin III is unique because it regulates neurotransmitter release in a neurotransmitter-specific manner (J. Neurosci. 28:10835; 30:9762; 36:6742). These effects must be caused by the J domain, a motif found only in synapsin III. To examine the function of the J domain, we first injected a peptide from the J domain of synapsin III into squid giant presynaptic terminals. This peptide reversibly inhibited synaptic transmission, while a scrambled version did not; thus, the inhibitory effect is sequence-specific. Analysis of the kinetics of synaptic depression during high-frequency stimulus trains (50 Hz) revealed that J domain peptide inhibits synaptic transmission both by reducing the size of the readily-releasable pool (RRP) and by slowing mobilization of vesicles from the reserve pool (RP) to the RRP. This effect is regulated by phosphorylation: pseudophosphorylating the J domain peptide at a MAPK phosphorylation site (S470D) inhibited synaptic transmission, while a non-phosphorylatable version (S470N) did not. To further clarify J domain function, we examined excitatory synapses of microisland-cultured mouse hippocampal neurons. Expressing recombinant J domain did not affect the amplitude of autaptic EPSCs. However, during repetitive stimulation (10 Hz), J domain expression slowed the time constant of synaptic depression and increased RRP size. Further, a phospho-null mutant J domain (S470A) further slowed depression and increased RRP size, while pseudophosphorylated J domain accelerated the rate of synaptic depression and reduced RRP size. Thus, in both squid and mouse neurons, phosphorylation of the J domain controls the ability of synapsin III to regulate mobilization of RP vesicles. In summary, our results indicate that interactions mediated by the J domain of synapsin III determine the dynamics of both RRP and RP vesicles, thereby regulating neurotransmitter release. Further, this regulation is gated by MAPK phosphorylation.

Disclosures: S. Song: None. **G.J. Augustine:** None.

Poster

559. Presynaptic Organization

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Topic: B.05. Neurotransmitter Release

Support: MH052804

Title: Neurexins mediate clustering of voltage-gated Ca^{2+} channels at presynaptic active zone

Authors: *F. LUO[1,2], M. JIANG[2], T. L. SÜDHOF[2]
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Abstract: The spatial organization of voltage-gated Ca^{2+} channels and synaptic vesicles at the presynaptic active zone are tightly regulated and play essential role in synaptic function. However, the molecular mechanisms underlying the tight regulation remain much unknown. Here we examined the function of neurexins, the central organizer of synapse formation and function, at the calyx of Held synapse. We found that deletion of all neurexins remarkably reduces synaptic strength at the calyx of Held. The functioning of Ca^{2+} channels and release machinery remain intact; however, the coupling between Ca^{2+} channel and synaptic vesicle was surprisingly impaired. These results together suggest a novel function of neurexins in organizing presynaptic active zone by facilitating tight coupling between Ca^{2+} channels and readily-releasable synaptic vesicles.

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Poster

559. Presynaptic Organization

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Topic: B.05. Neurotransmitter Release

Support: NIH Grant R01 MH080046

Title: Nanoscale organization of RIM-BP2 at the mammalian active zone

Authors: *T. B. TARR, T. A. BLANPIED
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Abstract: Effective communication within neural circuits requires the precise control of neurotransmitter release in response to a presynaptic action potential. Indeed, perturbation of release can trigger circuit malfunction that leads to cognitive and psychiatric disorders. Functional precision arises from the proper subsynaptic organization of the proteins involved in synaptic transmission. Previous work from our lab has established that evoked vesicle fusion preferentially occurs at subdomains of the AZ with the highest density of Rab3-interacting molecule (RIM), a protein that is necessary for synaptic vesicle priming and for the recruitment of Ca^{2+} channels to the AZ. These RIM "nanoclusters" are also aligned with postsynaptic nanoclusters of receptors and PSD-95, suggesting this presynaptic organization helps maximize synaptic strength by aligning sites of release with high density clusters of receptors. Considering the importance of subsynaptic protein organization, we sought to determine the nanoscale organization of RIM-binding protein 2 (RIM-BP2), another functionally relevant presynaptic protein that is crucial for the reliability and fidelity of synaptic transmission. Like RIM, RIM-BP2 binds Ca^{2+} channels, particularly P/Q-type Ca^{2+} channels, and thus is suspected to localize them close to vesicles through this direct interaction as well as indirectly through its binding to RIM. Using super-resolution STORM imaging of cultured hippocampal neurons, we have observed that RIM-BP2 is tightly clustered within small AZ subdomains that bear a striking resemblance to the RIM nanoclusters. Since the overall clusters of RIM-BP2 are similar to the nanoclusters of RIM, RIM-BP2 may be playing an important role in synaptic function by influencing the nanoscale organization of RIM. To test this, we are currently performing STORM imaging of RIM and PSD-95 at synapses that are expressing RIM-BP2 mutants that cannot bind to RIM, and at synapses in which RIM-BP2 has been knocked down. Not only will these experiments help determine the influence of RIM-BP2 on the nanoscale organization of RIM, but they will also provide insight into the effect, or lack thereof, of presynaptic perturbations on postsynaptic protein organization.

Disclosures: T.B. Tarr: None. **T.A. Blanpied:** None.

Poster

559. Presynaptic Organization

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Topic: B.05. Neurotransmitter Release

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DFG SPP 1757
DFG SCHO 820/4-1
DFG SCHO 820/6-1
DFG DI 853/3-2
DFG DI 853/7-1

BONFOR

Title: RIM4γ deficiency causes alterations in cerebellar Purkinje cell function

Authors: *E. M. SCHÖNHENSE, K. MICHEL, H. T. KIM, A. J. BECKER, D. DIETRICH, S. SCHOCH
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Abstract: RIMs (Rab3-interacting molecules) are multidomain proteins, enriched at presynaptic active zones. The large isoforms (RIM1α/β, RIM2α/β) have been shown to be important for mediating presynaptic active zone function by coupling synaptic vesicles to voltage-gated calcium channels (VGCCs) and by regulating neurotransmitter release as well as presynaptic plasticity. The functional role of the small RIM isoforms, RIM3γ and RIM4γ, in particular in vivo has so far remained unresolved. To address this open question we have generated constitutive RIM4γ knock-out (KO) mice. Starting after weaning these RIM4γ KO mice exhibit spontaneous episodes of strong hind limb impairments with rapid uncontrolled movements accompanied by weight loss. In order to uncover if this phenotype resulted from dysfunction in the cerebellum, we crossed conditional RIM4γ KO mice with a Purkinje-cell specific Cre-driver line (Pcp2-Cre) resulting in Pcp2-cre(Mpin)-RIM4γ KO mice. Behavioral experiments with these mice confirmed deficits in fine motor coordination and less exploration in a novel environment found in the constitutive KO line. Furthermore, it was also possible to induce the motor phenotype by injections of ethanol or caffeine.
Interestingly, morphological analyses of the cerebellum and of individual Purkinje cells revealed changes in size and branching of the dendritic tree. Juxtacellular recordings of Purkinje cells in the presence of blockers of synaptic transmission revealed in Pcp2-RIM4γ KO mice an overall reduced spontaneous firing frequency of Purkinje cells and an almost complete lack of bursting cells. In addition, a population of Purkinje cells firing tonically at 10-15 Hz is strongly diminished in Pcp2-RIM4γ KO mice.
Taken together, our data for the first time reports that RIM4γ in cerebellar Purkinje cells is required to maintain normal electrophysiological properties and to establish proper dendritic morphology. In turn, RIM4γ deficiency results in a phenotype resembling human dyskinesias.

Disclosures: E.M. Schönhense: None. **K. Michel:** None. **H.T. Kim:** None. **A.J. Becker:** None. **D. Dietrich:** None. **S. Schoch:** None.

Poster

559. Presynaptic Organization

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Topic: B.05. Neurotransmitter Release

Support: NIH Grant R01NS078179
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NIH Grant R01NS061914

Title: Acute homeostatic challenge induces a rapid active zone cytomatrix-dependent increase in synaptic calcium channel levels

Authors: *S. J. GRATZ[1], J. J. BRUCKNER[2], R. X. HERNANDEZ[3], K. KHATEEB[5], G. T. MACLEOD[4], K. M. O'CONNOR-GILES[5]
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Abstract: Communication in neural circuits depends on neurotransmitter release at specialized domains of presynaptic terminals called active zones. Neurotransmitter release properties vary significantly, even between neighboring active zones of the same neuron. To investigate the role of voltage-gated calcium channels in determining diverse release properties, we combined endogenous tagging of the sole *Drosophila* Ca_V2 channel Cacophony with functional imaging at motor synapses. We find that calcium channels levels vary between individual active zones and robustly predict synapse-specific release probability. We next turned to a well-studied paradigm to investigate calcium channel clustering during homeostatic plasticity. Upon exposure to the glutamate receptor antagonist philanthotoxin, *Drosophila* presynaptic motorneurons increase neurotransmitter release to precisely offset reduced glutamate receptor function. It has previously been shown that increased presynaptic calcium influx is required for this homeostatic response. However, how this occurs has remained an open question. Surprisingly, we find that active zone calcium channel levels are increased in as little as 10 minutes during acute presynaptic homeostatic potentiation. Rapid channel accumulation depends on the core active zone cytomatrix protein ELKS/CAST/Bruchpilot, whose levels increase in parallel. Thus, the active zone cytomatrix is dynamically reorganized to cluster more calcium channels and maintain circuit function in response to homeostatic challenge.

Disclosures: S.J. Gratz: None. **J.J. Bruckner:** None. **R.X. Hernandez:** None. **K. Khateeb:** None. **G.T. Macleod:** None. **K.M. O'Connor-Giles:** None.

Poster

559. Presynaptic Organization

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DFG SCHO 820/6-1
DFG DI853/3-2
DFG DI853/7-1
BONFOR

Title: Role of serine arginine protein kinase 2 (SRPK2) on composition and function of the active zone

Authors: *J. BETZIN[1], J. A. MÜLLER[1], A. OPRIŞOREANU[1], E. M. SCHÖNHENSE[1], K. ENGHOLM-KELLER[2], M. GRAHAM[2], A. J. BECKER[1], D. DIETRICH[1], S. SCHOCH[1]
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Abstract: The presynaptic active zone (AZ) is composed of multiple proteins mediating the release of synaptic vesicles (SVs). One major presynaptic scaffolding protein is RIM1α, the most abundant isoform of the RIM family. RIM1α is involved in the regulation of SV exocytosis, the recruitment of voltage-gated Ca^{2+}-channels and synaptic plasticity. Posttranslational modifications, like phosphorylation, play a role in controlling protein-protein interactions and thereby synaptic transmission and plasticity. RIM1α has been identified as a phosphoprotein, however, it is still unresolved how phosphorylation affects RIM1α function. As a first step in this direction new phosphorylation-dependent binding partners of RIM1α were identified using affinity chromatography/mass spectrometry. This screen identified the SR protein kinase 2 (SRPK2) as a novel RIM1α interacting protein. The homolog of SRPK2 in *D. melanogaster* (SRPK79D) plays an important role for T-bar assembly and the transport of the AZ protein Bruchpilot to the presynapse, however its role in mammalian synapse function is to date still unresolved. Here, we analyzed the role of SRPK2 in murine AZ formation and function. Overexpression (OE) and knock-down (KD) of SRPK2 was induced in hippocampal neuronal cultures by transduction with rAAV particles. To study the influence of SRPK2 expression on AZ components, we established a method to quantify the abundance of synaptic proteins in bead units based on immunofluorescence labeling. Overexpression of SRPK2 resulted in increased levels of RIM1 within synapses. Furthermore, the impact of SRPK2 on AZ formation and structure at the nanoscale was analyzed using direct stochastic optical reconstruction microscopy (dSTORM). Functionally, the effect of SRPK2 expression on synaptic release was investigated using the genetically encoded glutamate sensor iGluSnFr and FM4-64 dye-release assays. Overexpression of SRPK2 resulted in a two-fold increase in release probability. Intriguingly, OE of SRPK2 could not potentiate release probability in RIM1/2 knock-out cells, indicating that the release stimulating effect of SRPK2 requires RIMs. In addition, a phosphoproteomic analysis was used to identify novel SRPK2 target proteins at the presynapse and the specific phosphorylation sites of these proteins. Amongst other targets, this analysis revealed several putative phosphorylation sites in RIM1. Our data identifies SRPK2 as a kinase that by phosphorylating multiple presynaptic proteins affects AZ composition and synaptic transmission.

Disclosures: J. Betzin: None. **J.A. Müller:** None. **A. Oprişoreanu:** None. **E.M. Schönhense:** None. **K. Engholm-Keller:** None. **M. Graham:** None. **A.J. Becker:** None. **D. Dietrich:** None. **S. Schoch:** None.

Poster

559. Presynaptic Organization

Location: SDCC Halls B-H

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Program #/Poster #: 559.09/E37

Topic: B.05. Neurotransmitter Release

Support: DFG SPP 1608
DFG SFB 1134

Title: Synaptic maintenance in the absence of synaptic activity in the auditory brainstem

Authors: *C. KÖRBER[1], L. EBBERS[2], S. HOPPE[1], H. G. NOTHWANG[2]
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Abstract: The maintenance and integrity of synapses are thought to rely on the presence of neuronal activity. This includes the release of synaptic vesicles (SVs) at presynaptic active zones (AZs), either in response to an action potential or spontaneously. SV release is inhibited by bacterial neurotoxins which cleave neuronal SNARE proteins thereby preventing the assembly of the crucial SNARE complex. One of these neurotoxins is tetanus toxin (TeNT), which cleaves the SNARE protein synaptobrevin/VAMP2. We expressed TeNT in the bushy cells of the ventral cochlear nucleus (VCN) in the auditory brainstem using a specific Cre-driver mouse line (Math5). The globular bushy cells of the VCN give rise to the calyx of Held in the contralateral medial nucleus of the trapezoid body (MNTB), a giant axo-somatic synapse that comprises 300-700 individual AZs. The expression of TeNT at this specific synapse led to a gradual decrease of SV release with the virtual absence of neurotransmission by P15. However, we did not observe any alterations in the MNTB, neither on the number and size of the MNTB principal cells, nor on the morphology of calyx of Held synapse. Moreover, TeNT expression did not lead to a reduction in AZ number or a loss of SVs from AZs, albeit the number of "docked" SVs close to the plasma membrane was strongly reduced. We therefore conclude that synaptic activity is not necessary for the maintenance of this synapse but rather contributes to the remodeling of synapses in order to meet the current requirements of the neuronal circuit.

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Poster

559. Presynaptic Organization

Location: SDCC Halls B-H

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Program #/Poster #: 559.10/E38

Topic: B.05. Neurotransmitter Release

Title: A potential mechanism for locus coeruleus-dependent dopamine signalling

Authors: *A. SONNEBORN, R. W. GREENE
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Abstract: The dorsal hippocampus is essential for the consolidation of episodic memories, a process that is highly dependent on the activation of dopamine D1/D5 receptors. Initially the source of dorsal hippocampal dopamine (DA) was thought to be the ventral tegmental area (VTA), but several recent studies have established that the majority of this dopamine is actually released from locus coeruleus (LC) terminals. By selectively activating or suppressing LC terminal activity in CA1, these studies also determined a physiologically relevant role of LC DA in learning and memory. However, the mechanisms by which this dopamine can be released have not yet been explored. Here we combine optogenetics, electrophysiology, and pharmacology in order to show that one possible release mechanism is by reverse transport through the norepinephrine transporter. We also provide evidence that DA release in the dorsal hippocampus is mediated by presynaptic NMDA receptors on the terminal boutons of catacholaminergic projections. Since the LC is known to be involved in arousal, attention, and multiple types of memory, these experiments will provide new insight into how attentional processes can influence circuit-level memory formation

Disclosures: A. Sonneborn: None. **R.W. Greene:** None.

Poster

559. Presynaptic Organization

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Topic: B.05. Neurotransmitter Release

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FWF W1205-B09

Title: Structural correlates of transmitter release and readily releasable pool at hippocampal mossy fiber synapses

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Abstract: The readily releasable pool (RRP) is a key parameter that determines efficacy and dynamics of synaptic transmission. However, the structural correlate of this pool remains unclear. It is often thought that the RRP is identical to the pool of docked vesicles. However, it has been also suggested that the RRP represents only a subset of docked vesicles, or that it may include also vesicles added by rapid recruitment (Kaeser and Regehr, 2017). In order to test whether the docked vesicle pool can be fully depleted, we implemented and further developed on the recently described "Flash and Freeze" technique (Watanabe *et al*, 2013, Nature 504:242-247). "Flash and Freeze" combines optogenetics with high-pressure freezing (HPF): a light pulse activates genetically expressed channelrhodopsin (ChR2) in targeted cells, leading to action potential (AP) initiation and vesicle fusion at the active zone (AZ) membrane. During light stimulation, the tissue is kept at physiological temperature, and subsequently frozen by HPF with a pre-set timed delay after onset of stimulus, thus allowing for capture of events at different time points after synaptic transmission onset. We applied "Flash and Freeze" to assess docked vesicle pool dynamics at the mossy fiber-to-CA3 pyramidal cell synapse in organotypic slice culture and acute slices of mouse hippocampus. We expressed ChR2-H134R in granule cells and characterized direct light-evoked responses, as well as postsynaptic responses from CA3 pyramidal neurons. We tested different frequencies (0.1-100 Hz) and pulse duration (1-10 ms) to characterize granule cell AP initiation probability. We found that with 1-5 ms (n = 5) pulses, APs fire reliably, with up to 1-ms delay from stimulus onset. We also found stable postsynaptic responses; with a maximum reliable frequency of 20-30 Hz and median delay 4.6 ± 0.14 ms from stimulus onset. Analysis of AZ reconstruction from serial sections showed that the average area of AZs in putative mossy fiber boutons is 0.10 ± 0.01 μm^2 (n = 7) and mean number of docked vesicles is 67 per μm^2, corresponding to ~8 docked vesicles per AZ. After brief trains of stimuli at moderate frequency we observed vesicle pits, similarly to those described during ultrafast endocytosis and at the same time-scale, between 50-250 ms after stimulus. Furthermore, AZ profile analysis of light-stimulated tissue showed a significant decrease in the number of docked vesicles after a prolonged stimulus (1.6 in control to 0.2 vesicles per profile after 100x 20 Hz, $p < 0.00001$), presumably fully depleting the docked pool. Depletion of docked vesicles during activity suggests that docked vesicles represent the releasable vesicle pool.

Disclosures: C. Borges-Merjane: None. **O.S. Kim:** None. **P. Jonas:** None.

Poster

559. Presynaptic Organization

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Support: Swiss National Science Foundation (CRETP3_166815)
Swiss National Science Foundation (31003A_170085)

Title: Single-cell RNA-seq data reveals developmental specification of alternative splicing profiles of synaptic molecules

Authors: *D. LUKACSOVICH, J. WINTERER, W. LUO, L. QUE, C. FOLDY
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Abstract: Synaptic cell adhesion molecules (CAMs) are responsible for the complex and varied connectivity properties of cells. Understanding the pattern of CAM expression can help us to understand the logic of neural connectivity in the brain. While most recent studies utilizing RNA-seq focus on gene expression levels, alternative splicing has a large impact on the properties of many synaptic molecules. Therefore we looked at alternative splicing at the cell type level to develop a deeper and more fundamental level of understanding of the logic of CAM expression. Here, we utilized single cell RNA-seq data from the NCBI GEO online database to generate a dataset of over 1,600 cells from 45 cell types and, after quality control, performed statistical analysis to explore alternative splicing in hippocampal and cortical cell types. Searching for broad and encompassing trends in the logic of alternative splicing, we found a strong developmental origin-dependent pattern of alternative splicing of CAMs. As an example, while Neurexin genes were not differentially expressed across different cell types, they exhibited significant developmental origin-dependent alternative splicing. Alternative splicing may mute the effect of disorder related genetic mutations that are located on alternatively spliced exons. In this manner our results potentially identify which cell types may be rendered into a pathological state by such mutations. Scaling and automatization of our approaches and pipeline will make it possible to simultaneously analyze a multitude of molecules across vast cellular networks.

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Poster

559. Presynaptic Organization

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Program #/Poster #: 559.13/DP03/E41

Topic: B.05. Neurotransmitter Release

Support: NASA NAG13AL99G

Title: Architectural heterogeneity among ribbon synapse complexes in utricular hair cells

Authors: *L. F. HOFFMAN[1], I. LOPEZ[1], R. SCHIALEK[2], M. TERASAKI[3], J. W. LICHTMAN[2], F. E. SCHWEIZER[4]
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Abstract: Presynaptic complexes within inner ear sensory epithelia hair cells are characterized by synaptic ribbons, which are electron-dense structures exhibiting various morphologies surrounded by neurotransmitter vesicles. Ribbon synapses are also found in retinal rods and cones, lateral-line neuromast hair cells, and pinealocytes. The architectures of ribbon complexes exhibit diversity across the cells in which they are found, and some evidence suggests this heterogeneity may have physiologic correlates. However, the 3D ultrastructure of ribbon complexes in any sensory epithelia have not been extensively explored, and therefore a comprehensive understanding of ribbon architypes represents an important step in understanding potential contributions to processing within sensory epithelia. We addressed this problem through serial ultrastructural analyses to elucidate the 3D architecture of ribbon complexes in murine utricular hair cells. Utricles from C57Bl/6 mice were harvested, fixed (4% paraformaldehyde, 2% glutaraldehyde), and prepared for ATUM SEM (automatic tape-collecting ultramicrotomy and scanning electron microscopy) as previously described (Terasaki et al. 2012, Kasthuri et al. 2014). Sections were obtained at 30 or 50 nm thickness, respectively, and were imaged at either 4 or 3 nm/pixel, respectively. Ribbon complexes in utricular hair cells conformed to two general architectures. As previously described, individual micrographs revealed simple architectures characterized by a single ribbon body that exhibited spheroid, ellipsoid, or bar morphologies surrounded by clear vesicles. While some spheroid or ellipsoid ribbons conformed to the “expected” structure in serial reconstructions, ribbons that appeared as bars in 2D were, in fact, sections of a plate, resembling ribbons found in retinal rods. Such plates extended more than 0.5µm along the presynaptic membrane. In one case the plate extended approx. 1µm into the hair cell cytoplasm. Cluster architectures were formed by multiple ribbons exhibiting mixed morphologies (bar/plates or spheroid/ellipsoids). Neighboring ribbons were separated by a single “sheet” of vesicles. Ribbon clusters were distinguished by the regions of

close presynaptic membrane apposition. For some clusters the regions were very limited, but in others it was extensive. In most cases presynaptic contact was made by only 1-2 ribbons of a cluster, implying the existence of mechanisms to shuttle vesicles from distal ribbons to the active zone. The heterogeneity in ribbon architectures in vestibular hair cells may underlie differential contributions to the dynamic diversity exhibited by afferent neurons.

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Poster

559. Presynaptic Organization

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Topic: B.05. Neurotransmitter Release

Support: ERC Consolidator Grant "NeuroMolAnatomy"

Title: Molecular anatomy of the average hippocampal neuron

Authors: *S. JÄHNE[1], M. HELM[2], H. WILDHAGEN[2], S. MANDAD[2], M.-T. WEIL[3], W. MÖBIUS[4], S. O. RIZZOLI[2]
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Abstract: Neuronal function has been analyzed extensively both *in vivo* and *in vitro*, in the form of primary cultures. Cultured hippocampal neurons have served for decades as tools for the neurosciences, and are the primary *in vitro* tool for neuronal physiology studies. Nevertheless, their detailed molecular anatomy is still poorly understood. We aim here to increase our knowledge of neuronal morphology and physiology by using an integrative approach to study the physical parameters, compartmentalization, and molecular composition of hippocampal neurons. We employed large-field fluorescence microscopy in combination with genetically-encoded fluorescent membrane proteins to determine the average number of neurites, their branching, length, diameter, and volume. Using confocal microscopy, we determined the volume of the neuronal soma, as well as the distribution and proportion taken up by the organelles of the secretory pathway, by mitochondria, peroxisomes, and ribosomes. Finally, focused ion beam scanning electron microscopy and direct stochastic optical reconstruction microscopy (STORM) were used to determine the exact volumes and shapes of the aforementioned organelles, allowing for an accurate description of the cellular compartmentalization. We also determined the copy numbers of 3000 proteins per average neuron, and we are currently integrating super-resolution microscopy and comparative imaging to map the distribution of the most important neuronal proteins (around 200).

Combining the experimentally-generated parameters we aim to produce a 3D model of an average hippocampal neuron and its functional compartments. We will be able to explore this model to increase our knowledge on the organization of functional pathways, such as synaptic vesicle biogenesis. The model also serves as an ideal basis for a molecular nanomap that comprises the numbers and localizations of the most important neuronal proteins.
The resulting molecular nanomap of an average hippocampal neuron will be a comprehensive structural description of a neuronal cell that can function as a database, which colleagues in the field will be able to use as a basis for comparing different cell types, for unraveling functional pathways, and for studying structural differences caused by neurodegenerative diseases, such as Alzheimer's disease.

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Poster

559. Presynaptic Organization

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Topic: B.05. Neurotransmitter Release

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Title: The intra/perisynaptic CB_1 cannabinoid receptor pool tonically controls GABA release at mouse hippocampal synapses

Authors: *B. BARTI[1,2], B. DUDOK[3], K. KENESEI[1], V. MICZÁN[1,4], M. LEDRI[5], I. SOLTESZ[3], I. KATONA[1]
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Abstract: While recent evidence highlighted that distinct molecular machineries are segregated into functionally different nanodomains to mediate anterograde neurotransmission, the nanoarchitecture of retrograde synaptic signaling has remained elusive. Endocannabinoid signaling is a canonical retrograde pathway, and regulates synaptic transmission and plasticity at most synapse types throughout the brain. Notably, CB_1 cannabinoid receptors can control neurotransmitter release in both tonic and phasic manner. To determine the nanoscale organization of the underlying mechanisms behind these forms of synaptic cannabinoid

signaling, and retrograde synaptic signaling in general, we developed a novel approach, which makes the direct correlation of nanoscale molecular imaging data with the respective morphological and physiological synaptic parameters possible even at the single synapse level in brain circuits. First, paired whole-cell patch-clamp electrophysiological recordings were made between presynaptic CB_1-positive GABAergic interneurons and postsynaptic CA1 pyramidal cells in the mouse hippocampus. After anatomical reconstruction of both cells, pairs linked with one or two synaptic connections were further analyzed by correlated confocal and 3-D Stochastic Optical Reconstruction Microscopy (STORM). The nanoscale molecular distribution of presynaptic CB_1 receptors in relation to the active zone visualized by bassoon immunolabeling was quantified at the identified synaptic connection and the molecular data were correlated with the electrophysiologically and anatomically measured synaptic parameters. Our data revealed that the success rate of synaptic events was inversely correlated with the ratio of intra/perisynaptic CB_1 receptors/bassoon-positive voxels, but not with extrasynaptic CB_1/bassoon ratio. The correlation was not observed in the presence of the CB_1 receptor inverse agonist AM251. There was also no correlation between intrasynaptic CB_1/bassoon ratio and phasic endocannabinoid signaling. These observations indicate that tonic cannabinoid signaling fine-tunes GABAergic neurotransmission primarily via the intra/perisynaptic CB_1 receptor pool.

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Poster

559. Presynaptic Organization

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Topic: B.05. Neurotransmitter Release

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TRR166 TPB06

Title: Large volume dSTORM imaging of presynaptic active zones

Authors: M. PAULI[1], M. M. PAUL[1], S. PROPPERT[1], F. REPP[1], P. KOLLMANNSBERGER[2], M. SAUER[3], M. HECKMANN[1], *A.-L. SIREN[4]
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Abstract: Active zones (AZs) are complex molecular machineries that mediate and regulate neurotransmitter release from presynaptic terminals. Among different neuronal tissues AZs display a large variety and variability in size ranging e.g. in mice from about 100 nm at endplates to several hundred nanometers in hippocampal mossy fiber boutons. The architecture of synaptic

sub-compartments has been revealed by EM with serial sectioning or array tomography studies. Superresolution light microscopic imaging of synaptic protein distributions has been restricted to cultured cells or to tissue sections less than a few micrometers from the coverslips. So far imaging of AZs in intact tissue blocks at nanoscopic resolution remained challenging.
Here we present a novel superresolution light microscopic method using 3D *direct* stochastical optical reconstruction microscopy (*d*STORM) that allows mapping AZs with molecular resolution in intact tissue blocks without mechanical or optical cutting.
We applied 3D *d*STORM with a custom-built microscope, a high NA water immersion lens and optimized staining procedures in standardized sections to map protein distributions. In up to 25 µm thick cryosections we recorded *en bloc* thousands of neuronal sub-compartments aberration-free in volumes up to 28 x 30 x 9.5 μm^3. Using highly specific anti-Bassoon antibodies we measured protein clusters with distinct size, number and density in mouse hippocampus. Sequential imaging with the fluorophore Alexa 647 in brain sections of Thy1-mEGFP (Ls1) mice identified a total of 8826 Bassoon clusters in the imaged tissue volume of 5701 μm^3; 185 clusters could be localized in 8 identified CA3-mossy fiber boutons. The volume (0.0102 ± 0.0204 μm^3) of Bassoon clusters in individual mossy fiber boutons was larger than the volume (0.0058 ± 0.0152 μm^3) of all Bassoon clusters recorded in the imaged tissue volume.
3D single-molecule localization microscopy using *d*STORM with far red fluorophores opens new possibilities for quantitative tissue imaging at the molecular level. Because imaging can be assumed to be free of depth-dependent spherical aberrations, it is feasible to stack through a complete presynaptic terminal, count the number of AZs, and map their architecture with molecular resolution.

Disclosures: M. Pauli: None. **M.M. Paul:** None. **S. Proppert:** None. **F. Repp:** None. **P. Kollmannsberger:** None. **M. Sauer:** None. **M. Heckmann:** None. **A. Siren:** None.

Poster

559. Presynaptic Organization

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 559.17/E45

Topic: B.05. Neurotransmitter Release

Support: NSF Grant 1346826

Title: Characterization of the role of *Drosophila melanogaster* Vwa8 in the regulation of synaptic growth and transmission

Authors: *D. BEELER[1], F. L. LIEBL[2], J. E. RICHMOND[3], D. E. FEATHERSTONE[4]
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Abstract: Proper function of excitatory synapses is necessary for learning and memory formation and misregulation of these synapses is common in several neurological disorders. Thus, understanding the mechanisms that regulate excitatory synaptic function is critical. Using the neuromuscular junction (NMJ) of *Drosophila* as a model excitatory synapse, a novel, highly conserved protein, VWA8, was identified as a regulator of synaptic growth and transmission. VWA8 is the founding member of a novel protein family, most closely related to midasins and dyneins, and contains three ATPase domains and a von Willebrand factor A domain. SNPs in the human homolog of this gene have been linked to bipolar disorder, autism spectrum disorders, and migrane disorders through genome-wide human disease association studies. To further characterize this gene a *Vwa8* null mutant was generated using CRISPR genome editing. Additionally UAS-VWA8 flies were made for studying the effects of targeted overexpression of VWA8. Analysis of VWA8 expression in *Drosophila* third instar larvae determined the presence of protein presynaptically at the NMJ and in the larval brain. Electrophysiology studies of *Vwa8* null mutants display a decreased evoked amplitude and quantal content as well as increased mini frequency, suggesting a presynaptic deficit. Additionally, locomoter studies revealed *Vwa8* null mutant larvae crawl more slowly than controls, travel less distance, and have a higher angular velocity. Current results have shown that both the knockout and overexpression of *Vwa8* show a synaptic overgrowth phenotype. An increased number of both synaptic and ghost boutons is observed at the mutant NMJs. Continuing studies will examine the affects of VWA8 reduction and overexpression on presynaptic vesicle release proteins and further explore interacting partners.

Disclosures: D. Beeler: None. **F.L. Liebl:** None. **J.E. Richmond:** None. **D.E. Featherstone:** None.

Poster

559. Presynaptic Organization

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 559.18/E46

Topic: B.05. Neurotransmitter Release

Title: Analysing the 3D nanotopolgy of active zones at the *Drosophila* neuromuscular junction

Authors: *N. EHMANN[1], J. SCHERBEL[2], M. PAULI[2], P. KOLLMANNSBERGER[3], F. REPP[2], S. PROPPERT[2], M. M. PAUL[2], R. J. KITTEL[1], A.-L. SIRÉN[4], M. HECKMANN[2]
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Abstract: Communication between neural cells relies on accurate neurotransmitter release at special sites termed active zones (AZs). Here, tightly packed proteins provide the platform for the spatially and temporally precise fusion of transmitter-laden synaptic vesicles (SVs) with the

presynaptic membrane. Bruchpilot (Brp) is an integral component of *Drosophila* AZs that helps to tether SVs to the AZ cytomatrix and cluster AZ calcium channels [1–3]. Using super-resolution microscopy, previous work has contributed to resolve Brp's elongated conformation at the AZ and to clarify how its copy number and spatial arrangement impacts synaptic short-term plasticity [2–5]. Ehmann et al. (2014) studied the 2D nanotopolgy of Brp in wildtype and brp^{nude}. Lacking merely the last 1 % of the C-terminal amino acids (17 of 1740) of Brp, these mutants show altered SV tethering and enhanced synaptic depression. To gain further clarification on structure, function and diversification of the Brp protein we now compare wildtype and brp^{nude} in 3D.

To this end an approach was established to maintain the 3D organisation of AZs within their native environment. Employing *d*STORM [*direct* stochastic optical reconstruction microscopy; [6]], we were able to reconstruct super-resolved 3D images up to 10 µm depth that cover whole individual boutons.

By visualising the localizations, molecules were grouped into clusters and assigned to specific structures based on their spatial arrangement. To analyse larger fields of view, which can easily contain millions of localizations, automated clustering methods are used to perform detailed analyses of Brp at different levels of organisation.

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Poster

559. Presynaptic Organization

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 559.19/E47

Topic: B.05. Neurotransmitter Release

Support: NIH Grant R01NS078214
NIH Grant R01AG051470
K-INBRE postdoctoral award P20 GM103418
MEXT Japan

Title: Super-resolution microscopy analysis of neuromuscular junction reveals degeneration of active zones in ALS model mice

Authors: Y. BADAWI[1], K. SHIGEMOTO[2], *H. NISHIMUNE[1]
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Abstract: Presynaptic active zones play an essential role as synaptic vesicle release sites for synaptic transmission. We used stimulated emission depletion (STED) microscopy to reveal the molecular architecture of active zones at sub-diffraction limited resolution. We identified an unexpected finding of non-overlapping localization of the active zone proteins Bassoon and Piccolo in mouse neuromuscular junctions (NMJs). Piccolo puncta sandwiched a Bassoon punctum in a side-by-side pattern, which could not be resolved using conventional confocal microscopy. P/Q-type voltage-gated calcium channel (VGCC) puncta colocalized with Bassoon puncta. We aimed to reveal the distribution patterns of additional key active zone proteins in mouse NMJs using STED nanoscopy. We demonstrate that Munc13 and RIM2 active zone proteins distribute in discrete, punctate patterns at the active zone units formed by Bassoon and Piccolo in NMJs of adult wild-type mice. Based on the knowledge obtained from wild-type NMJs, we also aimed to elucidate how active zone proteins are altered in amyotrophic lateral sclerosis (ALS). ALS is a neurodegenerative disorder in which denervation occurs before the death of neuronal cell bodies in the spinal cord, suggesting a “dying-back” neuropathy. The mechanisms underlying NMJ denervation in ALS remain unknown. Changes in protein levels, which are important for the maintenance of NMJ active zones and regulation of neurotransmission may play a role in the pathogenesis of ALS. For this purpose, we analyzed active zone proteins in NMJs of a rodent ALS model $SOD1^{G93A}$ mice at an early, pre-symptomatic stage (P85) and a symptomatic stage (P140). Interestingly, we found that the quantity of active zone proteins Bassoon, Piccolo and PQ- type VGCC decreased in innervated NMJs of ALS mice. Bassoon and PQ-VGCC puncta intensity and density decreased in NMJs of P85 $SOD1^{G93A}$ mice compared to age- and sex-matched wild-type mice. This decrease became more significant as the disease progressed to the P140 symptomatic stage. Decreases in Piccolo quantity in active zones became significant at P140 in $SOD1^{G93A}$ mice NMJs. Impairments in presynaptic function are likely to contribute to NMJ denervation. In summary, this study revealed the distribution of previously unresolved active zone proteins in wild-type mice and described the progressive degeneration mechanism of active zone proteins in NMJs of $SOD1^{G93A}$ mice.

Disclosures: Y. Badawi: None. **K. Shigemoto:** None. **H. Nishimune:** None.

Poster

559. Presynaptic Organization

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 559.20/E48

Topic: B.05. Neurotransmitter Release

Support: NIH Grant 1F31 NS100488-01

Title: Complexin regulates spontaneous synaptic transmission in a sub-population of active zones

Authors: *H. R. ASTACIO[1], A. VASIN[2], M. BYKHOVSKAIA[2]
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Abstract: Neuronal transmitters are released via the fusion of synaptic vesicles with the neuronal plasma membrane at specialized sites termed active zones (AZs). Vesicle fusion can occur in response to action potentials or spontaneously, and the latter component is important for neuronal development and homeostasis. We took advantage of the transgenic *Drosophila* line expressing the Ca^{2+} - sensitive fluorescent marker GCaMP5 tagged to the postsynaptic reticulum to investigate spontaneous fusion at individual AZs at the larval neuromuscular junction (NMJ). We were able to detect optical events corresponding to spontaneous vesicle fusions at individual AZs at a spatial resolution of 0.2-0.3 μm. Statistical analysis enabled us to subdivide the entire AZ ensemble into two distinct sub-populations: low activity AZs (LAZs) which obeyed the Poissonian statistics and constituted over 90% of the entire AZ population, and a small sub-population of AZs with the activity which was higher by an order of magnitude (HAZs). Since spontaneous transmission in *Drosophila* is drastically elevated by the deletion of a protein complexin (*cpx*), which was implicated to serve as a fusion clamp, we asked whether all the AZs are equally sensitive to *cpx* deletion. To address this question, we generated a heterozygous $cpx^{+/-}$ line postsynaptically expressing the GCaMP5 sensor. The overall spontaneous activity in this line was approximately twice higher than in the control line. Strikingly, we found that only the activity of HAZs was selectively increased at the $cpx^{+/-}$ NMJ, while the activity of LAZs remained unchanged. This result suggests that spontaneous synaptic transmission is controlled by a heterogeneous ensemble of AZs, with a smaller population being regulated by *cpx* and having prominent activity, and a larger population representing a Poissonian ensemble, which is not sensitive to *cpx*.

Disclosures: H.R. Astacio: None. **A. Vasin:** None. **M. Bykhovskaia:** None.

Poster

560. Mechanisms of Seizure Generation and Epilepsy

Location: SDCC Halls B-H

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Program #/Poster #: 560.01/E49

Topic: B.10. Epilepsy

Support: NIH/NINDS (Y1-O6-9613-01)
USAMRICD (A120-B.P2009-2)

Title: Automated electroencephalogram analysis for identifying and measuring spontaneous recurrent seizures and epileptiform activity following soman exposure in mice

Authors: *P. DUBEE, C. E. ARDINGER, P. M. BODNER, E. N. DUNN, M. R. EISEN, K. M. HAINES, D. L. NGUYEN, K. T. PAGARIGAN, A. N. SANTORO, H. S. MCCARREN, P. M. MCNUTT
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Abstract: Exposure to organophosphorus nerve agents like soman (GD) can elicit severe neurological responses, including initial *status epilepticus* (SE) that progresses to persistent epileptiform activity and latent spontaneous recurrent seizures (SRS). Many studies have examined the progression and treatment of acute nerve agent-induced SE, but ensuing chronic neurological abnormalities are poorly understood. Our lab has begun a study to evaluate the therapeutic effectiveness of clinically approved antiepileptic drugs in treating SRS in adult male C57BL/6 mice following GD exposure. This study utilizes long-term cortical electroencephalogram (EEG) recordings to characterize SRS in adult male mice following GD-induced SE.
Twenty C57Bl/6J male mice, age 12-14 weeks, were used in this study. Three weeks prior to GD exposure, mice were implanted with telemetry transmitters to record EEG activity. The mice were then exposed to GD and given a drug regimen and supportive care to promote survival for ~42 d. The mice were observed for onset of convulsions, and the EEG was monitored for SE onset.
Custom automated analysis was developed using Neuroscore and verified against trained manual scoring, and used to quantify the duration and average spike rate of abnormal neurological manifestations. Two principal events were identified: SRS, defined as spiking with a rate greater than 5 Hz for at least 10 s; and low-frequency epileptiform events (LFE), defined as at least 10 spikes occurring with 2-59 s between spikes.
Ten of twenty GD-exposed mice survived the study. Each demonstrated both SRS and LFE, with large inter- and intra-animal variability in the number and duration of each event. Seven mice showed significant increases in SRS duration over time, and two showed significant changes in SRS spike rates. The longest LFE lasted for 10 d, though analysis of cumulative LFE activity is

still ongoing. Sex differences were not assessed. These data suggest that abnormal neurological effects arise shortly after acute SE and can persist for months. Automated characterization of SRS and LFEs is currently being used to evaluate the efficacy of FDA-approved antiepileptic drugs in mitigating chronic neurological symptoms in GD-exposed mice.

Disclosures: P. Dubee: None. **C.E. Ardinger:** None. **P.M. Bodner:** None. **E.N. Dunn:** None. **M.R. Eisen:** None. **K.M. Haines:** None. **D.L. Nguyen:** None. **K.T. Pagarigan:** None. **A.N. Santoro:** None. **H.S. McCarren:** None. **P.M. McNutt:** None.

Poster

560. Mechanisms of Seizure Generation and Epilepsy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.02/E50

Topic: B.10. Epilepsy

Support: NIH Grant RO1 NS075366
DOD Grant PR161864

Title: A predictive epilepsy index based on probabilistic classification of interictal spike waveforms

Authors: J. A. PFAMMATTER[1], *R. A. BERGSTROM[3], E. P. WALLACE[1], R. K. MAGANTI[2], M. V. JONES[1]
[1]Dept. of Neurosci., [2]Dept. of Neurol., Univ. of Wisconsin, Madison, WI; [3]Biol., Beloit Col., Beloit, WI

Abstract: Automated algorithms for the analysis of interictal spikes (IISs) aim to standardize and speed detection. Still, wide-scale application of these algorithms is limited by lack of a universal definition of spikes, thereby potentially biasing each algorithm based on assumptions about what constitutes a spike. Ideally, algorithms should be fast, bias-free and reveal aspects of IISs that are predictive and guide mechanistic studies. Here, we developed a principal components (PC)-based algorithm that is agnostic in classification of events, provided that they 'stand out' from the background. We applied the algorithm to EEG records from mice treated with saline (SA, i.p., n=7) or with an epileptogenic stimulus (KA, kainate i.p., n=15 mice). First, we detected events using a two-threshold-crossing method to define event start and end. Detected waveforms were projected onto the first three PCs and clusters of spike morphologies were identified by a Gaussian mixture model. Probability scores were assigned to clusters based on the odds-ratio of events from KA versus SA within each cluster. Some spike morphologies were more frequent in KA, whereas others occurred in both groups. We created a novel index by assigning each event its probability score, summing these values and dividing by the record duration to yield "equivalent epileptic spikes per second". This index predicted whether an

animal received an epileptogenic treatment (i.e., KA) even if a convulsive seizure was never observed. We used this method to define and track different spike morphologies in five KA animals monitored for ~1 month. The magnitude of the predictive index increased over time in a subset of animals and revealed longitudinal changes in the prevalence of spikes with specific morphologies. Importantly, in both the longitudinal data and in our development data, the three animals that had convulsive seizures also had a relatively high predictive index. This analysis is fast (i.e., minutes per 24-hour EEG record), unbiased and provides information regarding the salience of different spike morphologies for disease progression. Future refinement will allow a better understanding of how exactly interictal spikes should be defined in quantitative and unambiguous terms.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.03/E51

Topic: B.10. Epilepsy

Support: T32MH020017-19
T32GM007753-37
Harvard MSTP Fellowship
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Bertarelli Fellowship

Title: A highly sensitive and specific generalized linear model for seizure detection using a rat model of epilepsy

Authors: *S. EBRAHIM[1], N. F. FUMEAUX[2], A. KADAMBI[3], M. F. MORAES[5], E. Y. KIMCHI[4], M. ABOU JAOUDE[3], S. B. NAGARAJ[3], S. ARROYO[3], S. S. CASH[6]
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Abstract: Objective: Rodent models of epilepsy are indispensable for probing disease circuits and testing novel therapies due to their genetic and structural similarities to the human brain. However, chronic epilepsy models utilize prolonged EEG recordings that generate substantial amounts of data, resulting in a time-intensive manual labelling process. Previously published automated detectors yield a prohibitively low positive predictive values (PPV), with a false discovery rate (FDR) on the order of 0.5/hour. To address this challenge, we introduce a

generalized seizure detection model for automated evaluation of large EEG data sets.
Methods: Young male SD rats (2-3 mo, n = 12) were implanted with surface electrodes, EMG pads and intrahippocampal depth electrodes bilaterally. Unilateral intrahippocampal injections of kainic acid were administered to induce epilepsy, while video and EEG recordings were recorded continuously for 3 months. Three-channel EEG data was analyzed by computing standardized features in time, frequency, and synchronization domains for 5-second windows, and seizure segments were also manually labelled by an expert. PCA was used for dimensionality reduction and maximally discriminating features identified by computing Fisher scores. Generalized linear classifiers were built with lasso regularization using these features to classify seizures versus interictal EEG segments.
Results: The generalized and individualized classifiers all achieved an AUROC > 0.99 on test data, and at a threshold of 0.1, the classifier had a sensitivity of 0.99, specificity of 0.83 and an overall PPV of 0.37 with an FDR of 0.08/hour. The mean AUROC of our leave-one-out general classifier, in which no data from the test subject was included in training, was 0.88. Our PCA visualizations reflect the separability of the features along the axes constructed.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

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Program #/Poster #: 560.04/F1

Topic: B.10. Epilepsy

Support: This research was supported by a CounterACT inter-agency agreement between NIH/NINDS (Y1-O6-9613-01) and USAMRICD (A120-B.P2009-2).
This research was supported in part by appointments to the Postgraduate Research Participation Program at the U.S. Army Medical Research Institute of Chemical Defense administered by the Oak Ridge Institute for Science and Education.

Title: Optimizing a mouse model of severe nerve agent intoxication for long-term survivability, incidence of neuropathology, and emergence of spontaneous recurrent seizures

Authors: *H. S. MCCARREN, C. ARDINGER, P. BODNER, P. DUBEE, E. N. DUNN, M. R. EISEN, K. M. HAINES, D. L. NGUYEN, A. SANTORO, P. M. MCNUTT
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Abstract: Recently, nerve agents have been used in both widespread and targeted attacks on individuals. These deadly chemicals inhibit acetylcholinesterase, leading to signs of cholinergic

crisis such as excessive secretions, convulsions, and seizures that progress to generalized status epilepticus (SE). Research into nerve agent antidotes has largely focused on the acute phase of intoxication, but latent neurological defects resulting from brain damage sustained during SE can follow. The goal of this study was to optimize a mouse model of SE induced by the nerve agent soman (GD) for long-term survivability and development of neuropathology. Male C57Bl/6J mice (n=80) received 50 mg/kg HI-6, followed five minutes later by 172 µg/kg GD. Animals were assigned to one of five experimental paradigms: saline (SAL) one minute post-GD, 2 mg/kg atropine methyl nitrate (AMN) one minute post-GD, or 2 mg/kg AMN one minute post-GD followed by 5 mg/kg diazepam (DZP) at 30, 60, or 120 minutes post-onset of convulsions. Survival, weight, and nesting behavior were observed for 14 days, followed by H&E neuropathological scoring. Animals in the AMN group displayed the highest survival rate (81% survival), while other groups ranged from 56-63% survival. All groups except the SAL group returned to baseline body weight by the end of the experiment, but required supplemental care. There was an inverse relationship between nesting and severity of neuropathology. The AMN group displayed the highest incidence of neuropathology (76%), followed by the SAL and 120 min DZP groups (70% and 67%). Given the high survival rate and high incidence of neuropathology, the AMN paradigm was then applied to mice that had been implanted with EEG telemetry units (DSI, ETA-F10), with the ultimate goal of observing and characterizing spontaneous recurrent seizures (SRS) in these animals. Survival was substantially poorer in implanted animals, with only 40% survival at Day 14 and 13% survival at Day 28. Alternately, implanted animals that received DZP 120 minutes after SE onset had a Day 14 survival rate of 65% and a Day 28 rate of 55%, which was similar to that in non-implanted animals. All animals in both groups that survived to the end of the study demonstrated SRS, and the incidence of pathology among survivors was 100% for AMN and 72% for 120 minute DZP. Thus, continued efforts will use the 120 min DZP paradigm to characterize SRS after GD-induced SE and test the efficacy of FDA-approved antiepileptic drugs in preventing and treating them.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.05/F2

Topic: B.10. Epilepsy

Title: Electrographic changes in the brain after trauma in wild-type and aquaporin-4 knockout mice

Authors: *J. SZU, D. PATEL, C. JONAK, S. CHATURVEDI, J. LOVELACE, D. BINDER
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Abstract: Posttraumatic epilepsy (PTE) refers to the development of recurrent spontaneous seizures after a traumatic brain injury (TBI). Current models of PTE have focused on testing seizure susceptibility pharmacologically and injured animals were shown to be more predisposed to generalized seizures. These studies, however, lack chronic detailed EEG analysis. Here, we aim to detect electrographic changes in the posttraumatic brain in wildtype (WT) and aquaporin-4 knockout (AQP4 KO) mice to determine the role of AQP4 in the development of PTE.
TBI was induced in the right frontal cortex using controlled cortical impact (CCI) injury device. Sham animals received a craniotomy only and naïve mice did not receive an injury or a craniotomy. 10 days before each experimental endpoint (14, 30, 60, and 90 days) mice were implanted with an indwelling electrode in their ipsilateral hippocampus. After 3 days of recovery, mice underwent continuous video-EEG recording to monitor for spontaneous seizures and *in vivo* electrical intrahippocampal stimulation was performed for the quantitative assessment of electrographic seizure threshold (EST) and duration (ESD) at each experimental endpoint.
Spontaneous non-convulsive seizures were observed in injured animals only. A significant increase in delta, theta, and alpha powers were observed 14 days after TBI in WT mice and a significant increase in delta and theta powers were observed 14 days after TBI in AQP4 KO mice. Electrical stimulation revealed a significant increase in ESD in AQP4 KO mice compared with WT mice in both sham and TBI groups.
Our data suggest that AQP4 plays a critical role in epileptogenesis after TBI. The increase in EEG power at 14 days after TBI in both genotypes suggests that mice may be more excitable at this time point after injury. Additionally, increased ESD after injury alludes to impaired water homeostasis which prolongs seizure activity suggesting impaired seizure termination in AQP4 KO mice. Histological studies for AQP4 and Kir4.1, astrocyte molecules known to modulate excitability, are also performed to correlate protein expression levels with electrographic changes at each time point. Furthermore, studies utilizing a 30-channel multi electrode array (MEA) after TBI are currently underway to localize seizure onset, propagation, and termination.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.06/F3

Topic: B.10. Epilepsy

Support: NSERC

Title: Kindled seizures accelerate forgetting of previous context fear memory

Authors: L. E. BRANDT, C. COLE, A. KALININA, H. LEHMANN, *N. M. FOURNIER
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Abstract: Epilepsy is associated with a range of cognitive impairments, includes those related to memory. One type of memory deficit that has received increasing attention is accelerated forgetting, which is characterized by impaired recall of newly acquired information over long-delays (e.g., days, weeks) despite normal recall following short delays. Despite the prevalence of memory retention deficits in patients with epilepsy, the neurobiological mechanisms contributing to these problems remain obscure. There is strong evidence that high rates of neurogenesis can promote forgetting of hippocampal-dependent memories acquired at earlier time points. One explanation for this phenomena is that the continual maturation and synaptic integration of new neurons remodel hippocampal circuits thereby reducing the probability that information stored in these circuits can be easily accessed. Several studies have shown that epileptic seizures dramatically increase rates of neurogenesis in the rat hippocampus. A significant proportion of seizure generated neurons develop and function abnormally thereby contributing to the formation of faulty circuits that promote hyperexcitability and interfere with hippocampal function. Based on these observations, we hypothesize that seizure-induced neurogenesis promotes aberrant remodeling of hippocampal circuits that can interfere with the retrieval of previously acquired memories. To test this possibility, rats were trained in a contextual fear memory task. Following training, one group of rats underwent chemical kindling for 2-weeks with the chemoconvulsant pentylenetetrazole (PTZ). PTZ kindled rats showed significantly less freezing compared to saline-treated controls when re-exposed to the training context 4 days after their last seizure suggesting that seizures induced forgetting of the fear memory. Importantly, PTZ seizures did not induce markers of neuronal degeneration or gliosis in the hippocampus, but increased immature neuronal (DCX) and proliferation (Ki67) markers. Similar behavioural findings were observed with a low dose treatment with kainic acid. These experiments offer support that aberrant remodelling of hippocampal circuits can interfere with the retrieval of previously acquired fear memory. <!--EndFragment-->

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.07/F4

Topic: B.10. Epilepsy

Support: NHMRC Project Grant (1065638)

Title: Tracking brain state changes during epileptogenesis with seizure dynamics and probing

Authors: *D. N. CRISP[1], W. CHEUNG[3], A. LAI[4], D. R. FREESTONE[4], D. B. GRAYDEN[3,4,5], M. J. COOK[4], W. C. STACEY[1,2]
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Abstract: Our recent work has demonstrated that seizures can be classified according to the dynamics of their onset transitions (Saggio & Crisp et al., Under Review). However, the direct practicality of this taxonomy is still being explored. One analytical approach, analyzing evoked responses from periodic electrical probing, enables the collection of more robust features to assist in brain state tracking and seizure prediction frameworks (Freestone et al. 2011). Our objective is to begin delineating the potential clinical impact of this seizure dynamics taxonomy by pairing its seizure onset classifications with independent evoked response analysis. This was performed by analyzing seizure onsets and evoked responses from periodically perturbed, long-term (~10 weeks), DC coupled, epidural EEG recordings from an intrahippocampal tetanus toxin rat model of temporal lobe epilepsy. This model produces frequent, stereotyped, electro-clinical events that spontaneously remit after some weeks. Six rats were studied, each of which exhibited thousands of seizures, providing ample data for robust analysis. To track the evolution of epileptogenesis, we also investigated cumulative seizure duration as a function of time since toxin administration. In all cases, the distribution of cumulative seizure duration was sigmoidal in shape, characterized by an initial period of increasing seizure frequency, an inflection point, and finally leveling out as seizure freedom was achieved. In all six rats, seizure dynamics and features of the evoked responses underwent major alterations at the inflection point (~week 3), demonstrating correlation between the underlying seizure dynamics and the system's response to perturbing stimuli. This suggests that these methods may be uncovering novel biomarkers of ictogenesis, and could lead to further insights into underlying mechanisms.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.08/F5

Topic: B.10. Epilepsy

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Title: Glucose metabolism but not neuroinflammation during epileptogenesis correlates with chronic seizure outcome in a rat model of temporal lobe epilepsy

Authors: *I. JAHREIS[1,2], P. BASCUÑANA[1], A. POLYAK[1], T. L. ROSS[1], W. LÖSCHER[2], F. M. BENGEL[1], J. P. BANKSTAHL[1], M. BANKSTAHL[2]
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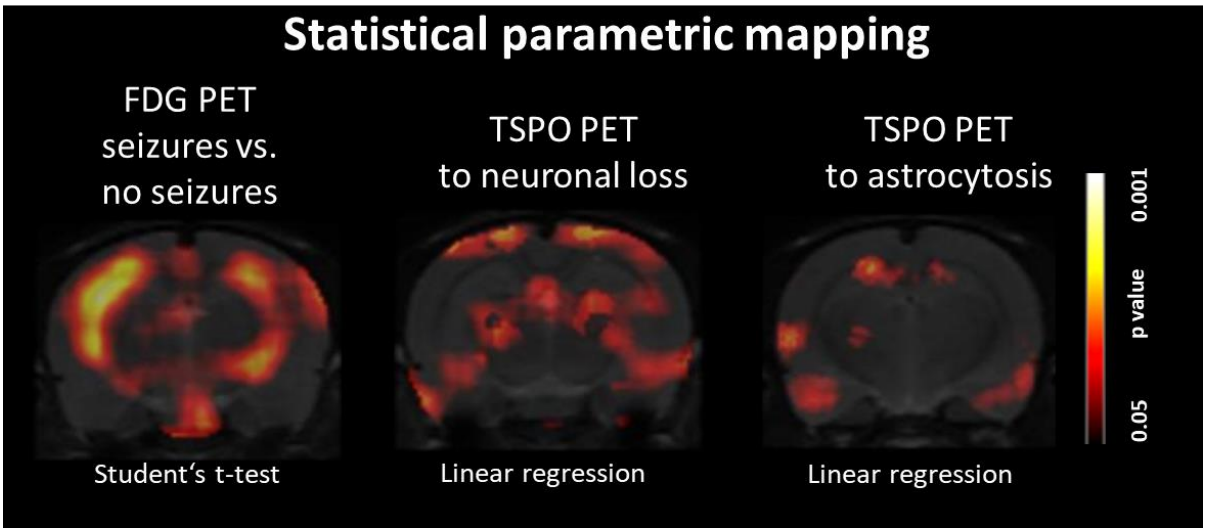
Abstract: The lithium-pilocarpine rat model is well-established to evaluate insult-induced epileptogenesis. Effective multi-target termination of status epilepticus (SE) results only in a proportion of chronically epileptic rats. Here, we aimed to evaluate the value of positron emission tomography (PET) imaging for prediction of epilepsy after SE.
SE was terminated by a combination of diazepam, phenobarbital, and scopolamine. Female Sprague-Dawley rats (22 post SE, 6 sham) underwent PET scans with the glucose analogue ^{18}F-fluordeoxyglucose (FDG) and the TSPO ligand ^{18}F-GE180 targeting neuroinflammation at 5d and 12-14d post SE, respectively. Video/EEG seizure monitoring was performed during weeks 22-24 after SE. Regional neuronal loss or astrocytosis were assessed by histological analysis. Potential correlations were evaluated using statistical parametric mapping (SPM) analysis.
Compared to sham, post-SE rats showed decreased FDG uptake (-9%, $p=0.033$) and increased ^{18}F-GE180 volume of distribution (V_t ,+37%, $p=0.046$) in the hippocampus. Lower hilar neuronal density (-25%, $p=0.001$) and mild astroglial activation were found 26 weeks post SE. Chronic epilepsy was present in 72% of the rats. SPM (Student's t-test, $p<0.05$) indicated a decrease in hippocampal FDG uptake in non-epileptic vs. epileptic animals. SPM did not reveal differences in TSPO V_t. Additionally, SPM (linear regression, $p<0.05$) demonstrated a correlation between chronic hippocampal neuronal loss and higher ^{18}F-GE180 signal during epileptogenesis in multiple epilepsy-associated brain regions, but not the hippocampus. By contrast, chronic astrogliosis correlated with early TSPO expression mainly in the hippocampus and piriform cortex.
Although glucose hypometabolism discriminates epileptic from non-epileptic rats, it may not be suitable as a clinical predictive biomarker since epileptic animals did not deviate from controls. Early inflammation is associated with reduced neuronal density and scar formation but is not correlated to seizure development.



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Poster

560. Mechanisms of Seizure Generation and Epilepsy

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Program #/Poster #: 560.09/F6

Topic: B.10. Epilepsy

Support: R01-NS089698

Title: The possible role of spontaneous seizures on epileptogenesis

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Abstract: Spontaneous recurrent seizures (SRS) are characteristic of epilepsy. Temporal lobe epilepsy (TLE) is a subtype of acquired epilepsy that might develop after stroke, traumatic brain injury or *status epilepticus*. Current hypotheses propose that a brain injury can trigger the transformation of a normal brain into an epileptic one in a complex process known as epileptogenesis. A less explored alternative is that SRS themselves might promote epileptogenesis. Pathologic activation of calpain, a calcium dependent protease ubiquitously expressed in neurons, has been observed in epilepsy. Calpain activation after a brain injury triggers a series of neurotoxic signaling cascades that results on the cleavage of proteins that negatively affect neuronal function and promote neuronal death. Here, we investigated if calpain activation can be detected after occurrence of SRS and if recurrent calpain activation might

contribute to epileptogenesis. To investigate if SRS themselves promote calpain activation and to evaluate if calpain activation exacerbates cellular and molecular abnormalities typically found in epileptic tissue, we used continuous EEG/video to monitor occurrence of seizures and evaluated if calpain activation can be detected in rats enduring SRS. We were able to detect both calpain activation and the loss of proteins required to maintain inhibitory drive. These studies aim to uncover the molecular mechanisms promoting occurrence of SRS and the possible role of SRS on epileptogenesis.

Disclosures: P.M. Lam: None. **M.I. Gonzalez:** None.

Poster

560. Mechanisms of Seizure Generation and Epilepsy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.10/F7

Topic: B.10. Epilepsy

Title: Involvements of tryptophan metabolism in the pathogenesis of epilepsy

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Abstract: Objective: Despite a long history of nonclinical and clinical research on the significance of tryptophan metabolism in epilepsy, the mechanistic implication of this amino acid in the pathogenesis of epilepsy is still unclear. In this study, we assessed tryptophan metabolisms in EL mice modeling genetic epilepsy by in vivo positron emission tomography (PET) with a radiolabeled tryptophan analog and mass spectrometry of brain tissues to pursue the kinetics and metabolism of tryptophan. Mechanistic associations of tryptophan with the epileptogenesis were also examined by treating these mice with tryptophan. **Method:** Male EL and age-matched control ddY mice were used. PET scans with [^{11}C]1-methyl-L-tryptophan([^{11}C]1-MT) were conducted in EL mice (n=6) in an interictal state and control ddY mice (n=10) during the period from 5 weeks (prior to the seizure onset) to 6 months (long after the seizure onset) of age. We also preformed mass spectrometry to measure concentrations of tryptophan, serotonin and related metabolites in the hippocampus of EL and ddY mice at 9 weeks of age antecedent to the seizure onset (n=8 in each group). Furthermore, we treated EL mice with either standard diet containing 0.1% tryptophan or diet containing 5% tryptophan from 5 weeks of age prior to the seizure onset. Epileptic seizures of EL mice were stimulated by tail suspension once a week, and we determined the latency to the first seizure and frequency of

seizures in these mice. **Results:** The uptake of [^{11}C]1-MT in the EL mouse brain significantly increased (by 70%) compared to control ddY mice from 5 weeks to 6 months of age. The content of tryptophan in the EL mouse hippocampus was significantly higher than the control level. Increased levels of serotonin and other related metabolites were also observed in EL mice. The diet containing 5% tryptophan induced seizures in EL mice at an earlier age than the standard diet. **Conclusion:** Our results indicate that an enhanced uptake of tryptophan in the brain provokes initiation of the epileptogenesis in EL mice. PET imaging with [^{11}C]1-MT potentially offers a means for diagnosing and subtyping epilepsy on a pathophysiological basis.

Disclosures: S. Hashimoto: None. **J. Maeda:** None. **Y. Takado:** None. **H. Takuwa:** None. **M. Shimojo:** None. **M. Takahashi:** None. **T. Urushihata:** None. **K. Kumata:** None. **Z. Ming-Rong:** None. **T. Suhara:** None. **M. Higuchi:** None.

Poster

560. Mechanisms of Seizure Generation and Epilepsy

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Program #/Poster #: 560.11/F8

Topic: B.10. Epilepsy

Support: NSERC

Title: Inhibition of seizure-induced hippocampal neurogenesis by the chemotherapy drug temozolomide rescue cognitive deficits after kindling

Authors: *T. J. FRANCIS, B. S. REIVE, K. BLEWETT, K. POST, J. REID, H. LEHMANN, N. M. FOURNIER
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Abstract: Cognitive impairments, such as memory loss, are a frequent and devastating co-morbidity associated with epilepsy. The neurobiological mechanisms through which recurrent seizures induce cognitive impairments are not well understood. Recent studies have shown that seizure activity stimulates the birth of new neurons in the adult hippocampus. Many of these new neurons develop abnormal morphological and functional characteristics that promote network hyperexcitability and hippocampal dysfunction. Previously, we found that kindling dramatically increases the rate of neurogenesis at early stages of seizure development, followed by a long-term suppression at later stages. These changes in the rate of cell proliferation coincides with aberrant modifications in the migration, excitability, and functional integration of these new neurons. It has been suggested that the long-term consequences of seizure-induced neurogenesis contributes to the development of cognitive impairment seen in chronic epilepsy. However, direct experimental evidence has been limited. To explore this question, we inhibited neurogenesis by administering the DNA-alkylating agent temozolomide (TMZ) in rats that

underwent long-term amygdala kindling. Kindled rats began treatment with TMZ (25 mg/kg, i.p.) after their 30th stimulation—a time point that corresponds with increased neurogenesis—and kindling proceed until 99 stimulations were delivered. We found that TMZ reversed seizure-induced deficits in contextual fear learning and context discrimination. In addition, we found that TMZ did not adversely impact exploratory behaviour, anxiety, and contextual discrimination learning in non-epileptic rats. Our findings suggest that suppressing neurogenesis improves memory impairments seen after kindling, and helps to further establish that targeting aberrant neurogenesis can serve as a novel approach for reducing cognitive deficits associated with epilepsy.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.12/F9

Topic: B.10. Epilepsy

Title: Diving responses elicited by nasopharyngeal irrigation compared to seizure-associated central apneic episodes in a rat model

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Abstract: Epileptic seizures spreading to respiratory and autonomic regions in the brainstem of rats can elicit episodes of obstructive apnea or central apnea. These episodes can be associated with significant oxygen desaturation (Nakase et al., Epilepsy Res. 128: 126-139, 2016). Obstructive apnea in these animals is due to intense adduction of laryngeal muscles as a result of seizure spread to the recurrent laryngeal nerve, a branch of the vagus nerve that controls both abduction and adduction of the vocal folds, and is lethal (ibidem). Central apneic events in the same animals, in contrast, are typically brief (1-30 seconds) and transient. Central apneic events are concomitant with (1) respiratory rhythm reset and (2) suppression of breathing behavior, which, we argued, was due to seizure spread into brainstem regions to activate the efferent limb of the diving reflex (Villiere et al., Neurobiol. Dis. 101: 8-15, 2017). In this study, we sought to explore the similarities of seizure-induced central apneic episodes to apneic episodes occurring as part of the diving reflex. We induced the diving response in urethane-anesthetized animals with or without kainic acid-induced seizure activity to (1) demonstrate diving-reflex properties in our rat model and (2) form a basis for comparison with spontaneous seizure-induced central

apneic events. Nasopharyngeal irrigation with cold water or mist elicited the typical diving response with apnea and significant bradycardia. When evoked during ongoing seizure activity, bradycardia was associated with decreased seizure activity. Repeated irrigations led to a dissociation of the apneic episodes (which always occurred) from the bradycardia (which became less pronounced with repetition). This dissociation of apnea from the cardiovascular components of the diving response supports the idea that seizure-associated central apnea and the diving response share a common neural basis. Further, the coupling or uncoupling of respiratory and cardiovascular elements of the diving response is dependent on the physiological state of the animal (e.g. levels of autonomic tone at baseline or set by ongoing seizure activity, recent history of similar episodes, etc.) at the onset of the diving response or a seizure-associated central apneic episode.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

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Topic: B.10. Epilepsy

Support: Natural Sciences and Engineering Research Councilof Canada (NSERC); Grant number: 222912 (to L.E.K.)
NSERC Canada Graduate Scholarship and a Savoy Foundation Stu-dentship (to J.J.B.).

Title: Calbindin immunoreactivity may be related to cognitive dysfunction in the epileptic brain

Authors: *L. E. KALYNCHUK[1], N. NOGOVITSYN[2], J. J. BOTTERILL[3], H. CARUNCHO[1]
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Abstract: Although temporal lobe epilepsy is primarily characterized by recurring seizure activity, it can also include profound interictal behavioral and cognitive comorbidities, such as anxiety, depression, and cognitive impairments. The neural mechanisms that contribute to these behavioral comorbidities are largely unknown. Interestingly, recent studies have suggested that alterations in expression of the calcium-binding protein calbindin within the dentate gyrus may be important for hippocampal plasticity and cognitive dysfunction. In this experiment, we used the kindling model of temporal lobe epilepsy to investigate the relationship between cognitive function and calbindin expression following repeated seizure activity. Kindling refers to the gradual development and intensification of elicited motor seizures resulting from electrical

stimulation of a discrete brain site. We kindled 25 male rats over a 6.5 week period, with a total of 99 stimulations delivered 3 times per day, 5 days per week. We delivered electric stimulations into two limbic brain regions — the basolateral amygdala and dorsal hippocampus-- and one non-limbic brain region - the caudate nucleus. Following kindling, rats were subjected to a trace fear conditioning paradigm to assess cognition. Rats were then sacrificed and their brains collected for immunohistochemical analyses of calbindin and Arc, which is an immediate early gene that signals neuronal activation. We found that kindling had no effect on the acquisition of hippocampal-dependent fear conditioning, but the amygdala- and hippocampal- kindled rats showed impaired retrieval of fear memories. This was accompanied by decreased Arc expression in both the subgranular zone/granule cell layer and hilus and reduced calbindin in the subgranular zone and granule cell layer. Our results provide the first demonstration of a relationship between kindling-induced cognitive deficits and reduced calbindin expression and suggest that novel therapeutics that normalize calbindin may be effective against the behavioral comorbidities associated with temporal lobe epilepsy.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

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Topic: B.10. Epilepsy

Support: NIH/NINDS 7R01NS036692-16
NIH/NINDS 7R01NS082851-04

Title: Brain-derived neurotrophic factor may contribute to ictogenesis in a mouse model of viral infection-induced temporal lobe epilepsy

Authors: *D. C. PATEL[1], E. G. THOMPSON[1], H. SONTHEIMER[2]
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Abstract: Epilepsy resulting from CNS infection is often refractory to established anti-seizure drugs. C57BL/6J mice infected with Theiler's murine encephalomyelitis virus (TMEV) show acute behavioral seizures between 3-7 days post-infection (dpi), exhibit clinically relevant pathological and physiological changes in the hippocampus, survive the infection and later develop chronic epilepsy after approximately two months of infection. Therefore, this is an appropriate model to study mechanism(s) underlying epileptogenesis for infection-induced limbic epilepsy. Numerous studies have implicated brain-derived neurotrophic factor (BDNF)

signaling in the development of epilepsy. BDNF is released from neurons in an activity-dependent manner, but reactive glia, which are prevalent in the TMEV-infected mice, can also contribute to its synthesis and release. In addition to its effects on the expression and cellular trafficking of excitatory and inhibitory receptors, BDNF may cause hyperexcitation by impairing chloride homeostasis and GABAergic inhibition by downregulating K^+-Cl^- cotransporter (KCC2). Therefore, we hypothesized that increased BDNF signaling impairs GABAergic inhibition via cation chloride cotransporters that may underlie network hyperexcitability and seizures following viral infection. Mice were injected with either TMEV or PBS (sham) and monitored for acute seizures. In one group of mice, the hippocampi were dissected at 1, 3, 5, 14 and 60 dpi (n=5-6) and the protein expression of BDNF was quantified by ELISA and that of KCC2, phosphorylated KCC2 and NKCC1 by gel electrophoresis and western blot. In a second group of mice, the membrane expression of KCC2 was measured with a cell surface biotinylation assay in acute hippocampal slices (n=5-6) at 5 dpi. The BDNF level in the hippocampus from TMEV-infected mice with seizures was increased at the onset of acute seizures compared to the sham group. BDNF continued to increase during the peak acute seizure, latent and chronic phases of epilepsy and more than doubled at 60 dpi. We found no change in the expression of NKCC1, whereas the expression of KCC2 and phosphorylated KCC2, as well as the surface level of KCC2, were significantly decreased at 5 dpi in the hippocampi from TMEV-infected mice. Therefore the ratio of NKCC1 to KCC2 was reduced, which may favor accumulation of chloride intracellularly and may contribute to hyperexcitability by reversing GABA-mediated inhibition. Further investigation will seek to probe the causal relationship between BDNF signaling and epileptogenesis following viral infection.

Disclosures: D.C. Patel: None. **E.G. Thompson:** None. **H. Sontheimer:** None.

Poster

560. Mechanisms of Seizure Generation and Epilepsy

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Program #/Poster #: 560.15/F12

Topic: B.10. Epilepsy

Support: Sylics BV
Dept Functional Genomics

Title: Epistatic interaction between Stxbp1 and Snap25 genes produces super-additive effects on synaptic transmission and epileptic seizures

Authors: *J. KOVACEVIC[1], K. D. B. WIERDA[2], J. B. SORENSEN[3], M. VERHAGE[1,4]
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Abstract: De novo, heterozygous mutations in *STXBP1/Munc18* and *SNAP25* genes cause largely overlapping symptoms: neurodevelopmental delay, intellectual disability and epilepsy. Because these two genes work together in neurosecretion, the pathogenic mechanisms for mutations may also be similar. We tested this hypothesis at the synaptic, system and behavioral level using single and double heterozygous *Stxbp1*$^{+/-}$ and *Snap25*$^{+/-}$ mice. Epistatic interactions were observed at the synaptic and system level, but not behavioral level: Patch clamp recordings of synaptic transmission in single neuron micro dot cultures revealed 70% reduced evoked and 80% reduced spontaneous excitatory synaptic transmission in *Stxbp1*$^{+/-}$*Snap25*$^{+/-}$ neurons, while synaptic transmission was (virtually) normal in single *Stxbp1*$^{+/-}$ and *Snap25*$^{+/-}$ neurons. *Stxbp1*$^{+/-}$ *Snap25*$^{+/-}$ mice showed a variety of behaviorally- and EEG- detected seizures, including clonic seizures and generalized epileptic attacks, that were not observed in *Stxbp1*$^{+/-}$and *Snap25*$^{+/-}$ mice. Excessive cortical cFos activity was detected in *Stxbp1*$^{+/-}$*Snap25*$^{+/-}$ mice and it was higher than previously detected cFos activity in *Stxbp1*$^{+/-}$ mice. At the behavioral level, *Stxbp1*$^{+/-}$*Snap25*$^{+/-}$ mice mimic phenotypes of single *Stxbp1*$^{+/-}$ mice. Double heterozygous mice showed impairment in several cognitive domains: impaired contextual and cued learning in fear conditioning and impaired behavioral flexibility. Additionally, *Stxbp1*$^{+/-}$*Snap25*$^{+/-}$ mice showed lower spontaneous activity during the light phase in the home cage and anxiety-related behavior in the elevated plus maze test. All these behavioral phenotypes were similar to single *Stxbp1*$^{+/-}$ mice and were not observed in single *Snap25*$^{+/-}$ mice. Taken together, these results provide clear evidence for epistatic interactions between *Stxbp1* and *Snap25* genes, at least at the synaptic and systems level, suggesting that the pathogenic mechanisms for patients carrying mutations in these genes have shared, but also unique features. This presynaptic gene set, not only *Stxbp1* and *Snap25* but also *Stx1b* and *Syt1*, may be considered together for future treatment design.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.16/F13

Topic: B.10. Epilepsy

Support: IBRO

Title: Focal motor seizure model in mouse

Authors: *T. SINGH, A. BRODOVSKAYA, J. M. WILLIAMSON, J. KAPUR
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Abstract: Focal motor can cause injury and secondarily generalized tonic clonic seizures are associated with sudden unexpected death in epilepsy (SUDEP). However circuits underlying these seizure have not been delineated. In order to get insight into neuronal circuits and plasticity underlying focal motor seizures, we translated cobalt model of focal motor seizures from rats to mice. We implanted cobalt wire in supplementary motor (M2) area of the right prefrontal cortex (AP, 2.6 mm; ML, 1.8 mm) of mice. Four doses were administered 0.44 mg, 0.66 mg, 0.88 mg, and 1.75 mg. Animals were monitored by video and EEG, 30 min after the surgery continuously for 7 days. Seizure frequency, latency to first and last seizure, and total number were characterized. After 7 days, animals were sacrificed, and brains were sectioned and processed for nissl staining to evaluate the lesion size. Also, to understand layer specific activation of neurons in the cortex due to cobalt induced seizures two kinds of transgenic mice were used. Transgenic mice that express EGFP under the control of tetracycline repressor and early immediate gene, cfos (Mayford) were maintained on high doxycycline diet until 24 hours prior cobalt implantation. We also used TRAP (targeted recombination in activated population of neurons) mice. Immunohistochemistry was performed on the brain sections with different cortical layer-specific antibodies (Brn2, Tbr1, and Ctip2) in transgenic mice after peak occurrence of seizures. All doses triggered motor seizures, which were first observed within 8 to 12 h; the peak seizure frequency was at 24 h (0.44 mg, n=8), 28 h (0.66 mg, n=13), 36 h (0.88 mg, n=12), and 24h (1.75 mg, n=12). Seizures dissipated by 72 h in all animals. Total seizures increased as a function of Cobalt dose. Mean seizure ranged from 3.9±0.29 (0.44 mg, n=8) to 13.91±2.22 for four doses. Histological examination also depicted increased lesion size with increased cobalt dose. Initial studies of transgenic mice indicate activation of principally layers II/III and also of V/VI in the motor and somatosensory cortices. Further studies to map the seizure propagation employing combination of imaging and electrophysiology to elucidate the cortical seizure spread pathway are in progress.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.17/F14

Topic: B.10. Epilepsy

Title: Pharmacological augmentation of on-demand 2-arachidonoylglycerol (2-AG) signaling in the brain modulates epileptic seizures in rodents

Authors: *A. VIADER, J. L. BLANKMAN, A. R. COPPOLA, R. A. HERBST, A. KNIZE, J. S. WARBURG, C. GRICE, G. O'NEIL, A. EZEKOWITZ, J. R. CLAPPER
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Abstract: Epilepsy is a common, debilitating neurological disease, comprising a spectrum of syndromes with diverse etiologies, and often categorized as acquired forms of epilepsy (e.g. temporal lobe epilepsy), those with genetic origin (e.g. Dravet syndrome), and status epilepticus. Despite etiological diversity, epilepsy is unified by the spontaneous occurrence of seizures caused by excessive and hypersynchronous neuronal activity in the brain. The endocannabinoid system (ECS) is now recognized as a ubiquitous, retrograde lipid signaling system, central to activity-dependent synaptic modulation and able to prevent excessive network excitability. 2-arachidonoylglycerol (2-AG), a principal ligand for the ECS, is produced and released from postsynaptic neurons in an activity-dependent manner to act retrogradely on presynaptic cannabinoid receptors (CBRs) and dampen aberrant neurotransmitter release. As such, modulators of the ECS and of 2-AG represent promising novel antiepileptic therapies. 2-AG signaling is tightly regulated by this lipid's principal biosynthetic and degradative enzymes in the nervous system, diacylglycerol lipase-α (DAGLα) and monoacylglycerol lipase (MGLL), respectively. In the present study we leverage recently-developed pharmacological inhibitors of DAGLα and MGLL to examine whether modulation of 2-AG signaling can regulate seizure activity in preclinical rodent models of epilepsy. We found that PTZ-induced seizures were exacerbated in mice with depleted brain 2-AG levels through pharmacological inhibition of DAGLα. Remarkably, the observed seizures were more severe following DAGLα inhibition than CB1 inverse agonism, emphasizing the importance of on-demand 2-AG production in suppressing abnormal brain network activity. Conversely, enhancement of 2-AG signaling through inhibition of MGLL, significantly decreased the occurrence of PTZ-induced seizures in rats. Together, these results highlight a critical role for 2-AG as a natural brake to excessive neuronal excitability and support the potential use of MGLL inhibitors for the treatment of epilepsy syndromes.

Disclosures: A. Viader: A. Employment/Salary (full or part-time):; Abide Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Abide Therapeutics. **J.L. Blankman:** A. Employment/Salary (full or part-time):; Abide Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Abide Therapeutics. **A.R. Coppola:** A. Employment/Salary (full or part-time):; Abide Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Abide Therapeutics. **R.A. Herbst:** A. Employment/Salary (full or part-time):; Abide Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Abide Therapeutics. **A. Knize:** A. Employment/Salary (full or part-time):; Abide Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Abide Therapeutics. **J.S. Warburg:** A. Employment/Salary (full or part-time):; Abide Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual

funds); Abide Therapeutics. **C. Grice:** A. Employment/Salary (full or part-time):; Abide Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Abide Therapeutics. **G. O'Neil:** A. Employment/Salary (full or part-time):; Abide Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Abide Therapeutics. **A. Ezekowitz:** A. Employment/Salary (full or part-time):; Abide Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Abide Therapeutics. **J.R. Clapper:** A. Employment/Salary (full or part-time):; Abide Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Abide Therapeutics.

Poster

560. Mechanisms of Seizure Generation and Epilepsy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.18/F15

Topic: B.10. Epilepsy

Support: CIHR #125984
CIHR #153111
NSERC CREATE

Title: Effects of the type 1 cannabinoid receptor positive allosteric modulator GAT211 on absence seizures and the anxiety-like phenotype of Genetic Absence Epilepsy Rats from Strasbourg

Authors: *A. J. ROEBUCK[1], M. ALAVERDASHVILI[1], Q. GREBA[1], M. ANDERSON[1], W. N. MARKS[2], S. M. CAIN[3], T. P. SNUTCH[4], S. GURAI[5], G. A. THAKUR[5], R. B. LAPRAIRIE[1], J. G. HOWLAND[6]
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Abstract: Absence epilepsy is characterized by recurring seizures that lead to brief lapses of awareness and a variety of comorbid complications. The most widely accepted treatments for absence epilepsy are ethosuximide, which can produce drowsiness and confusion; and valproic acid, which displays hepatotoxicity. The type 1 cannabinoid receptor (CB1R) is considered a potential therapeutic target for many forms of epilepsy, including absence seizures. In a series of experiments, we tested the effects of the CB1R positive allosteric modulator (PAM) GAT211 (10 mg/kg; i.p.) for its potential to reduce absence seizures and behavioural comorbidities in the

Genetic Absence Epilepsy Rats from Strasbourg (GAERS). Using a within-subjects design adult males (n = 3) were implanted with recording electrodes in sensorimotor cortex and hippocampus and treated with both GAT211 and a vehicle treatment. In the hour following GAT211, the number of cortical-recorded seizures decreased from an average of 65 seizures per hour to an average of 45 seizures per hour. Spike frequency was also decreased from an average of 7.5 Hz to an average of 6.5 Hz after GAT211. In the second experiment, male and female GAERS and non-epileptic controls (NECs; total n = 76) were treated with either vehicle or GAT211 in a between-subjects design using an acoustic startle task. Both sexes of GAERS demonstrated a significantly higher startle response than NECs. Treatment with GAT211 reduced the startle response in female GAERS and NECs. Results from these experiments suggest that GAT211 may be effective in reducing seizure severity and may also reduce the anxiety-like phenotype previously identified in GAERS and NEC animals. However, potential interactions between strain and sex must be further investigated. In conclusion, these results suggest that CB1R PAMs may be a therapeutically effective target for ameliorating absence seizures, as well as their comorbidities such as anxiety.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.19/F16

Topic: B.10. Epilepsy

Title: Anticonvulsant effect of cannabidiol in female rats during two phases of estrous cycle on a PTZ-induced convulsive seizure model

Authors: *N. L. JANISSET[1], B. M. LONGO[2]
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Abstract: Cannabidiol (CBD) is one of the major cannabinoids present in *Cannabis sativa* with great therapeutic potential. According to evidence from preclinical and clinical studies, CBD was shown to have anticonvulsive effect and has recently been proposed for the treatment of epileptic seizures. Approximately 70% of women with epilepsy face additional challenges on seizures exacerbation due to hormonal changes that occur during the menstrual cycle. Given the impact of hormonal influences on seizure activity and potential complications of treatments, the goal of the present study was to investigate the anticonvulsive effect of CBD in seizures induced by PTZ in two phases of the estrous cycle of female rats. Rats in the estrus (E) and diestrus (D) phases were treated with vehicle (CTRL) or CBD (50mg/kg). One hour after CBD treatment, acute

generalized seizures were induced by administration of PTZ (100mg/kg), and the following parameters were recorded: mortality, latency, duration and frequency of seizures. After 24h, half of animals from each group were perfused and their brains processed by immunohistochemistry for the microglial marker Iba-1. In the other half of animals, blood was collected for the analysis of the pro-inflammatory interleukin *IL-1β levels*. CTRL animals from estrus and diestrus groups presented a 12.5% and 50% of mortality rate, respectively, whereas there was no mortality in groups treated with CBD. Microglial quantification was reduced in the hippocampus (P= 0.05), and *IL-1β blood levels* decreased in CBD-E group when compared with CTRL-E group (P= 0.05). Our preliminary data suggest that CBD has an anticonvulsive effect in catamenial epilepsy, reducing the inflammatory response that occurs after an acute seizure. The present results indicate that, this protective effect is influenced by hormonal variations, showing prominent effect during the estrus phase. Our study may help to clarify some issues related to the therapeutic potential of CBD in catamenial epilepsy and may contribute to the development of new therapies in the future.

Disclosures: N.L. Janisset: None. **B.M. Longo:** None.

Poster

560. Mechanisms of Seizure Generation and Epilepsy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.20/F17

Topic: B.10. Epilepsy

Support: DCR - CNPq/FAPEG Grant
CNPq - Universal Grant

Title: Seizures frequency modifies cardiovascular responses *ex vivo* and cardiac tissue in rats submitted to electric amygdala kindling model of epilepsy

Authors: *A. P. PANSANI[1], P. P. GHAZALE[3,1], E. G. DOS SANTOS[1], K. S. BORGES[1], K. P. GOMES[1], C. Q. DE LIMA, Jr.[1], P. P. P. BRAGA[1], B. P. DE SOUZA[1], C. H. DE CASTRO[1], C. H. X. CUSTÓDIO[1], E. P. MENDES[1], F. C. A. DOS SANTOS[2], M. F. BIANCARDI[2], F. A. SCORZA[3], D. B. COLUGNATI[1]
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Abstract: Cardiovascular alterations have been identified as the main cause of sudden unexpected death in epilepsy. We aimed to evaluate the impact of the number of seizures on heart function and morphology and on aortic vascular reactivity in rats submitted to the electrical amygdala kindling model. Male Wistar rats were fitted with electrodes on the right amygdala for stimulation and two surface electrodes for recording cortical EEG. The amygdala was stimulated

electrically once a day, and seizure evolution was classified according to Racine's scale (R1-R5). Kindled rats were submitted to 5 seizures R5 (Low Seizure - LS) or to 10 seizures R5 (High Seizure - HS). At the end of this protocol, rats were decapitated, and heart and aortic rings were dissected. The heart was submitted to Langendorff technique and challenged by ischemia/reperfusion protocol. Vascular reactivity of aortic ring (with and without endothelium) was assessed by organ bath technique. After *ex vivo* analysis, the heart was fixed and histologically processed. Cardiomyocyte size, interstitial fibrosis, perivascular fibrosis, and ventricular mass index were evaluated. Compared to sham group (rats with implanted electrodes without stimulation), LS group presented decreased basal values of the following parameters: intraventricular systolic pressure, positive and negative dP/dt. During ischemia, all groups had a reduction on developed intraventricular pressure and positive and negative dP/dt, but with less magnitude in LS group. During reperfusion, only LS group did not recover its basal parameters. There were no alterations among groups in both intrinsic heart rate and ventricular fibrillation after reperfusion. The aortic ring with endothelium had higher contraction induced by phenylephrine (PHE) in LS group than in both SHAM and HS group. The PHE-induced contraction curve of aortic rings without endothelium was shifted to the left in HS group. Concerning morphological analysis, LS group had larger cardiomyocyte size and percentage of interstitial fibrosis than both sham and the HS groups. The perivascular fibrosis was also higher in LS group than in HS group, which in turn has a smaller fibrotic area compared to the sham group. No alterations were observed on the ventricular mass index. So, rats with low seizures frequency had worst ventricular function and cardiac tissue alterations than those with high seizures frequency. Epileptic rats had greater aortic contraction reactivity, regardless of the number of seizure. Therefore, seizure frequency interferes with cardiovascular parameters.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.21/F18

Topic: B.10. Epilepsy

Support: PR100040
EP150033

Title: Validity of post traumatic epilepsy outcome following lateral fluid percussion injury

Authors: *S. M. TATUM[1], Z. Z. SMITH[2], A. BERNIER[2], D. POULSEN[3], D. BARTH[2]
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Abstract: Post-traumatic epilepsy (PTE) is characterized by recurrent spontaneous nonconvulsive or convulsive seizures, typically emerging after a "latent period" of months to years following injury. Presumed epileptogenesis during the latent period may provide a therapeutic window for intervention. Yet, the successful exploration of potential anti-epileptogenesis compounds and intervention strategies relies directly on valid animal models of PTE. For the past 10 years, a leading rat model of PTE has used lateral fluid percussion injury (LFPI) to simulate closed head injury. This model has been reported to result in frequent and brief (seconds) bouts of spike-wave discharges (SWDs) that are claimed to reflect spontaneous complex partial non-convulsive seizures (CPSs) within weeks of injury. Yet, except for the distinction of focal onset near the injury site, the SWDs characterizing CPSs are indistinguishable from absence-like (genetic) seizures recordable in both injured and control rats. We hypothesized that if post-injury, focal-onset CPSs are in fact distinct from absence-like seizures, they should be resistant to the established anti-absence medication, ethosuximide, which is ineffective in treating human CPSs. We performed chronic video/EEG recording in rats with severe LFPI for 6 months post-injury. A subset of rats displayed SWDs of focal onset ipsilateral and not contralateral to injury. These were intermixed with bilaterally synchronous SWDs typically characterizing absence-like seizures in the rat model. Ethosuximide transiently (4-6 hr) but completely suppressed all SWDs (focal or bilaterally synchronous). Conversely, carbamazepine, used to effectively treat CPSs in humans, had no effect on either focal or bilaterally synchronous SWDs. These results suggest that SWDs, whether focal or generalized, do not present a viable outcome measure for exploration of the mechanisms or treatment of PTE in the rat LFPI model.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.22/F19

Topic: B.10. Epilepsy

Title: Intracerebral application of pilocarpine induces progressive limbic epilepsy in Wistar rats

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Abstract: Introduction: Temporal lobe epilepsy (TLE), the most common adult human pharmacoresistant epilepsy, involves limbic networks and is progressive. Systemic application of pilocarpine in rodents, a model of TLE, induces epilepsy with variable efficiency and mortality. The main goal of this study was to detect limbic seizures and their evolution after induction of status epilepticus (SE) by intra-amygdala application of pilocarpine in rats. **Methodology:** Following ARRIVE standards, pilocarpine (0.9mg/μL; 1μL) or sterile saline solution (0.9%; 1μL) were injected into the right amygdala of experimental (n=7) and control (n=6) groups of male Wistar rats, respectively. Anticonvulsant therapy was applied four hours after the SE-onset or the intra-amygdala injection of saline solution. Beginning at the time of the intracerebral injection, animals were individually monitored by a CCTV video system during the SE-day and along a period from the 30th to the 120th days. Rat's behavior was qualified in a blinded way using the Racine's scale. **Results:** Along the experiment, the behavior was normal in control rats while all experimental group rats displayed SE followed by spontaneous recurrent seizures (SRS). Limbic seizures corresponding to the SE, with a total duration of more than 120 min, were mainly generalized in three animals, partial in two animals and one rat showed generalized and partial seizures with similar duration. Additionally, one rat died during the SE. In the chronic period, all experimental rats displayed spontaneous recurrent seizures (SRS). However, frequency of seizures occurred with two profiles: 1) rats with frequent seizures (total number of seizures > 80) and 2) rats with few seizures (less than 80 seizures). The frequency of SRS was directly associated with severity of SE. Two months after SE, rats with frequent seizures showed higher proportion of partial than generalized seizures. This proportion was inverted by the fourth month. In contrast, rats with few seizures presented similar proportion of partial and generalized seizures throughout the analyzed period. **Conclusion:** Generalized limbic SE, induced by intra-amygdala application of pilocarpine, is associated to progressive limbic epilepsy. In contrast, partial SE seems to be followed by non-progressive and less severe epilepsy. Additionally, animal mortality associated to this experimental paradigm is low.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.23/F20

Topic: B.10. Epilepsy

Support: NINDS Grant 1U54NS079202

Title: Combination intramuscular allopregnanolone and perampanel in the treatment of acute diisopropylfluorophosphate (DFP)-induced status epilepticus in rats

Authors: *A. DHIR[1], D. J. TANCREDI[2], M. A. ROGAWSKI[1]
[1]Neurol., [2]Pediatrics, Univ. of California, Davis, Sacramento, CA

Abstract: Organophosphate (OP) nerve agent intoxication may cause lethal status epilepticus (SE) in animals and humans. These seizures are often refractory to first line, standard-of-care benzodiazepines, especially when therapy is delayed. In this study we assessed the combination of the neurosteroid allopregnanolone, a positive allosteric modulator of synaptic and extrasynaptic GABA-A receptors, and perampanel, an AMPA receptor antagonist, in the treatment of status epilepticus induced by the OP nerve agent surrogate DFP. AMPA receptors, the main mediators of excitatory glutamate neurotransmission, were considered a potential treatment target since excessive glutamate levels are believed to be a mechanism of toxicity in OP poisoning. The combination was studied as we previously found that AMPA receptor block alone does not terminate DFP seizures. To mimic a typical clinical treatment scenario, the combination treatment was administered following the benzodiazepine midazolam, which was administered at a time of benzodiazepine resistance. For comparison, we studied valproate, a marketed antiseizure agent available in a parenteral formulation, that could be used in the treatment of benzodiazepine-refractory OP intoxication. Status epilepticus was induced in male SD rats with DFP (4 mg/kg, SC). One minute later, animals were injected with atropine (2 mg/kg, IM) and pralidoxime chloride (25 mg/kg, IM) to avoid peripheral side effects. Forty min after DFP, animals received midazolam (1.8 mg/kg, IM) followed by either (a) allopregnanolone (6 mg/kg, IM) plus perampanel (2 mg/kg, IM) or (b) valproate (200 mg/kg, IP). High-amplitude epileptiform discharges occurred within a few minutes after DFP treatment that were resistant to midazolam. Treatment with the combination of allopregnanolone and perampanel resulted in rapid cessation of behavioral and electrographic status epilepticus. Valproate reduced the EEG power but did not eliminate spikes and electrographic seizures in the EEG. The results indicate that allopregnanolone/perampanel combination treatment is more effective than valproate in terminating benzodiazepine-refractory OP-induced SE.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

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Program #/Poster #: 560.24/F21

Topic: B.10. Epilepsy

Support: Lily's Fund Grace Grant
DOD
CURE

Title: Effects of sleep deprivation on tonic GABAergic inhibition in the hippocampus

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Abstract: Sleep is critical for effective homeostasis. Disturbances in sleep patterns can impact cognition and memory. For people with epilepsy, for whom problems with sleep are common, sleep deprivation is one of the most potent triggers for seizures. However, the mechanisms for these detrimental effects remain poorly understood. Having been implicated in memory as well as seizure generation in some epilepsies, and displaying specific sleep-related activity, the hippocampus may be involved in mediating numerous detrimental effects of sleep disruption. Pathologies in the hippocampal trisynaptic circuit are common in temporal lobe epilepsy. We hypothesized that brief disruptions in sleep may affect hippocampal neurotransmission, increasing excitability, which may heighten susceptibility for epileptiform activity in individuals with epilepsy.

Here, we evaluated the effect of 4-hour sleep deprivation (4SD) on GABAergic neurotransmission. Building on evidence that hippocampal mRNA expression of alpha-5 and delta subunits of $GABA_A$ receptors (that mediate tonic inhibition) decrease after acute sleep disruption, we used whole-cell voltage-clamp (-70 mV, room temp.) to investigate GABAergic currents in CA1 and DG of hippocampal slices prepared from sleep-deprived and control C57bl/6 mice (P37±4). Total sleep deprivation occurred ZT0-4 using novel object methods, ensuring continuous locomotion and exploration. Tonic current density was measured as the change in holding current upon application of 100 µM bicuculline methiodide, divided by membrane capacitance. Sleep deprivation significantly decreased tonic current density in both CA1 pyramidal cells (control: 0.25±0.02 pA/pF, n=14, 4SD: 0.13±0.03 pA/pF, n=11, $p<0.01$, unpaired t test) and DG granule cells (control: 0.44±0.08 pA/pF, 4SD 0.17± 0.03 pA/pF, $p<0.01$). In both cell types, we saw no significant alteration of miniature inhibitory postsynaptic current amplitude or frequency.

Sleep deprivation could therefore contribute to hyperexcitability and seizure susceptibility, in part, by reducing tonic inhibition. Future experiments will aim to determine the underlying mechanisms by evaluating changes in GABA receptor expression and function, as well as regulation of ambient GABA concentration, after acute sleep deprivation.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

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Topic: B.10. Epilepsy

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Innovate Perú Nro.135-PNICP-PIAP-2015

Title: Abnormal hypersynchrony of neuronal activity and its relation to neuroinflamation, number and location of cysts in rats with neurocysticercosis

Authors: *A. D. DELGADO[1], R. P. CARMEN[2], R. H. GILMAN[3], F. ANCAJIMA[2], L. E. BAQUEDANO[2], R. H. CELIZ[2], D. G. DAVILA[2], M. R. VERASTEGUI[2]
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Abstract: Neurocysticercosis (NCC) is caused by the larva of the taenia solium located in the central nervous system (CNS). In endemic countries as Peru, it is the main cause of late epilepsy. Our group has developed a rat model to study the electrophysiology of the waveforms of seizures in neurocysticercosis. In preliminary studies we have observed that our model allows the development of viable cysticerci in the brain, and the presence of generalized tonic clonic seizures, which allows us to have a model similar than humans. Our objective is to relate changes in the electroencephalogram recording with neuroinflammation, number and location of cysts in rats with neurocysticercosis. Male Holtzman rats received intracranial infection with activated T. solium oncospheres between 12-15 days of birth, after 3 months of infection MRI T2 were performed in order to detect the presence of the cysticercus in the rat brain. Selected Infected rats (n=14) and not infected rats (n=8), were continuously recorded by telemetric electroencephalography (tEEG) to monitor the brain activity and detect seizures for five weeks. Abnormal hypersynchrony of neuronal activity was observed in the tEEG recording associated with generalized tonic clonic seizures in 15% (n = 2), with an average duration of 120 seconds per seizure. These rats had the highest number of parenchymal cysts in the group of infected rats, immunohistochemistry studisn were performed to observe neuroinflammation.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

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Program #/Poster #: 560.26/F23

Topic: B.10. Epilepsy

Support: FAPEG
CNPq

Title: Carbamazepine and lamotrigine revert cardiovascular alterations in rats submitted to the pilocarpine model of epilepsy

Authors: *D. B. COLUGNATI[1], B. P. DE SOUZA[1], K. P. GOMES[1], P. P. P. BRAGA[1], C. Q. DE LIMA, Jr[1], F. C. A. DOS SANTOS[1], M. F. BIANCARDI[1], P. P. GHAZALE[1,2], E. P. MENDES[1], C. H. DE CASTRO[1], F. A. SCORZA[2], A. P. PANSANI[1]
[1]Physiological Sci., Univ. Federal De Goiás, Goiânia, Brazil; [2]Univ. Federal de São Paulo, São Paulo, Brazil

Abstract: Sudden Unexpected Death in Epilepsy (SUDEP) is responsible for approximately 15% of all deaths in individuals with epilepsy and 50% in refractory epilepsy, presenting an incidence among young epileptic population between 1:500 and 1:1000 patient-years. In fact, cardiovascular alterations have been often demonstrated in association with epilepsy. Therefore, it is believed that cardiovascular alterations may be one of the main causes for SUDEP. Regarding risk factors for SUDEP the antiepileptic drugs (AEDs) has been extensively evaluated. With respect to specific AEDs, sodium channel-blocking drugs such as carbamazepine (CBZ) and lamotrigine (LTG) may be related with heart rhythm alterations. So, we sought to evaluate cardiovascular parameters in epileptic rats treated with CBZ or LTG. The epilepsy was induced by pilocarpine model in male Wistar rats. After the first spontaneous recurrent seizure, the rats were treated with CBZ (150mg/day) or LTG (150mg/day) or vehicle for 60 days. Than, systolic blood pressure (SBP); Diastolic blood pressure (DBP); Mean arterial pressure (MAP) and heart rate (HR) were recorded. We also performed a baroreflex test with *bolus* administration of phenylephrine (PHE - 5μg) and sodium nitroprusside (NPS - 10μg) via cannulation of femoral vein. After *in vivo* protocols the animals were euthanized and the heart were prepared for histological analyzes. The epileptic rats treated with vehicle (EP) had higher HR, SBP, DBP and MAP when compared to control rats (CNT) and epileptic rats treated with CBZ (EP-CBZ). Also, the treatment with LTG reduced resting HR. No differences were observed regarding the baroreflex. Furthermore, we observed that the EP rats had a greater cross-sectional area of cardiomyocytes when compared to the other groups and an increased deposition of perivascular collagen compared to both CNT and EP-CBZ groups. It is important to note that neither CBZ nor LTG reduced the seizure frequency compared to EP. However, both CBZ and

LTG had a beneficial effect on cardiovascular parameters and cardiac remodeling of rats with epilepsy. So, CBZ and LTG at doses studied were cardioprotective, with no modification of seizure frequency.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.27/F24

Topic: B.10. Epilepsy

Title: Hippocampal oxygen levels during the development and expression of epilepsy in two status epilepticus models: Intrahippocampal kainate and perforant path electrical stimulation

Authors: *M. D. WOLFF[1], M. H. SCANTLEBURY[2], G. C. TESKEY[3]
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Abstract: We determined that following the cessation of brief focal seizures, a long-lasting severe hypoperfusion/hypoxic event occurs in the brain regions involved in the seizure. We reasoned that there might also be changes in local oxygen levels during and following focal status epilepticus (SE). Current animal models of epilepsy employ the use of either the infusion of a chemoconvulsant or electrical stimulation to induce an epileptic state in rodents. We aimed to compare both models with the goal to prevent generalized SE, limit lethality, and ultimately produce self-generating seizures. We hypothesized that; 1) during SE there will be drastic changes in hippocampal oxygen levels and 2) following the termination of SE spontaneous hippocampal seizures will produce episodes of postictal hypoxia.
A dose of urethane was administered to induce sedation before the induction of SE to sequester electrical activity and prevent generalized SE. In the intrahippocampal kainic acid model, kainic acid was infused directly into the rat ventral hippocampus. In the electrical stimulation model, a 24-hour stimulation protocol of the performant path was used. In both groups oxygen levels and EEG were recorded in dorsal hippocampus throughout the first 24 hours. Immediately after the 24-hour induction period, rats were transferred to a 24/7 video-EEG monitoring unit. Hippocampal EEG was monitored continuously for 4-6 weeks. We have found that prolonged stimulation of the performant pathway results in a long-lasting severe hyperoxia in the hippocampus during the 24-hour stimulation period. Animals infused with kainic acid on the other hand experience mild hyperoxia during bouts of seizure activity that lasts for approximately 2-4 hours. Following epilepsy induction, both groups experience immediate epileptiform activity during the first week, which eventually matures into self-generating

seizures. We have also found that these self-generating seizures are followed by severe postictal hypoxia. This study advances our current understanding of epilepsy models in relation to local oxygen levels. These discoveries may lead to the development of new treatments or preventative strategies for people with epilepsy.

Disclosures: M.H. Scantlebury: None. **G.C. Teskey:** None.

Poster

560. Mechanisms of Seizure Generation and Epilepsy

Location: SDCC Halls B-H

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Program #/Poster #: 560.28/F25

Topic: B.10. Epilepsy

Support: NIH/NINDS grant R41NS107148
a seed grant from EpiC, the University of Kentucky Epilepsy Research Center
A. Ajwad received scholarship support from the Higher Committee of Education in Iraq

Title: A noninvasive screening method for seizures and related behaviors in small animal models

Authors: A. AJWAD[1], H. WANG[1], F. YAGHOUBY[1], D. M. HUFFMAN[1], *B. F. O'HARA[2], S. SUNDERAM[1]
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Abstract: Animal models of epilepsy and other seizure disorders require careful monitoring to characterize phenomena and assess the effect of experimental therapies. Some contributing factors are: latency to development of spontaneously recurring seizures (in models of acquired epilepsy); types of seizures & the spectrum of behaviors associated with them; the difficulty in distinguishing seizures from baseline behavior; and the sporadic & unpredictable nature of seizure recurrence. Invasive EEG is often required to detect & verify seizures, with associated costs and need for skilled personnel. Visual observation or retrospective video analysis is labor-intensive and potentially inaccurate. Thus, convenient noninvasive automated methods of seizure analysis are needed to remove some of these limitations. In prior work, we demonstrated how a piezoelectric motion sensor (Signal Solutions LLC) placed on the cage floor can be used to discriminate sleep-wake states. In the present study, we examine the use of this sensor for noninvasive seizure detection in rodent disease models. C57BL/6 mice were injected with pilocarpine i.p. to induce acute seizures. Seizures subsided in 1-2 hrs and after a latent period of weeks led to spontaneously recurring seizures, a sign of chronic epilepsy. Animals were surgically instrumented for EEG recording & monitored for 4-5 weeks. Overt seizures (grade 4-5 on the Racine scale) were detected from EEG and used as test data to assess feasibility of noninvasive seizure detection from the piezo signal. 160 seizures were identified in 5 mice. A

simple algorithm based on comparison of instantaneous line length in a moving window of the piezo signal with a threshold, after adaptive correction for changes in baseline state, was used for seizure detection. The performance of the algorithm was evaluated against previously accumulated seizure data using conventional metrics of sensitivity & precision. Analysis of the data using a 5-fold cross-validation scheme showed that the piezo seizure detection algorithm had a sensitivity of 88% and precision of 29% on average. This implies that only 1 in 10 seizures are likely to be missed while about 1 in 3 are likely to be true events. The performance in this preliminary study gives confidence that the piezo sensor method will enable significant savings in time and effort with only a moderate proportion of candidate events that may need to be reviewed retrospectively on video. Our ongoing studies are focused on expanding the range of events to include more subtle seizures in both mouse & rat models and finer characterization of seizure-related motion and respiratory distress, which is also enabled by the piezo sensor.

Disclosures: A. Ajwad: None. **H. Wang:** None. **F. Yaghouby:** None. **D.M. Huffman:** None. **B.F. O'Hara:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solutions LLC. **S. Sunderam:** None.

Poster

560. Mechanisms of Seizure Generation and Epilepsy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.29/F26

Topic: B.10. Epilepsy

Support: Start-up Funds

Title: Effect of the gut microbiome on seizure susceptibility in murine models of epilepsy

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Abstract: Epilepsy is a devastating neurological disorder that currently affects over 3 million Americans. Although multiple anti-epileptic drugs (AEDs) with diverse mechanisms of action are available, over 30% of patients suffering from epilepsy experience seizures that do not respond to AED treatment. Ketogenic diets are administered as alternatives to AEDs for seizure management in patients with drug-resistant epilepsy, but the anti-epileptic mechanism of action of ketogenic diets remains to be elucidated. Over the past decade, an increasing body of evidence has grown to support the idea that the trillions of bacteria residing in the gut, termed the gut microbiome, have widespread effects on neural functions. Given that ketogenic diets lead to marked changes in the composition of the gut microbiome, we hypothesize that alterations in the

gut microbiome can affect seizure susceptibility. To address this hypothesis, we altered the microbiomes of several murine models of epilepsy with a cocktail of antibiotics chronically administered through drinking water. The electrophysiological properties of neurons from microbiome-altered and un-altered mice will be evaluated. Additionally, continuous recordings of electroencephalography (EEG) activity in microbiome-altered mice and un-altered mice will be evaluated to determine whether alteration of the microbiome affects seizure activity. Elucidating specific changes in gut microbes that alters seizure susceptibility in epilepsy models could lead to potential therapies for patients with epilepsy that do not respond to currently available treatments.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.30/G1

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH intramural awards to CJM and KAZ

Title: A comprehensive investigation of human hippocampal mossy fiber transmission and plasticity

Authors: K. A. PELKEY, D. CALVIGIONI, R. CHITTAJALLU, K. A. ZAGHLOUL, *C. J. MCBAIN
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Abstract: Computational models, based primarily on evidence from rodents, predict that synaptic connections between hippocampal dentate gyrus (DG) granule cells and CA3 pyramidal cells are essential for encoding contextual memories. In support of this, disruption of the mossy fiber (MF) pathway connecting DG and CA3 impairs spatial memory encoding while false memories can be generated by optogenetically driving MF transmission through memory engram-bearing granule cells of one context in a novel context. The ability of MF-CA3 synapses to support memory encoding is considered to relate to their somewhat peculiar specialized synaptic properties. Indeed a rich literature describes several unique structural/functional properties of MF-CA3 synapses including sparse innervation by large multi-release site presynaptic terminals supporting a remarkable frequency-dependent dynamic range of transmission onto the most proximal dendrites of CA3 pyramids, dramatic susceptibility to presynaptic modulation (particularly cAMP levels), rapid AMPAR dominated kinetics with minimal NMDAR-mediated contribution, “detonator” capabilities, and presynaptically expressed

NMDAR-independent long-term plasticity. Ultimately, the goal of examining synaptic function in experimental models to such exquisite detail is to gain insight into how a given circuit may function in the human brain. Thus, the translation and relevance of rodent MF findings to their role in human hippocampal function demands validation that human MF transmission displays similar unique properties. We comprehensively evaluated the basic synaptic properties of MF-CA3/4 pyramidal cell connections within the human hippocampus obtained from tissue resected for treatment of epilepsy. Remarkably, human MF-CA3/4 pyramidal cell transmission exhibits the same hallmark features described in the rodent including AMPAR dominated synapses with small contributions from NMDARs and KARs, large dynamic range with strong frequency-facilitation, NMDAR-independent presynaptically expressed long-term potentiation, strong cAMP sensitivity of presynaptic release engaged by group II mGluRs. While interpretation of our findings could be confounded by the diseased nature of the resected tissue the astonishing congruence of core features shared between rodent and human MF synapses argues that the basic properties of MF transmission reported in animal models (including studies in non-human primate) are also critical to human MF function. Further investigation will compare/contrast human MF transmission with other "model" hippocampal synapses such as Schaffer collateral-CA1 pyramidal cell synapses.

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Poster

560. Mechanisms of Seizure Generation and Epilepsy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 560.31/G2

Topic: B.11. Glial Mechanisms

Title: Multiparametric screening of compound-induced seizure risk

Authors: *J. KANERVA[1], Y. CHEN[1], C. CARROMEU[2], O. GUICHERIT[2], J. MCDUFFIE[1]
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Abstract: Approximately 30% of candidate drugs are attrited in Phase I clinical trials due to seizure liability. The assessment of compound seizurogenic activity is currently limited to functional measurements such as electroencephalography, observation of clinical signs and brain histopathology in animal models, which are often called into question due to limited translatable evidence of neurotoxicity. To address this issue, we have developed *in vivo* and *in vitro* approaches to screen compounds for seizure risk associated with perturbed glia-neuron interactions. First, we developed different kainate-, 4-aminopyridine-, pentylenetetrazole-, and undisclosed proprietary compound-induced rat seizure models to identify candidate genomic/proteomic biomarker panels for neuronal/astrocytespecific toxicities. Second, we

characterized two human induced pluripotent stem cell (hiPSC)derived neuronal models (microBrain® 2D and 3D), which are comprised of cortical neurons (GABAergic and glutamatergic) and astrocytes, exhibit sustained viability, and possess functionalities comparable to the human brain. Functionally, the microBrain® 2D platform shows spontaneous firing on lowthroughput microelectrode array (MEA), while the microBrain® 3D platform shows spontaneous synchronized calcium transient oscillations on highthroughput Fluorescent Imaging Plate Reader (FLIPR™). Third, we examined MEA and FLIPR™ responses from the two physiologicallyrelevant hiPSC models post-exposure to seizurogenic or non-seizurogenic compounds, as well as the *in vitro* context of use for astrocyte toxicity biomarkers that were first detected following recurrent seizures in rats. Elucidating the mechanistic role of astrocytes in compound-induced seizures in rat as well as the 2D/3D human microBrains® may help bridge the gap in species translatability. These data support the use of a novel, integrated, multiparametric *in vitro*/*in vivo* screening paradigm for de-risking compound seizurogenicity.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 561.01/G3

Topic: B.10. Epilepsy

Support: NIH R01 NS066974
R01 NS096088

Title: Limbic seizures depress cortical activation via subcortical pathways

Authors: J. POK[1], L.-A. SIEU[1], L. FENG[1], C. MA[1], C. W. ZHAO[1], J. CARDIN[1], *H. BLUMENFELD[1,2,3]
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Abstract: Temporal lobe epilepsy is the most common type of focal seizure disorder and is usually accompanied by loss of consciousness in its complex partial form. Despite its prevalence however, the specific neural pathways by which this occurs are still poorly understood. Previous intracranial EEG studies have shown that focal limbic seizures depress cortical activity, as marked by deep sleep-like slow wave activity in cortex and reduced cholinergic arousal. In addition, electrostimulation of the lateral septum (LS), a subcortical region with connections to

the hippocampus, was found to induce slow waves in cortex and reduce choline release. Given this knowledge and understanding that the nucleus basalis (NB) has cholinergic outputs to cortex, we propose that partial limbic seizures arising from the hippocampus could potentially inhibit cholinergic neurotransmission from NB to cortex through subcortical pathways. First, we examined any potential functional pathways between the NB and LS to better understand how partial seizures arising from the hippocampus could result in cortical deactivation. To this end, we developed an optogenetic rat model to restore cholinergic arousal in NB neurons during electrically-induced hippocampal seizures. Our results showed that the delta wave oscillations (0.5-2 Hz) found in cortex during seizure were converted to fast waves following optogenetic stimulation of the cholinergic neurons, suggesting that cholinergic arousal from NB plays a role in cortical arousal. To confirm any neuronal connections between the two regions, we did neuroanatomical retrograde and anterograde tracing studies. Our histology showed evidence of direct connections between the aforementioned regions. Moreover, we observed connections from NB and LS to midline thalamic nuclei; specifically, the paratenial region of the thalamus. To better understand what role, if any, this region has in the circuit, we recorded multiunit activity (MUA) and local field potentials (LFP) during electrically induced hippocampal seizures. Our recordings showed that during seizure, multiunit activity in PT was suppressed. These findings suggest that a possible pathway by which cortical depression occurs may be that PT receives inhibitory inputs from LS, thereby decreasing excitatory output to NB, leading to decreased cholinergic arousal. More thematically, our findings show that partial seizures arising from the temporal lobe affect several subcortical networks to induce loss of consciousness, suggesting that further investigation into these networks may bring about novel therapeutic targets aimed at improving cortical arousal during and after seizures.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 561.02/G4

Topic: B.10. Epilepsy

Support: NIH R01 NS066974
R01 NS096088

Title: Mouse model of electrically inducible focal seizures with impaired consciousness

Authors: *L.-A. SIEU[1], S. SINGLA[1], C. MCCAFFERTY[1], M. VALCARCE-ASPEGREN[1], A. NIKNAHAD[1], Q. PERRENOUD[2], J. CARDIN[2], H. BLUMENFELD[1,2,3]
[1]Neurol., [2]Neurosci., [3]Neurosurg., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Focal temporal lobe seizures impair cortical function and often result in a loss of consciousness. Clinical studies showed that temporal lobe seizure is accompanied by an increase in cortical slow-waves activity in intracranial EEG recordings and a decreased cerebral blood flow (CBF). These results are replicated in a previous anesthetized rat model where partial limbic seizures are induced electrically. Further work in this model revealed increased CBF in lateral septum (LS), and reduced cholinergic input to cortex from subcortical arousal systems during seizures. Electrostimulation of LS resulted in both cortical slow oscillations and a decrease of cholinergic neurotransmission as seen during seizures suggesting that limbic partial seizures cause impaired cortical function through depressed subcortical arousal, possibly via LS. While the rat model provides insight to potential networks responsible for impaired consciousness, it is limited by the poor availability of genetic tools and the impossibility to assess behavior due to its anesthetized state. Therefore, mechanisms underlying depressed subcortical arousal, e.g. inhibition or removal of excitation, have not been investigated. The genetic techniques available and the possibility to do awake head-fixed experiments makes a mouse model much more desirable. However, most mouse models of chronic temporal lobe epilepsy develop spontaneous seizures, which limit the use of optogenetics and calcium imaging techniques. Here, we present a model of electrically inducible focal seizures in awake, behaving mice. Partial seizures were unilaterally induced and recorded from the dorsal hippocampus with a 60 Hz 2 s bipolar stimulus, while local field potential signals (LFP) were recorded from lateral orbitofrontal cortex (LO). Focal seizures were 5-10s in length, were repeatable for several weeks (n=40 seizures, 7 animals) and were associated with increased slow wave activity in the frontal cortex as observed in patients and rats. To assess behavioral responses during seizures, water-restricted mice were trained to lick a spout in response to a sound (0-50kHz noise, 12ms) every 10-15s while head-fixed on a running wheel. Response to sound decreased during seizures with reduced number of licks (n=7 animals) with increased lick latency (n=7 animals). Interestingly, response to sound was often normal during seizures suggesting consciousness was not always impaired as seen in patients. Overall, this mouse model shares characteristics seen in both human and in rat while offering new possibilities to investigate the mechanisms underlying loss of consciousness.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 561.03/G5

Topic: B.10. Epilepsy

Support: IBRO travel grant to present in the SfN annual meeting

Title: *In vitro* anticonvulsant activity of pterolobium stellatum extracts

Authors: *S. S. SALILE[1,2], H. J. LEE[2], J. V. RAIMONDO[2], T. A. ORJINO[1]
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Abstract: Though there are conventional antiepileptic drugs in use ,one third of cases are refractory to treatment which underscores the need for new anticonvulsant agent. While four-fifths of the potential market for antiepileptic drugs is in the developing world, up to 90% of people with epilepsy in developing countries receive no treatment at all. In Ethiopia, many diseases are treated using traditional medicines. *Pterolobium stellatum* is often used to treat epilepsy. The whole plant juice is given orally for one month. The aim of this study is to investigate the anticonvulsant activity of *P. stellatum* extracts using the *in vitro* 0 Mg^{2+} model of seizures in mouse hippocampal brain slices.

Methods

Plant material was collected and extracted using standard methods. The crude hydroalcholic extracts of *P. stelatum*: petether, chloroform, butanol and water extracts with 0.7 mg/ml concentration were tested for anticonvulsant activity. Extracellular field potential recordings were performed in coronal hippocampal slices from P14-P21 of C57BL16 mice.The $0Mg^{2+}$model of seizures was utilized. Baseline recordings were made for 600s with normal artificial cerebrospinal fluid(aCSF) before $0Mg^{2+}$aCSF was washed in for 3000s in order to induce seizure-like activity. The 0 Mg^{2+} solution either contained plant extract or solvent as a control. The presence of seizure-like events was compared in treated versus untreated control. The Chi square test with $P<0.05$ was used to determine statistical difference between groups.

Results

The crude extract had a statistically significant anticonvulsant activity compared to control($P=0.0153$). The chloroform and water extracts were also shown to have significant anticonvulsant activity as compared to control ($P=0.0008$ and $P= 0.0001$ respectively). The petether and buthanol extract activity was not statistically significant compared to control ($P=0.4760$ and $P=0.4637$ respectively). A positive control using the known anticonvulsant diazepam(3µM), showed significant anticonvulsant activity ($P= 0.0118$).

Discussion and recommendations

Our results demonstrate that *P. stellatum* has anticonvulsant activity. Active compounds are likely in both the water fraction and the chloroform fraction of the extracts as these both demonstrated good anticonvulsant activity. Further chemical studies are required to isolate the active compounds from these fractions. The mechanism of action of the active compounds in terms of their targets will also require further elucidation. This work demonstrates the utility of harnessing Africa's indigenous knowledge and rich biodiversity to identify novel anticonvulsant therapies based on natural compounds.

Disclosures: S.S. Salile: A. Employment/Salary (full or part-time):; Arbaminch University, Ethiopia. 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.; Addis Ababa University,Ethiopia. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); University of Cape Town, South Africa. **H.J. Lee:** None. **J.V. Raimondo:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); University of Cape Town, South Africa. **T.A. Orjino:** 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.; Addis Ababa University,Ethiopia.

Poster

561. Models of Developmental Epilepsies and Seizure Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 561.04/G6

Topic: B.10. Epilepsy

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Title: Suppression of HCN channel function in thalamocortical neurons prevents spontaneous and pharmacologically induced absence seizures

Authors: F. DAVID[1], N. CARCAK YILMAZ[2], S. FURDAN[3], F. ONAT[4], T. GOULD[5], A. MESZAROS[3], G. DI GIOVANNI[6], V. M. HERNANDEZ[7], S. CHAN[8], M. L. LORINCZ[3], *V. CRUNELLI[5]
[1]INSERM, Lyon, France; [2]Istanbul Univ., Istanbul, Turkey; [3]Univ. of Szeged, Szeged, Hungary; [4]Dept. of Pharmacol. and Clin. Pharmacol., Marmara Univ. Sch. of Med., Istanbul, Turkey; [5]Cardiff Univ., Cardiff, United Kingdom; [6]Physiol. and Biochem., Univ. of Malta, Msida, Malta; [7]Northwestern Univ., Chicago, IL; [8]Dept. of Physiol., Northwestern University, Feinberg Sch. of Med., Chicago, IL

Abstract: Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels and the I_h current they generate contribute to the pathophysiological mechanisms of absence seizures, but their precise role in neocortical and thalamic neuronal populations, the main components of the network underlying absence seizure generation remains controversial. In diverse genetic absence seizure models, I_h amplitude is smaller in neocortical neurons and either larger or unchanged in thalamocortical neurons compared to non-epileptic strains. A lower expression of neocortical

HCN subtype 1 channels is present in genetic absence seizure-prone rats and HCN2 Knock-Out mice exhibit absence seizures. Furthermore, whereas many studies have characterized I_h contribution to "absence-like" paroxysmal activity *in vitro*, no data is available on the specific role of cortical and thalamic HCN channels in behavioural seizures.
We have now performed experiments showing that the pharmacological block of HCN channels with the antagonist ZD7288 applied via reverse microdialysis in the ventrobasal thalamus of freely moving male Genetic Absence Epilepsy Rats from Strasbourg decreases TC neuron firing and abolishes spontaneous absence seizures. A similar effect is observed on γ-hydroxybutyric acid-elicited absence seizures in normal male Wistar rats. Moreover, thalamic knockdown of HCN channels via virally-delivered shRNA into the ventrobasal nucleus of male Stargazer mice, another genetic model of absences, decreases spontaneous absence seizures and I_h-dependent electrophysiological properties of ventrobasal nucleus thalamocortical neurons.
Overall, these findings provide the first evidence that the block of HCN channels of thalamocortical neurons prevents absence seizures. Moreover, they suggest that any potential anti-absence therapy that targets HCN channels should carefully consider the opposite role for cortical and thalamic I_h in the modulation of absence seizures.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

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Topic: B.10. Epilepsy

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Title: Cortical drive and thalamic feed-forward inhibition control thalamic output synchrony during absence seizures

Authors: C. P. MCCAFFERTY[1], F. DAVID[2], M. VENZI[3], M. L. LORINCZ[4], F. DELICATA[5], Z. ATHERTON[8], G. RECCHIA[8], G. ORBAN[6], R. C. LAMBERT[9], *G. DI GIOVANNI[7], N. LERESCHE[9], V. CRUNELLI[8]

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Abstract: Absence seizures, the most common form of generalized seizure, are periods of apparent impaired consciousness accompanied by distinctive spike-and-wave discharges on the electroencephalogram (EEG). Syndromes for which these seizures constitute the primary symptom (include the archetypal Childhood Absence Epilepsy (CAE)) feature significantly decreased quality of life, with developmental and psychosocial impairments common. Furthermore, current first-line pharmacological treatments have approximately 50% efficacy and non-seizure symptoms may persist even after seizure suppression. To better understand the neuronal and network mechanisms of absence seizures, we investigated the cortical and thalamic mechanisms of seizure synchrony using ensemble extracellular unit recordings and delivery of T-type Ca2+ channel (T-channel) blockers by reverse micro dialysis in a freely behaving polygenic rat model of absence. In contrast to previously prevailing hypotheses of seizure generation, we found that somatosensory thalamocortical (TC) neurons rarely expressed T-channel dependent burst firing during seizures, and that the pharmacological antagonism of these T-channels prevented neither seizure expression nor synchronous thalamic output during seizure. Rather, we found that the firing times of TC neurons appeared to be determined by a combination of strong inhibition from reticular thalamic (NRT) neurons, which persisted through the majority of each spike-and-wave cycle, and rhythmic cortical excitation, which preferentially elicited tonic (rather than burst) TC firing. This cortical excitation, in fact, was also the primary driver of the NRT neurons, thus expressing a novel form of feed-forward inhibition of TC cells. Despite this strong inhibition, and the relative paucity of T-channel dependent bursts, somatosensory TC neurons still provided synchronous and reliable reciprocal excitation of the cortex at the population level, potentially contributing to seizure perpetuation.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 561.06/G8

Topic: B.10. Epilepsy

Title: Audiogenic seizure modeling SUDEP (sudden and unexpected death in epilepsy): comparative study between four inbred strains of mice

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Abstract: Sudden and Unexpected Death in Epileptic Patients (SUDEP) rate is about three times that of the general population. Several hypothesis are suggested to explain the mechanisms of SUDEP, most of them underlying a post-ictal respiratory dysfunction secondary to affecting cardiac functions. Mouse models of SUDEP use audiogenic seizures (AGS), which are seizures induced with a sound stimulation. Immediately after the sound presentation, the mouse manifests a stereotyped behavior for which one can identify successively a wild running, clonic seizures, a tonico-clonic seizure when the mouse falls on its flanks and a tonic seizure with an extension of the limbs toward the tail, followed or not by death. AGS in mice represents one of the greatest models for SUDEP since one can observe death after respiratory arrest and a cerebral shutdown as observed in SUDEP. Moreover, the seizure is the consequence of a non-invasive induction without any pharmacologic or electric component. Only a few inbred strains of mice are AGS prone and the vast majority of studies involve DBA/2 or DBA/1 strains. These strains are ideal for basic experiments but, due to their fragile constitution, it remains very difficult to use them for experiments requiring surgery. With the goal to offer a larger panel of mice available for AGS studies, we performed a comparative study of the variability in AGS responses in four inbred strains of mice, DBA/1, DBA/2, BALB/c and 129/SvTer. The experiments were conducted on independent groups of mice at different ages, from week 3 to week 17, and for each week, we scored the percentages of mice 1/ presenting no seizure or just the wild running, 2/ presenting clonic seizures without a tonic seizure, 3/ presenting a tonic seizure without death, 4/ presenting a tonic seizure and death. As mentioned previously, the tonic seizures can be followed by death or not, even in the same inbred strain. Hence, in a second experiment, we addressed the "determinism" component in death. In other words, if some mice present a determinism to die after a tonic seizure or not. Since one can "resuscitate" mice with a respirator after a lethal tonic seizure in AGS, we addressed the question of the determinism in testing mice during 5 consecutive days and we scored for each of the five days, the lethal versus non-lethal tonic seizures.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

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Program #/Poster #: 561.07/G9

Topic: B.10. Epilepsy

Support: NIH NINDS 1R21 NS096483

Title: Sleep-like slow-wave oscillations can drive epileptic spike-wave discharges in an idiopathic generalized epilepsy model with GABAR γ2 Q390X mutation

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Abstract: Idiopathic generalized epilepsy(IGE) influences large percentage of epileptic patients (2/3 of almost 3 million people in US). In patients epileptic activity is preferentially present during sleep or non-motion quiet-wake period, implying that sleep-related cortical activity may contribute seizure initiation. Thus we hypothesized that sleep-like slow-wave oscillations(SWOs) can drive epileptic spike-wave discharges (SWD) and seizure onset. Here we used heterozygous(het, GABAR $\gamma 2^{Q390X}$) IGE mice expressing halorhodopsin in cortical neurons by crossing het mice with Thy1-eNpHR2.0-EYFP mice (012332, Jackson laboratory). Following the Vanderbilt IACUC approved animal protocol, mouse surgery was performed for EEG headmounts, and cannula implantation(optic fiber (200 μm)(Thorlabs, Newton, NJ, USA) within somatosensory cortex. Tungsten electrodes were positioned in cortical layer V and the tips were below the cannula optic fiber ending. Animal behaviors were video-recorded and synchronized with EEG (band filtered 0.1~100 Hz)/multi-unit recordings (band filtered 300~2K Hz) (two Multiclamp 700B amplifiers and one DigiData1200, current clamp mode, sampling frequency 20K Hz). Sleep-like SWOs(0.5 Hz for 5~10 min) were induced by optogenetically activating halorhodopsin (590 nm, around 1.5 s)(DPSS Laser MGL-III-589-50) and intracortical stimulation (400~500pA, 20 ms, tungsten electrodes). In WT littermate mice (n=3), spontaneous SWOs(1~3 Hz) were present except a few spontaneous SWDs (16.33±4.51 per hour, 1.72±0.14s 4~10 Hz). In contrast, in het mice (n=4), spontaneous atypical slow-SWDs (4~5 Hz) and typical SWDs(6~10 Hz) were present (53.50±7.62 per hour, duration 2.13±0.22s). Moreover, in WT mice, sleep-like SWOs by optogenetic induction in cortex did not cause any epileptic behaviors and only SWOs and short multi-unit burst were present, and SWDs were slightly increased (24.67±8.60 per hour). However, in het mice, sleep-like SWOs by optogenetic induction in cortex caused dramatically increased slow-SWDs/SWDs (110.25±13.57 per hour, paired t-test $p=0.01$ pre *vs* post) and longer duration (3.28±0.15s, paired t-test $p<0.001$) (particularly after several repeats of sleep-like SWO induction in same mice) while mice exhibited behavioral rest/pausing. Moreover, accompanied with some slow-SWDs and SWDs, multi-unit activity in cortex layer V was also increased with longer duration. In conclusion, sleep-like SWOs *in vivo* could drive epileptic SWDs in het mice, suggesting one potential mechanism (due to hemostatic potentiation impairment of GABAergic currents) which can initiate seizures in this idiopathic generalized seizures model.

Disclosures: C. Zhou: None. **L. Ding:** None. **M.J. Gallagher:** None. **R.L. Macdonald:** None.

Poster

561. Models of Developmental Epilepsies and Seizure Disorders

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Program #/Poster #: 561.08/G10

Topic: B.10. Epilepsy

Support: DFG Grant HA7597
Excellence Cluster 'BrainLinks-BrainTools' (DFG grant EXC1086)

Title: Integration of dispersed CA2 pyramidal cells in the hippocampal network in a focal epilepsy model

Authors: *A. KILIAS[1,2,3], S. TULKE[3,5], N. BARHEIER[5], K. HEINING[1,2,3], U. EGERT[1,2,4], C. A. HAAS[1,5,4], U. HÄUSSLER[5,4]
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Abstract: Mesio-temporal lobe epilepsy is characterized by recurrent spontaneous seizures and extensive cell loss in the CA1, CA3 and hilar regions of the hippocampus. Granule cells and CA2 pyramidal cells, however, are mostly spared from cell death but their interconnection is pathologically altered. Dentate mossy fibers sprout within the dentate gyrus (DG) and into the pyramidal cell layer of CA2. How CA2 pyramidal cells are integrated into the hippocampal network after they lose their target neurons in CA1 and whether they participate in the generation of epileptic and non-epileptic activity patterns remained unclear. To investigate connectivity and activity of the surviving CA2 pyramidal cells in MTLE we unilaterally injected kainate (KA) into the hippocampus of Amigo-cre/ERT2 mice which express cre-recombinase in CA2 pyramidal cells and in littermates. NaCl injections served as controls. One subgroup of mice received an ipsilateral injection of a cre-dependent adeno-associated virus (phSyn1(S)-FLEX-tdTomato-T2A-SypEGFP-WPRE) into CA2 to trace axons of ipsilateral CA2 pyramidal cells. We found strong projections to CA1 and to the contralateral hippocampus in controls. While the projection to CA1 was lost in chronic epileptic mice in agreement with the loss of CA1 pyramidal cells, the projection to contralateral CA2 was preserved. A second subgroup of chronically epileptic and control mice was implanted either with wire electrodes bilaterally into the DG and CA2 or with a silicon probe into the ipsilateral CA2 region. Local field potentials recorded while mice were freely behaving showed alternating epileptic and non-epileptic activity patterns in the DG and CA2 of both hippocampi. Current source density analysis of epileptic activity patterns revealed that, analogous to dentate granule cells, CA2 pyramidal cells

participate in the generation of epileptic activity. Likewise, we found sinks/sources that were alternating at theta frequency in the somatic regions of CA2 during periods free of epileptic activity. Interestingly, the frequency of these theta oscillations was decreased bilaterally in CA2 of epileptic animals when compared to controls. This is in line with the theta frequency reduction reported for the entire DG and the MEC. We conclude that CA2 is an active part of the epileptic network and might contribute to the propagation of epileptic activity towards the contralateral hippocampus by its bilateral connection.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

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Program #/Poster #: 561.09/G11

Topic: B.10. Epilepsy

Support: DFG grant EXC1086

Title: Activity-dependent Arc expression is associated with synaptic plasticity of dentate granule cells during epileptogenesis

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Abstract: Remodeling of neuronal circuits is known to be largely activity-dependent. However, the relationship between neuronal activity and synaptic plasticity during the development of mesial temporal lobe epilepsy (mTLE) remains poorly understood. Therefore, the present study aimed to provide an integrated view on epileptic activity, activity-dependent gene expression and synaptic plasticity in the hippocampus during kainic acid-induced epileptogenesis in mice.We show that shortly after status epilepticus, seizure activity is present and persists throughout epileptogenesis in both, sclerotic and non-sclerotic regions of the hippocampal formation. The sclerotic hippocampus differed from non-sclerotic regions by displaying milder paroxysmal discharges, which increased in their severity over time. This increase was paralleled by the upregulation of the activity-related cytoskeleton protein (Arc) gene expression exclusively in dentate granule cells (DGCs) residing in the sclerotic hippocampus. Importantly, we found that Arc mRNA-upregulating DGCs exhibited an increase in their spine density and size within the

terminal field of entorhinal afferents. But at the same time the density of AMPA-type glutamate receptors decreased. In order to probe its functional significance in mTLE, we performed optogenetic stimulation of entorhinal synapses on DGCs in vivo and showed that seizure activity was evoked with higher probability under epileptic conditions. Moreover, optogenetically-induced seizures failed to induce dendritic translocation of Arc mRNA and further AMPAR attenuation only in sclerotic regions of the hippocampus, supporting the notion of a local breakdown of the dentate gate in mTLE. We conclude that during epileptogenesis epileptic activity emerges early and persists in the whole hippocampus, however, only the sclerotic part shows modulation of seizure severity accompanied by plasticity of DGC synapses. In this context, we identified *Arc* as a putative mediator between seizure activity and synaptic plasticity.
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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

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Topic: B.10. Epilepsy

Support: DFG (HA7597)
Excellence Cluster 'BrainLinks-BrainTool' DFG (EXC1086)

Title: Molecular and structural characterization of inhibitory innervation of the CA2 region in experimental epilepsy

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Abstract: Mesial temporal lobe epilepsy (MTLE) is often associated with extensive loss of excitatory neurons in the regions CA1, CA3 and hilus and inhibitory neurons throughout the hippocampus. In contrast, the majority of granule cells and CA2 pyramidal cells (PCs) survive and contribute to epileptic activity. We have shown before that sprouted mossy fibers not only induce recurrence between granule cells but also form aberrant synapses on CA2 PC somata (Häussler et al., 2016, Hippocampus). The molecular nature of synaptic inputs (mossy fibers and other terminals) innervating the CA2 region in MTLE is, however, still unclear. To characterize synaptic inputs to the CA2 region we induced MTLE with a unilateral injection of kainate (KA)

into the hippocampus of transgenic Thy1-EGFP mice which intrinsically express EGFP in a subset of adult granule cells and mossy fibers and in Rbp4-Cre mice expressing Cre-recombinase in granule cells. Rbp4-Cre mice received an adeno-associated virus (phSyn1(S)-FLEX-tdTomato-T2A-SypEGFP-WPRE) injection inducing tdTomato expression in somata and EGFP in mossy fiber synapses. At 21d after injection we performed *in situ* hybridization for glutamic acid decarboxylase 67 (GAD67) and immunohistochemistry for GAD65, both key enzymes for GABA production, vesicular GABA transporter (vGAT) and potassium-chloride-cotransporter 2 (KCC2) and localized CA2 PCs with PCP4 or RGS14, followed by Imaris-based reconstruction of synapses. We show only slight variations in somatic GAD67 mRNA expression in granule cells but strongly upregulated expression of GAD65 in mossy fiber terminals innervating CA2 and an increased fraction of EGFP+GAD65-expressing mossy fiber boutons contacting CA2 PC somata. Importantly, we did not detect any co-expression of GAD65 with vGAT in mossy fiber terminals, indicating that in case GABA is produced by GAD65 it is not loaded into synaptic vesicles, rendering classical synaptic GABA release unlikely. Yet, we found a substantial plexus of vGAT-positive fibers which did not express EGFP (neither intrinsically in Thy1-EGFP mice, nor AAV-driven in Rbp4-Cre mice) in CA2 indicating preservation of inhibitory nerve terminals. KCC2 was persistently expressed after KA injection indicating intact postsynaptic prerequisites for inhibition of CA2 PCs. Altogether, we hypothesize that despite expressing GAD67, sprouted mossy fiber synapses do not contribute to GABA-ergic transmission. Instead, CA2 PCs are still innervated by other GABAergic fibers and express KCC2 which might contribute to their resilience towards epileptogenicity.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

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Program #/Poster #: 561.11/H1

Topic: B.10. Epilepsy

Support: NIH R37NS100901

Title: Neural and hemodynamic mechanisms underlying variable consciousness impairment in rodent absence seizures

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Abstract: Absence seizures, characterized by behavioral impairment and a distinct rhythmic spike-and-wave electrographic signature, are associated with attentional deficits and developmental difficulties that have a major impact on quality of life. While clinical symptoms have been largely described, the mechanisms of how absence seizures impair cognition and behavior remain unknown, and therapy often fails. Human studies suggest that the degree of behavioral impairment varies, with accompanying hemodynamic and electrographic changes. Early data from our lab provide evidence that undrugged conditions may be necessary for the preservation of electrophysiology and hemodynamics during absence seizures in Genetic Absence Epilepsy Rats from Strasbourg (GAERS). This awake, non-medicated animal model of absence seizures provides the opportunity to investigate the neuronal mechanisms underlying behavioral impairments. Hemodynamics, electrophysiology and behavioral components of absence seizures were studied in awake adult female GAERS, aged 4-8 months. Rats were trained for awake body and head restraint, and were implanted with frontoparietal epidural EEG electrodes to electrically detect spike-and-wave discharges (SWDs). EEG was recorded with simultaneous local cerebral blood flow (CBF) and multi-unit activity (MUA). Rats were also trained on one of two behavioral tasks of increased complexities: a repetitive spontaneous licking task and a goal-oriented sensory detection task. For the spontaneous licking task, rats were encouraged to lick a spout intermittently by the presentation of a 20% sucrose water reward at varying intervals. In the sensory detection task, an 8KHz tone was used to signify reward availability. There were significant increases in CBF and MUA seen in deep layers of multiple regions of the cortex for the first 2 seconds of seizure activity, followed by a reduction from baseline for the remainder of the seizure duration. Larger CBF and MUA increases were associated with longer seizures. The increase in MUA appeared to be more dependent on seizure frequency than intensity of neural firing. SWDs in GAERS were accompanied by impaired performance in behavioral paradigms - repetitive spontaneous licking decreased upon SWD initiation, while stimulus responses were less reliable during SWDs. The degree of impairment varied significantly between seizures and was associated with electrographic changes. These findings may lead to better understanding of cellular mechanisms for variable severity in absence epilepsy and potentially guide improved therapy options.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 561.12/H2

Topic: B.10. Epilepsy

Support: NIH Grant R37NS100901

Title: Awake fMRI in a rat model of absence epilepsy

Authors: *Z. B. KRATOCHVIL[1], C. P. MCCAFFERTY[1], P. HERMAN[1], J. H. RYU[1], B. G. SANGANAHALLI[2], B. F. GRUENBAUM[1], P. ANTWI[1], W. ISLAM[1], E. A. JOHNSON[1], P. VITKOVSKIY[1], I. FREEDMAN[1], A. J. KUNDISHORA[1], A. DEPAULIS[3], F. HYDER[1], H. BLUMENFELD[1]
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Abstract: Absence epilepsy is the most common form of epilepsy in children and raises significant challenges for learning and quality of life. Current pharmacological therapies frequently fail to fully treat this condition or lead to side effects. Therefore, it is important to understand the underlying neural mechanisms towards the development of better therapeutic options. Such mechanistic investigation requires validated animal models. Previous work has focused on sedated animals, but has not replicated the behavioral components or hemodynamics of human absence seizures. We hypothesize that seizure hemodynamics and behavior in awake, drug free animals will be more consistent with those of human seizures. Here, we report head-fixed, awake, drug free functional magnetic resonance imaging (fMRI) of Genetic Absence Epilepsy Rats from Strasbourg (GAERS), an established rat model of absence epilepsy, with simultaneous electroencephalography (EEG) recordings. Animals were incrementally acclimated to the head-fixed apparatus and to a recording of sounds from the high field magnet (9.4T) over 3 weeks. They were then scanned in a total of 117 sessions across 18 animals, during which we monitored 1719 seizures. Echo-planar imaging (EPI) was used to acquire fMRI; T1- and T2-weighted anatomical images were obtained for signal localization. Preprocessing included estimation of motion, realignment, removal of motion and artifact epochs, functional to structural registration, registration of animals to a template, and spatial smoothing. Two analyses were performed: first, a voxel-wise general linear model comparing seizure to non-seizure periods and second, a region of interest (ROI) based time-course analysis. In the voxel-wise analysis seizures were associated with a decrease in blood oxygen level dependent (BOLD) signal in the primary somatosensory cortex and an increase in thalamic nuclei. This resembles the cortical decreases and thalamic increases seen in human fMRI during absence seizures. In the ROI based analysis, we also find different time-courses in the cortex vs thalamus for BOLD fMRI both during and

after seizures. We conclude that the hemodynamics of seizures in GAERS are like those in humans with absence epilepsy, supporting the application to humans of findings of electrophysiological recordings and behavioral testing from GAERS. We also conclude that like in human patients the BOLD signal in GAERS seizures has significant regional heterogeneity. This suggests that mechanistic investigations should take place in awake, drug free animal models to ensure translational validity and facilitate development of new therapies.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

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Program #/Poster #: 561.13/H3

Topic: B.10. Epilepsy

Support: NC 123240.1

Title: Increase of seizure activity by picrotoxin in thalamic reticular nucleus of the rat

Authors: *V. M. MAGDALENO-MADRIGAL, F. J. HIDALGO-FLORES, G. CONTRERAS-MURILLO, S. ALMAZÁN-ALVARADO
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Abstract: The deep brain stimulation (DBS) is used for the control of refractory epilepsy. In our laboratory we reported that DBS in the thalamic reticular nucleus (TRN) protects against seizures caused by Pentylenetetrazol (PTZ). The TRN contains inhibitory neurons that release GABA and is involved in the generation and control of spike-wave (SWD) and generalized tonic-clonic seizures (GTCS). The goal of this study was to analyze the behavioral and electroencephalographic (EEG) changes of the low-frequency stimulation (LFS) and the microinjection of picrotoxin (PTX) in the TRN. Wistar rats were implanted with a tripolar electrode and a cannula guide in the left TRN. The animals were randomly assigned to four groups: SS group, they received an ICV microinjection of saline solution (SS) and PTZ; PTX group, received an ICV microinjection of PTX and PTZ; SS/LFS group, received SS plus LFS and PTZ; PTX/LFS group, received PTX plus LFS and PTZ. Meantime, EEG recordings were done. The SWD number and latency were analyzed. In addition, the GTCS number, duration, latency, and severity. The results showed an increase in the number and duration of the GTCS of the groups PTX and PTX/DBS. The groups that received LFS showed a significant increase in the frequency of SWD and the amplitude. Our preliminary results suggest that the picrotoxin in

the TRN show a tendency to increase the GTCS effect that is facilitated with the LFS. Suggesting that the probable mechanism of the protective outcome of the DBS in the TRN it may be an increase in intrareticular inhibition.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

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Program #/Poster #: 561.14/H4

Topic: B.10. Epilepsy

Support: NIH Grant NS35439

Title: Experimental febrile status epilepticus increases seizure susceptibility in developing mice: A powerful experimental model?

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Abstract: Premise: Prolonged fever-invoked seizures (febrile status epilepticus; FSE) during infancy increases risk for epilepsy and cognitive deficits later in life, but the underlying mechanisms are incompletely understood. Experimental febrile status epilepticus (eFSE) in rats has successfully modeled human FSE, recapitulating epilepsy and cognitive problems seen in a subset of humans, yet rats do not enable the use of many genetic tools developed in mice. **Methods:** We used mice on the C57BL/6J background strain and examined if eFSE can be generated in immature mice and promote vulnerability to subsequent convulsant drugs. For eFSE, mice age P14-15 were maintained at temperatures between 40ºC to 40.9ºC for more than 30 minutes. We exposed naïve and eFSE-experiencing adult mice to subthreshold kainic acid dose (15 mg/kg) intraperitoneal. In a separate cohort, we assessed if eFSE induces epileptogenesis. We implanted electrodes into the hippocampus of control and eFSE experiencing mice and then recorded 24/7 video electroencephalogram and analyzed for seizures and spike series. **Results:** eFSE in immature mice increased susceptibility to kainic acid induced seizures, apparent from reduced latency and increased propagation, indicating an enduring change in the brain networks that are involved in limbic seizures. Studies of overt epileptogenesis are ongoing. **Conclusion**: EFSE is feasible in mice and provokes enduring pro-epileptogenic changes in the underlying brain circuits.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 561.15/H5

Topic: B.10. Epilepsy

Support: NSF Graduate Research Fellowship
NIH Research Grant R01 NS025704

Title: Hippocampal deletion of sodium channel Nav1.1 causes thermally evoked seizures and spatial learning deficits in a mouse model of Dravet Syndrome

Authors: *R. E. STEIN, J. S. KAPLAN, W. A. CATTERALL
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Abstract: Dravet Syndrome is a severe epileptic disorder with many debilitating comorbidities caused by haploinsufficiency of the *Scn1a* gene, which encodes the alpha-subunit of Nav1.1 voltage-gated sodium channels. Dravet Syndrome is characterized by treatment-refractory epileptic seizures that present before one year of age, followed by symptoms that include developmental delays, autism, and severe cognitive impairment. Mouse models of Dravet Syndrome closely mirror the mutations and phenotypes present in humans. Previous work has demonstrated that reduced sodium current due to heterozygous loss-of-function of Nav1.1 channels causes hypoexcitability of GABAergic interneurons, which is responsible for the core disease phenotypes of epilepsy, cognitive impairment, and social interaction deficits. However, the impact of reduced Nav1.1 expression in specific brain regions on epileptiform activity and co-morbidities is unknown. To elucidate this question, we used a floxed *Scn1a* mouse line and the Cre-Lox method to delete Nav1.1 in the hippocampus of C57BL/6 mice using targeted viral injections. The frequency of spontaneous inhibitory postsynaptic currents from GABAergic synapses onto dentate granule cells was impaired by this local gene deletion. Mice with local deletion of Nav1.1 experienced thermally evoked behavioral and electrographic generalized tonic-clonic seizures, which were similar in intensity to seizures in mice with global mutation of Nav1.1. Local gene deletion in the hippocampus also caused impairments in spatial learning and memory in the Barnes maze, but had no effect on novel object recognition or social interaction behaviors. Our results provide evidence that local Nav1.1 deletion in the hippocampus is sufficient to induce generalized tonic-clonic seizures and deficits in spatial learning and memory that are characteristic of Dravet Syndrome in mice and humans.

Disclosures: R.E. Stein: None. **J.S. Kaplan:** None. **W.A. Catterall:** None.

Poster

561. Models of Developmental Epilepsies and Seizure Disorders

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 561.16/H6

Topic: B.10. Epilepsy

Support: NIH Grant NS090843

Title: Characterization of kindled VGAT-Cre mice as a new animal model of pharmacoresistant epilepsy

Authors: J. STRAUB, A. GAWDA, P. RAVICHANDRAN, C. BURKE, J. KANG, I. VITKO, M. M. SCOTT, *E. PEREZ-REYES
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Abstract: Our long-term goal is to develop novel gene therapies for temporal lobe epilepsy (TLE) patients whose seizures are not controlled by drugs. Due to a lack of suitable animal models, there has been little progress in developing therapies for TLE. While testing the hypothesis that "inhibiting inhibitory neurons" was sufficient to induce seizures, we discovered that hippocampal kindling of VGAT-Cre mice leads to spontaneous seizures (protocol: twice a day, every day, @1.5x after-discharge threshold, ADT). Control experiments demonstrate electrical kindling was required to induce the epileptic phenotype. Spontaneous seizures developed ~10 days after kindling was complete (3 tonic-clonic seizures). These seizures resemble those in post-status epilepticus models of TLE in terms of both electrographic and behavioral components (tonic-clonic seizures). In contrast to post-status models, seizures in VGAT-Cre mice occur in the absence of neuronal death, absence of ectopic dentate granule cells, and with only a small increase in aberrant granule cell axon sprouting. These findings rule out changes that are commonly postulated as the cause of limbic seizures in post-status models. These mice express Cre recombinase under the control of the vesicular GABA transporter (VGAT), a gene that is specifically expressed in GABAergic inhibitory neurons. Loss or dysfunction of hippocampal GABAergic neurons has been linked to the development of TLE. Accordingly, we hypothesize Cre expression impairs the function of GABAergic neurons, leading to increased seizure susceptibility. Spontaneous seizures in kindled VGAT-Cre mice occur 1-2 seizures/day with little sign of clustering. We conclude kindled VGAT-Cre mice are an ideal model for screening novel anti-seizure and anti-epileptogenic drugs.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

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Program #/Poster #: 561.17/DP04/H7

Topic: B.10. Epilepsy

Support: Private Donations to TGen's Center for Rare Childhood Disorders
Michael and Robyn DeBell Grant

Title: Development of a zebrafish model to study childhood epileptic encephalopathy caused by dynamin 1 (DNM1) mutations

Authors: *G. C. MILLS, E. FRANKEL, J. DODSON, L. LLACI, R. GUPTA, B. GERALD, M. STRINGER, V. NARAYANAN, S. RANGASAMY
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Abstract: Next generation sequencing (NGS) technology has led to the identification of causal genes in epileptic encephalopathies. Recently, mutations in the DNM1 gene encoding dynamin 1 (OMIM: 602377) have been recognized to cause early infantile epileptic encephalopathy-31 (OMIM: 616346). While recent studies have provided insights into dynamin-1 structure and function, it is still unclear how the *de novo* missense mutation in DNM1—a core component of postsynaptic endocytosis machinery—leads to early epileptic encephalopathy (EE). It is critical to use valid animal models in our effort to understand the pathophysiology of EE caused by *DNM1* mutations. The zebrafish is an alternative model system with substantial benefits which is now widely used to study the pathophysiology of human Mendelian disorders, develop cost-efficient breeding, and practice *in vivo* drug discovery. Zebrafish Dnm1 is structurally similar (88%) to human DNM1 at the gene and protein levels. In this study, we utilized the zebrafish model to analyze the role of DNM1 in epilepsy and characterize disease-specific phenotypes using a reverse genetics approach. To create the animal model, wildtype AB zebrafish between six and 24 months old were bred to produce embryos which were then randomly assigned to treatments of the dynamin inhibitor (n = 417), the vehicle treatment, DMSO (n = 306), or left untreated (n = 376). At 72 hours post fertilization (hpf), zebrafish locomotion was captured through videography and behavior was analyzed for seizures and curvature. By creating a 2d-trace map (path length) of zebrafish motion, we were able to evaluate convulsant-like locomotor behaviors in the zebrafish. We found a significant increase ($p<0.001$) in locomotor pathlength between treatment and both the vehicle treatment and the untreated zebrafish at the same time interval. Two-tailed, heterozygous t-tests showed a significant increase ($p<0.001$) in seizure and curvature expression in 72 hpf zebrafish. Seizures were classified in this study as quick and involuntary movements in accordance to previous research. By targeting *dnm1a* using Morpholinos (Gene Tools, LLC.), we recapitulated the seizure-like activity in 48 hpf embryos

seen using DNM1 inhibitors, but the seizures were less severe than those produced by chemical inhibition of dnm1 in the zebrafish. Our preliminary data indicates that the disruption of Dnm1 leads to seizure like-activity in zebrafish, suggesting that this is a promising model system. This approach can potentially lead to the identification of novel therapies and treatment of epileptic encephalopathy caused by DNM1 mutation in humans.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

Location: SDCC Halls B-H

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Program #/Poster #: 561.18/H8

Topic: B.10. Epilepsy

Support: Dravet Syndrome Foundation Grant 201600552
NIH Grant GM119831

Title: Deletion of a key Scn1a regulatory element causes severe phenotypes in mice

Authors: *A. S. NORD[1], T. W. STRADLEIGH[1], I. ZDILAR[1], M. SRAMEK[1], A. NGUYEN[1], A. ADHIKARI[2], N. COPPING[3], J. L. SILVERMAN[4]
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Abstract: Dravet Syndrome (DS) is understood to be a disorder driven primarily by loss-of-function mutations in *SCN1A*, a gene encoding the $Na_V1.1$ sodium channel. We hypothesize that mutations to non-coding DNA regulatory elements (REs) represent a secondary causal mechanism in *SCN1A*-associated pathology. Changes to REs can produce strong phenotypes, and there are instances of in patients of large genomic deletions affecting the *SCN1A* without impacting coding sequence, yet the role of gene regulation in DS is not well understood. We used the Cas9/CRISPR system to generate a mouse model harboring a deletion of the h1b non-coding RE of *Scn1a*. Three genotypes were investigated at all developmental time points: wild type (WT), deletion carrier (*Scn1a*-REdel$^{+/-}$), and homozygous deletion (*Scn1a*-REdel$^{-/-}$). Mice expressing a homozygous deletion of RE demonstrated severely reduced survival after P28. No early lethality effects have been noted in heterozygous deletion carrier mice. *Scn1a* was the most significant differentially expressed gene identified via RNA-seq, with dosage-sensitive down-regulation in h1b deletion brain. We did not identify a strong global signature of differential expression at P7, but did find suggestive evidence of pathology-related changes even at this early stage of life. We are currently evaluating *Scn1a* transcription and global expression changes via

RNA-seq at later ages. WB analysis and IHC indicates Na_V1. 1 expression follows a dose-dependent relationship, with expression differences most pronounced at the later developmental time points (P21, P28). We demonstrate that targeting the h1b element with dCas9-p300 can drive increased *SCN1A* mRNA levels in human cells. These data suggest we have created a novel alternative mouse model of DS and *SCN1A*-associated developmental disorders that relies upon deletion of a non-coding regulatory element associated with *SCN1A*. This work and extension of the approach to characterize other *Scn1a* REs has the potential to generate new insights about pathology and guide diagnosis and treatment of DS and *SCN1A*-associated disorders in the future.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

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Program #/Poster #: 561.19/H9

Topic: B.10. Epilepsy

Support: NIH/NINDS grant R01-NS096976
NIH/NINDS grant R01-NS103139

Title: A novel zebrafish model of GABRB3-linked childhood epilepsy

Authors: *C. A. CARPENTER, B. P. GRONE, S. C. BARABAN
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Abstract: Epilepsy is a debilitating, chronic neurological disorder, where individuals experience spontaneous and recurrent seizures. Although a variety of anti-epileptic drugs (AEDs) are available, 20-30% of patients, many suffering with genetic childhood epilepsies, remain classified as pharmaco-resistant. To address this problem, there is need for further elucidation of the causative elements of epilepsy and improvements in the current AED discovery process. Reduced expression or function of $GABA_A$ receptor β3 subunit (GABRB3) is linked to the pathogenesis of a collection of early-onset epilepsies. *GABRB3* null mice exhibit seizures, impaired learning, poor motor performance and hyperactivity. Nonetheless, rodents are not an ideal species for drug discovery. Through this study we aim to establish the first zebrafish model of GABRB3 deficiency. Using CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas9 system, we generated mutant *gabrb3* zebrafish and our goal is to validate whether this model accurately recapitulates salient features of the human epileptic disorder. Here we describe our initial characterization of the behavioral, electrophysiological, pharmacological and morphological phenotypes of mutant *gabrb3*$^{-/-}$ zebrafish. In vivo electrophysiology experiments

at 5 days postfertilization (dpf) indicate that *gabrb3*$^{-/-}$ larvae exhibit spontaneous electrographic seizure events characterized by brief high-frequency interictal-like events and rare larger, long duration multi-spike discharges, which are absent in recordings from the wild-type control larvae. In locomotion assays, freely swimming *gabrb3*$^{-/-}$ larvae are hyperactive compared to age-matched wild-type control larvae at 5 dpf. We are also testing the effect of various AEDs in these behavioral and electrophysiological assays including, but not limited to, carbamazepine, valproate, ethosuximide, topiramate and diazepam. Taken together, our results already point to similarities between our *gabrb3*$^{-/-}$ zebrafish larvae and GABRB3-linked human epilepsies. We are therefore excited by the prospect of generating a novel, translational model that will help us better understand childhood epilepsies and discover new effective AEDs.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 561.20/H10

Topic: B.10. Epilepsy

Title: Status epilepticus induced in the infant rat by pentylenetetrazol and lithium pilocarpine promotes similar c-Fos expression in the hippocampus and the cerebellum

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Abstract: Consequences of *status epilepticus* (SE) in the developing brain can be assessed through different experimental models. SE can be induced by pentylenetetrazole (PTZ) characterized by clonic-tonic seizures, or by lithium-pilocarpine (Li-Pilo) characterized by complex partial seizures with secondary generalization. The goal of this study was to compare the effect of SE induced by PTZ (PTZ-SE) or LI-Pilo (Li-Pilo-SE) in infant rats on c-Fos protein expression in the hippocampus and cerebellum. SE was induced in fourteen-days-old Wistar rat pups (both sexes). PTZ-SE was produced by 55mg/kg of PTZ (n=6); Li-Pilo-SE was induced by 3mEq/kg of LiCl (on the day before the induction of SE) and 100mg/kg of pilocarpine hydrochloride (n=7). Control animals were given an equal volume of saline or LiCl followed by saline, respectively (n=6). 90 to 120 min after SE or control conditions, rats were anesthetized and transcardially perfused with 4% paraformaldehyde; the brain and the cerebellum were removed, cryoprotected in 30% sucrose and cut to obtain 40-µm-thick coronal (dorsal

hippocampus) or sagittal (medial vermis of cerebellum) sections. Colorimetric immunohistochemistry was performed to detect c-Fos immunoreactive (Fos-IR) cells in the hippocampal CA1, CA2 and CA3 pyramidal layer and dentate gyrus (DG) granular layer, as well as in the cerebellar granular layer of lobules I-X (ROI 30,000 um^2). Differences between SE models were analyzed with the U Mann Whitney test (data are expressed as the median); a multivariate clustering analysis was performed to identify similarities in neuronal activation after SE. Scarce or null c-Fos immunoreactivity was detected in controls. There was a higher number of Fos-IR cells in the CA2 area after Li-Pilo-SE (185) than after PTZ-SE (82; p=0.0082); no additional differences were observed in the hippocampus (PTZ-SE: CA1=49, CA3=85, DG=139; Li-Pilo-SE: CA1=146, CA3=113, DG=125). The number of Fos-IR cells after PTZ-SE (I=130, II=150, III=127, IV=137, V=114, VIa=130, VIc=137, VII=125, VIII=177, IX=186, X=147) or Li-Pilo-SE (I=119, II=116, III=122, IV=128, V=129, VIa=126, VIc=162, VII=191, VIII=185, IX=180, X=163) was similar in all the cerebellar lobules. The clustering analysis showed three brain regions with different c-Fos expression after SE (PTZ and Li-Pilo SE; Cohen coefficient=0.8586): 1) Hippocampus (including CA1, CA2 and DG), 2) Anterior lobe of cerebellum (including lobules I-V and VIa) and 3) Posterior lobe of cerebellum (including lobules VII-X). In conclusion, SE induced in the infant rat by two different experimental models promotes similar neuronal activation in the hippocampus and the cerebellum.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

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Program #/Poster #: 561.21/H11

Topic: B.10. Epilepsy

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The Ryan J. Murphy SUDEP Research Funds at UW
CURE 2017, Sleep and Epilepsy Award

Title: Contributions of excitatory and inhibitory neurons to epilepsy and sudden death susceptibility in Leigh syndrome

Authors: A. M. BARD[1], I. T. BOLEA[2], N. SAHAI[1], J. RAMIREZ[3], A. QUINTANA[4], *F. K. KALUME[1]
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Neurosci. and Dept of Cell Biology, Physiol. and Immunol., Univ. Autonoma De Barcelona, Bellaterra, Spain

Abstract: Epilepsy and premature mortality are common and prominent features of Leigh syndrome (LS) (OMIM: #256000). Leigh Syndrome (LS), or subacute necrotizing encephalopathy, is a debilitating, progressive, and neurodegenerative mitochondrial disorder of childhood. Mouse models of LS, generated by global or CNS-specific Knock-out (KO) of *Ndufs4* recapitulate several key clinical features of the disease in humans, including spontaneous seizures and premature death.
We examined the contribution of excitatory and inhibitory neurons to the development of epilepsy and sudden death phenotypes in the mouse model of LS.
Methods: Control mice and mice with *Ndufs4* knocked out selectively in either GABAergic interneurons (Gad2-specific KO mice) or in Vglut2-positive glutamatergic neurons (Vglut2-specific KO mice) were generated by crossing the *Ndufs4* floxed mice with the Gad2Cre or Vgut2Cre driver mice respectively. Thermal seizure susceptibility test, plethysmography, and video-EEG assessment of the mice were conducted as described in our previous work.
Results: All Gad2-specific KO (not control) mice exhibited spontaneous behavioral seizures and died prematurely. Series of myoclonic seizures, often preceding and following a generalized tonic-clonic seizure, were observed beginning on postnatal day (P) 32. Premature death occurred starting at P49, with none of the mice surviving past P82. All witnessed deaths occurred immediately following a Racine 5 generalized spontaneous seizure, suggesting they are precipitated by a seizure. Combined video-EEG recordings revealed generalized interictal epileptiform spikes during resting behavior or sleep as well as spontaneous and thermal seizures, marked by high-voltage spike and wave EEG discharges closely associated with hypermotor behaviors lasting 31 ± 9 seconds. In a striking contrast, Vglut2-specific KO mice did not show any behavioral or electrographic sign of predisposition to spontaneous or thermal seizures. Interestingly however, they develop respiratory disturbances and succumb to non-epilepsy related premature death, starting at ~P50, with none of the mice surviving past P200.
Conclusion: These results suggest for the first time that LS can lead to fatality via sudden unexpected death in epilepsy (SUDEP) or non-SUDEP. *Ndufs4* KO in GABAergic neurons is critical for the development of epilepsy and SUDEP in LS. Whereas the same mutation in excitatory neurons is central in the pathogenesis of respiratory abnormalities and non-SUDEP phenotype.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

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Program #/Poster #: 561.22/H12

Topic: B.10. Epilepsy

Support: ISF grant 1454/17
Fondation Jérôme Lejeune grant 1565
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Title: Using Dravet Syndrome mice to trace the progress of Dravet-associated comorbidities

Authors: *M. RUBINSTEIN[1], S. FADILA[2], Y. ALMOG[2], K. ANDERSON[2]
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Abstract: Dravet syndrome (Dravet) is an infantile epileptic encephalopathy with ominous course. Children develop normally during the first year of life but subsequently exhibit unusually severe febrile seizures that progress to prolonged refractory seizures and frequent episodes of status epilepticus. Following the onset of epilepsy, developmental delay becomes evident with cognitive decline, appearance of autistic features, hyperactivity and motor deficits. Importantly, while toward adolescence the epilepsy improves, the invalidating comorbidities persist. The onset of Dravet-associated comorbidities and its relationship to the recurrent seizure are unclear. Here, we conducted combined electrophysiological and behavioral studies of the Dravet mouse model in order to characterize developmental changes in Dravet phenotypes, examining neuronal changes and the presentation of Dravet-associated comorbidities. Electrophysical brain slice recordings from hippocampal interneurons demonstrated that at P14, prior to the onset of spontaneous seizures in mice, the excitability of wild-type and Dravet interneurons is similar. However, at P21, when spontaneous seizures appear and premature death is frequent, the excitability of hippocampal inhibitory neurons is reduced. These recordings suggest that the onset of epilepsy correlates with the onset of reduction in inhibition, supporting the view of disinhibition as the cause of Dravet. In contrast, motor deficits, characterized by lower performances on the rotarod and wider base of support in both front and hind paws, are evident already at P14, before the onset of epilepsy. Surprisingly, these deficits were improved at the onset of seizures. Thus, motor dysfunction precedes the onset of seizures, with transient improvement at the onset of seizures, and further decline at adulthood. However, hyperactivity assessment in the open field showed a different progression profile with later onset; while no changes were observed at P14 and P21, hyperactivity was evident at P35, after the period of instance seizures and frequent death. Together, these results indicate that Dravet-associated comorbidities change thorough development, with some appearing before the onset of epilepsy and others that are evident only later in life.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

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Topic: B.10. Epilepsy

Support: NIH NINDS K08 NS097633
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Title: Vasoactive intestinal peptide-expressing interneurons are impaired in a mouse model of Dravet syndrome

Authors: *K. GOFF[1], E. M. GOLDBERG[2]
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Abstract: Dravet syndrome (DS) is a severe neurodevelopmental disorder defined by intractable epilepsy and a high rate of autism spectrum disorder. It is caused primarily by *de novo* mutations in *SCN1A* which codes for the voltage gated sodium (Na+) channel alpha subunit Nav1.1. Nav1.1 is prominently expressed in GABAergic interneurons, and it is hypothesized that selective dysfunction of interneurons leads to impaired inhibition in the developing brain, which in turn leads to DS pathology. Both parvalbumin (PV-INs) and somatostatin expressing interneurons (SST-INs) are impaired in DS; however, the function of the third major group of interneurons - the vasoactive intestinal peptide (VIP) expressing interneurons (VIP-INs) - has yet to be specifically investigated. Here, we used *Scn1a*$^{+/-}$ mice crossed to VIP-Cre.tdTomato reporter mice to perform targeted whole cell recordings from VIP interneurons in layer 2/3 primary somatosensory cortex of acute brain slices prepared from male and female mice across development. We demonstrated that VIP-INs from *Scn1a*+/- mice exhibit evidence of hypoexcitability relative to age-matched wild-type littermate controls, with a depolarized action potential threshold, reduced steady state firing frequency, and prominent spike height attenuation in both post-natal day (P) 18-21 and P35-56 age groups, consistent with the presence of Nav1.1 in VIP-INs. Partial block of Na+ channels in VIP-INs with low concentrations of TTX mimics the *Scn1a*$^{+/-}$ phenotype. We then used single-cell PCR to investigate the expression of Nav1.1 isoforms, as well as other neural Na+ channels, in VIP interneurons. Finally, as VIP-INs are considered to serve a predominantly disinhibitory role in cortex via inhibition of SST-INs, we tested the function of this microcircuit in DS by optogenetically stimulating corticocortical motor afferents in the superficial layers of barrel cortex while measuring activation of VIP-INs and disynaptic inhibition of SST-INs. Our results show that VIP-INs express Nav1.1 and, along with PV and SST-INs, are dysfunctional in DS. This is an important step towards understanding how loss of Nav1.1 gives rise to the circuit abnormalities that underlie epilepsy and cognitive

dysfunction in DS. As VIP-INs are presumed to be disinhibitory, VIP-IN dysfunction may contribute to non-epilepsy comorbid conditions in DS.

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Poster

561. Models of Developmental Epilepsies and Seizure Disorders

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Program #/Poster #: 561.24/H14

Topic: B.10. Epilepsy

Support: NIH/NINDS U01 NS090340

Title: Interictal dentate gyrus hyperexcitation in a mouse model of Dravet syndrome

Authors: *I. AIBA, J. L. NOEBELS
Baylor Col. of Med., Houston, TX

Abstract: Dravet syndrome is an infantile onset genetic epilepsy that involves a variety of comorbidities including autonomic and cognitive dysfunction and an extremely high risk of premature sudden death (SUDEP). The majority of DS cases are caused by loss of function mutations in the SCN1A gene which reduces the excitability of inhibitory neurons and contributes to network hyperexcitability. Apart from this well characterized cellular mechanism, the neuroanatomical basis of the seizures and comorbidity of DS is not well understood. While the SCN1A gene is widely expressed in the CNS, it is not known which brain regions are particularly vulnerable to the mutation and whether specific single brain regions could contribute to epilepsy and individual comorbidities of the DS.
To address this question, this study screened for hyperexcitable brain regions in a mouse model of Dravet Syndrome (Scn1a+/R1407X) using the FosTRAP system which genetically labels neuronal populations during a ~12 hour time window after activation by tamoxifen administration. Scn1a mutant mice (P20-30) were crossed with FosTRAP as well as a tdtomato reporter line to fluorescently label the activated population during a period while cortical EEG activity was continuously recorded. In a series of such experiments, we detected robust and reproducible labeling within the dentate gyrus (DG) of juvenile Scn1a mutant mice. The same labeling pattern could be detected even in animals which did not show visible generalized convulsive seizures, suggesting the activation was likely due to localized subcortical hyperexcitation. Remarkably, this DG hyperexcitation pattern was restricted to a juvenile developmental stage when SUDEP incidence is high; thus DG activity labeling was most reliably detected in mutant mice younger than P30 when the majority of SUDEP cases are detected, but was never detected in animals older than P60 when SUDEP incidence is extremely rare.
These preliminary results suggest the presence of age-dependent, spatially restricted

hyperexcitation patterns in the hippocampal formation of DS mice. Because of the involvement of the DG in seizure gating, cognitive functions, and limbic output, this localized DG activation may define an early and reversible interictal network excitability defect contributing to epilepsy and comorbidities in this DS model.

Disclosures: J.L. Noebels: None.

Poster

561. Models of Developmental Epilepsies and Seizure Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 561.25/H15

Topic: B.10. Epilepsy

Support: IBACS UCONN
Munson Family foundation

Title: BRAFV600E expression in mouse neocortical progenitors is sufficient to induce glial activation, elevate neuronal excitability, and cause seizures in mice

Authors: *R. GOZ[1], J. J. LOTURCO[2]
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Abstract: The mechanisms by which low-grade neuroepithelial tumors (LNETs) associated with epilepsy cause hyper-excitability and hyper-synchrony of cortical tissue are not completely understood. Hypotheses include somatic mutations transforming the physiology of neurons, and mutations altering the physiology of astrocyte networks and function. BRAFV600E mutations have now been identified as a common somatic mutation in pediatric low grade glioma, and the most common mutation in ganglioglioma, two focal lesions that cause seizures. We found that introducing human BRAFV600E mutations into mouse neocortical progenitors by *in utero* electroporation and piggyBac transposition resulted in focal cortical developmental disruptions and behavioral and electrographic seizures in mice. The developmental alterations included a several fold increase in astrocytogenesis relative to neurogenesis in Glast+ progenitors and an opposite effect in Nestin+ progenitors, astrocytes activation, increased gene expression related to inflammatory responses including elevated expression in genes in the classic complement pathway. In whole-cell patch clamp recordings of cortical neurons in slices we found that BRAFV600E mutant neurons showed marked changes in intrinsic excitability relative to neighboring control pyramidal neurons. Elevated excitability in the current-clamp included more hyperpolarized threshold to Action Potential (AP) firing, and increased AP firing frequencies in response to depolarizing current pulses. Some BRAFV600E neurons also showed a distinct type of bursting behavior that was not observed in control pyramidal neurons. In addition, BRAFV600E expressing neurons had increased rebound excitation, and increased voltage

hyperpolarization induced SAG. In voltage clamp experiments, BRAFV600E neurons had increased Ih currents, and reduced sustained potassium currents. Early activation of potassium currents contributing to the sustained currents with retigabine decreased the AP firing frequencies. Unlike the effects of BRAFV600E, neurons induced to over-express human BRAF wild type (wt) displayed no significant changes in intrinsic excitability compared to controls. Based on these results we propose that the same somatic mutation arising in neocortical progenitors can both increase pyramidal neuron excitability cell autonomously and increase the numbers and activation of astrocytes.

Disclosures: R. Goz: None. **J.J. LoTurco:** None.

Poster

561. Models of Developmental Epilepsies and Seizure Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 561.26/H16

Topic: B.10. Epilepsy

Support: Dup15q Alliance
NIH Grant 5R21HD091541

Title: Transcriptomic and proteomic profiling in an epilepsy fly model reveals cell non-autonomous downregulation of synaptic proteins

Authors: *K. A. HOPE[1], D. JOHNSON[2], D. KAKHNIASHVILI[3], L. REITER[1,4,5]
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Abstract: Duplication 15q syndrome (Dup15q) is caused by maternally inherited duplications of chromosome 15q11.2-q13.1 and has a high rate of treatment resistant epilepsy. Previous research in mice focused on the neuronal overexpression of *UBE3A,* which is located within 15q11.2-q13.1, yet seizures were not observed in any of these models. Our lab recently generated a novel fly model that recapitulates the Dup15q seizure phenotype when *UBE3A* is overexpressed in glial cells, not neurons, implicating glia in Dup15q epilepsy. To investigate the effects of *UBE3A* overexpression in glia compared to neurons we employed proteomic analysis through liquid chromatography coupled to high-resolution mass spectrometry and transcriptome analysis through RNA-sequencing of whole fly head extract in glial *Dube3a* (fly *UBE3A* homolog) vs neuronal *Dube3a* overexpression. We measured approximately 2,500 proteins at both the transcript and protein level, allowing us to identify genes that were altered only at the transcript level, only the protein level, or both the transcript and protein level in glial or neuronal overexpressing lines. Gene ontology analysis revealed enrichment in synaptic proteins downregulated at both the transcript and protein level following overexpression of *Dube3a* in

glia (*repo>Dube3a*), including *synapsin, Sap47, Syx1a,* and *Nwk*. These synaptic proteins were relatively unchanged in neuronal *Dube3a* overexpression (*elav*>Dube3a), indicating synaptic proteins in neurons change in a cell non-autonomous manner upon glial overexpression of *Dube3a*. Additionally, we identified an upregulation of glutathione s-transferase (GST) genes in *repo>Dube3a* flies. GSTs are known to metabolize xenobiotics and may underlie the treatment resistant nature of Dup15q epilepsy. We are currently investigating whether downregulation of synaptic proteins and upregulation of GSTs is specific to our Dup15q epilepsy seizure model or if this is common across multiple "gliopathic" epilepsy types. In summary, cell non-autonomous downregulation of synaptic proteins may play a key role in Dup15q epilepsy, and an upregulation of GSTs may be common across multiple seizure types driven by glial abnormalities.

Disclosures: K.A. Hope: None. **D. Johnson:** None. **D. Kakhniashvili:** None. **L. Reiter:** None.

Poster

561. Models of Developmental Epilepsies and Seizure Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 561.27/H17

Topic: B.10. Epilepsy

Support: Thelethon Foundation, Italy, Grant GGP17176
Jérôme Lejeune Foundation

Title: Characterization of a novel antiepileptic therapy by targeting eEF2K/eEF2 pathway for Dravet syndrome

Authors: *C. SALA[1], L. GRITTI[1], L. PONZONI[2], S. BERETTA[1], P. SCALMANI[3], M. A. MANTEGAZZA[4], M. SALA[1], C. VERPELLI[1]
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Abstract: Eukaryotic Elongation Factor 2 Kinase (eEF2K) is a ubiquitous Ca2+/Calmoduline-dependent kinase that regulates protein translation by catalyzing the phosphorylation of eEF2 at Thr56. In neurons, eEF2K is activated by Ca2+ influx mediated by glutamate stimulation leading to an increased the expression of certain proteins involved in synapse formation and plasticity, whereas the general protein translation is decreased. We recently demonstrated that eEF2K activity regulates excitation/inhibition ratio in the brain. In particular, eEF2K-/- mice display enhanced GABAergic transmission and tonic inhibition by the upregulation of proteins involved in inhibitory synapses functioning and are less susceptible to epileptic seizures. Accordingly, to

these data, we propose eEF2K/eEF2 pathway as a possible target for antiepileptic therapies (Heise et al., 2017). We studied the effect of eEF2K deletion in Scn1a+/- mice, through a genetic approach. We generated a mouse model by crossing Scn1a+/- mice with eEF2K-/- mice. First, we found that eEF2K deletion protected Scn1a+/- mice from the onset of epileptic seizures either under basal condition or under thermal stress, a condition known to trigger seizures in Dravet syndrome patients as well as in Scn1a+/- mice. Also, motor coordination defect, memory impairments, and stereotyped behavior are reverted by eEF2K depletion. The analysis of spontaneous inhibitory postsynaptic currents (sIPSCs) suggested that the rescue of the pathological phenotype was driven by the potentiation of the GABAergic synapses. In addition, the analysis of eEF2 phosphorylation in samples from cerebral cortex and hippocampus of Scn1a+/- mice revealed that eEF2K/eEF2 pathway might play a role in the progression of the pathology. Heise C et al. (2017) eEF2K/eEF2 Pathway Controls the Excitation/Inhibition Balance and Susceptibility to Epileptic Seizures. Cereb Cortex 27:2226-2248.

Disclosures: C. Sala: None. **L. Gritti:** None. **L. Ponzoni:** None. **S. Beretta:** None. **P. Scalmani:** None. **M.A. Mantegazza:** None. **M. Sala:** None. **C. Verpelli:** None.

Poster

561. Models of Developmental Epilepsies and Seizure Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 561.28/H18

Topic: E.05. Brain-Machine Interface

Support: Malcolm Feist Weiler Research Seed fund

Title: The comparison of high-frequency oscillations in three types of limbic seizures

Authors: *H. SUN[1], C. R. STEPHENSONS, 71103-4228[2]
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Abstract: High-frequency oscillations (HFO) are involved with seizure genesis and spread. It has been demonstrated that different HFO patterns detected on intracranial electroencephalogram (EEG) are associated with different mechanisms of seizure onset. Here, we analyzed HFO patterns among three types of limbic seizures in mice: 1. Seizures induced by optogenetic stimulation of glutamnergic neurons in the hippocampus, 2. Spontaneous seizures detected in Kv1.1 knockout (KO) breeds, and 3. Seizures induced by systemic administration of 4-Aminopyridine (4-AP).In optogenetics-induced seizures, wild-type mice were injected with adeno-associated virus (AAV) in order to achieve hippocampal channelrhodopsin (ChR2) expression. Glutaminergic neurons were targeted using a CaMKIIA promotor. A custom, single-channel optic fiber-electrode (optrode) assembly was stereotactically implanted into the dorsal

hippocampus for stimulation and EEG recording. For Kv1.1 KO and 4-AP recordings, an EEG recording electrode was stereotactically implanted into the same location of the hippocampus. Each animal was then recorded for at least 30 minutes for possible seizure activities with multiple recording sessions. Custom MATLAB software, combined with an open-source software package, was used to perform analyses of HFO patterns on hippocampal recordings from each group. For reliable detection of an HFO, we set a minimum requirement for oscillatory event duration of 30 milliseconds.For this study, we included one mouse for each seizure induction method. Each mouse has undergone at least 4 recording sessions. In optogenetics-induced seizures, the ictal event is more likely preceded by appearance of fast ripple (250-500Hz) activity, while the spontaneous activity induced by 4-AP and associated with Kv1.1 KO animals are more likely preceded by gamma ripples (40-120Hz) and ripples (120-240Hz). It appears that optogenetics-induced seizures had the hypersynchronous (HYP) onset pattern, while both seizures from Kv1.1 KO animal and seizures induced by 4AP had low-voltage fast (LVF) onset pattern. These findings suggest that different neuronal types may be responsible for these different types of limbic seizures.

Disclosures: H. Sun: None. **C.R. Stephensons:** None.

Poster

561. Models of Developmental Epilepsies and Seizure Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 561.29/I1

Topic: B.10. Epilepsy

Support: NIH GRANT NS082570

Title: Hippocampal GLT1 regulation in the intrahippocampal kainic acid model of epilepsy

Authors: *A. R. PETERSON[1], D. BINDER[2]
[2]Biomed. Sci., [1]Univ. of California, Riverside, Riverside, CA

Abstract: Temporal lobe epilepsy (TLE) is the most common form of epilepsy worldwide. Current antiepileptic drugs (AEDs) primarily target neurons which can lead to cognitive slowing, incoordination and behavioral disorders. Therefore, new drugs for non-neuronal targets are an attractive alternative for the treatment of TLE.
Astrocytes are an essential component of the tripartite synapse playing an important role in the clearance of extracellular glutamate using Na^+-dependent transporters. Glutamate transporter-1 (GLT1) is responsible for the largest proportion of total glutamate clearance. GLT1 is downregulated in various neurological diseases which can lead to glutamate neurotoxicity. Extracellular glutamate homeostasis is essential to decrease neurotoxicity.
Previous results have shown reduced total expression of GLT1 in the hippocampus following an

intrahippocampal kainic acid injection model. Here we aim to characterize expression of GLT1 at the tripartite synapse using crude synaptosomal fractionation. In addition, we hope to investigate the therapeutic capacity of adeno-associated virus type 8 (AAV8)-Gfa2 vectors in the kainic acid model of temporal lobe epilepsy. The ability to increase the expression of GLT1 could attenuate the effects of glutamate neurotoxicity.

Disclosures: A.R. Peterson: None. **D. Binder:** None.

Poster

561. Models of Developmental Epilepsies and Seizure Disorders

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 561.30/I2

Topic: A.02. Postnatal Neurogenesis

Title: Are planarians a useful model organism for high throughput genetic and toxicological investigations of neurodevelopment?

Authors: *S. GUARIGLIA[1], J. GOTIANGCO[2], S. NARVAEZ[3]
[1]New York State Inst. for Basic Reserch, Staten Island, NY; [2]St. Joseph by the Sea High Sch., Staten Island, NY; [3]CUNY Col. of Staten Island, Staten Island, NY

Abstract: Toxicological studies in intact organisms are often time consuming and expensive. Modeling gene and environmental interactions become increasingly complex and take long periods of time to complete. Here, we discuss our work in standardizing high throughput assays to utilize planarians as an alternative model for such investigations. The genome of Schmidtea mediterranea has been characterized and is publically available, which facilitates the capacity of any small lab to create RNAi knockdown organisms using cost-effective commercially synthesized dsRNA constructs to target genes of interest that are introduced by simple feeding with meals. Although transgenic manipulations through feeding can be performed in Caenorhabditis elegans models, planarians offer an additional advantage as they regenerate an entirely new brain after head amputation within seven days, thus expediting neurodevelopmental studies. Furthermore, planarians can be made transparent using RNAi manipulations (albino), thus allowing for studies of cell proliferation, reactive oxygen species production and other manipulations in live animals. In combining these techniques with toxicology, there are endless combinations of genetic and environmental interactions that could be modeled, thus providing the groundwork for more guided studies in higher organisms. Additionally, we have worked to standardize behavioral assays that are robust and highly reproducible using behavioral paradigms from rodents that have been amended to planaria. Such assays include a locomotor assay for hyperactivity and anxiety using the rodent tracking software ANYmaze, an active avoidance memory task in combination with ANYmaze tracking and a three-chambered social, behavioral assay. We have standardized our assays in pharmacologically manipulated models for

hyperactivity using nicotine exposure, and we created scopolamine models for learning and memory. The results of our work provide evidence and highly reproducible protocols for examining cell proliferation and reactive oxygen species generation in intact organisms, as well as protocols for studying behaviors that are important in neurodevelopmental and neurodegenerative studies.

Disclosures: S. Guariglia: None. **J. Gotiangco:** None. **S. Narvaez:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.01/I3

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: FWO grant G0B2315N
BOF-OT/ KU Leuven grant OT14/00830

Title: Myeloid-macroglial crosstalk as a motor for optic nerve regeneration

Authors: *I. BOLLAERTS, J. VAN HOUCKE, L. ANDRIES, A. BECKERS, S. VANHUNSEL, L. DE GROEF, L. MOONS
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Abstract: Appropriate modulation of acute neuroinflammation upon central nervous system (CNS) damage is known to trigger a regenerative response, yet, the underlying cellular and molecular mechanisms remain largely elusive. In contrast to mammals, zebrafish retain high regenerative capacities into adulthood. As such, zebrafish form a powerful model to study the contribution of neuroinflammation to successful regeneration.
Firstly, we characterized the inflammatory response after optic nerve injury in zebrafish, using the transgenic fish lines *Tg(mpeg:GFP)* and *Tg(coro1a:eGFP; lyz:dsRed)*, to label microglia, macrophages and neutrophils. During the regenerative process, a timed induction and resolution of microglia/macrophages was observed in the retina, optic nerve and optic tectum. Secondly, we studied the effect of inflammatory stimulation on the course of optic nerve regeneration. Intravitreal injection of zymosan induced additional retinal inflammation and accelerated tectal reinnervation, as revealed by biocytin tracing. These data indicate that induced acute inflammation can stimulate optic nerve regeneration in fish, similar to what is observed in mammals. Interestingly, we disclosed that inflammatory stimulation also induces macroglial reactivity and proliferation of Müller cells ($GFAP^+/PCNA^+$), in both naive and injured retinas. As such, our data are suggestive for crosstalk between myeloid and macroglial cells in the retina, and it is conceivable that zebrafish Müller glia mediate an important part of the beneficial effect

of inflammatory stimulation to optic nerve regeneration, similar to their mammalian counterparts. We are currently performing pharmacological depletion of microglia/macrophages and/or inhibition of Müller glia, in order to disentangle the interactions between these cell populations, and gain insight into their respective contributions to successful optic nerve regeneration.
Conclusively, our data indicate that inflammatory stimulation is beneficial for optic nerve regrowth in the spontaneously regenerating adult zebrafish, and suggest a role for crosstalk between different neuroglial cell populations herein. Further characterization of the underlying cellular and molecular mechanisms might unveil new targets for the development of novel regenerative strategies in the mammalian CNS.

Disclosures: I. Bollaerts: None. **J. Van houcke:** None. **L. Andries:** None. **A. Beckers:** None. **S. Vanhunsel:** None. **L. De Groef:** None. **L. Moons:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.02/I4

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: FWO Grant G053217N

Title: Inflammatory stimulation as motor for axonal regeneration: Elucidating the underlying cellular and molecular players

Authors: *L. ANDRIES[1], L. DE GROEF[1], M. SALINAS-NAVARRO[1], I. BOLLAERTS[1], K. MOVAHEDI[2], L. MOONS[1]
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Abstract: Despite intensive research, induction of long-distance axonal regeneration and functional recovery of the damaged central nervous system (CNS) remain a challenge. The ever-innovating insights into the dichotomous role of neuroinflammation sprouted the idea that, instead of suppressing the inflammatory machinery, directing and instructing it may be a better therapeutic objective to trigger axonal regrowth. The overarching goal of this project is to unravel the underlying cellular and molecular players that link inflammation to axonal regeneration using optic nerve crush (ONC) (degeneration model) and ONC combined with inflammatory stimulation (IS) (regeneration model).
The responses of resident glia and invading macrophages during neurodegenerative and -regenerative processes are still controversial and insufficiently described. Therefore, we investigated the kinetics and ontogeny of myeloid cell influx in the retina and optic nerve, at

different time points after ONC and ONC+IS, in wild-type and *Cx3Cr1.CreERT2xR26.STOP.YFP* transgenic mice combining flow cytometry and immunohistochemical stainings. From 2dpi onwards, there was a large influx of monocytes, monocyte-derived macrophages (MDMs) and neutrophils after ONC+IS in both tissues, which was not found after ONC. In both injury models, we detected a significant increase in the number of microglia at 6 and 8dpi, but microglia became larger and more internally complex after ONC+IS, a sign of augmented activation. Later time points showed long-term engraftment of the infiltrating inflammatory cells in the microglia population, indicating an altered resident cell population. In addition, we investigated the expression profile of pro- and anti-inflammatory cytokines in the retina and optic nerve after ONC and ONC+IS. In the retina, the expression of TNFα, IFNγ, iNOS, IL-1β, ArgI, IL-10 and Ym1 was upregulated after ONC+IS compared to ONC alone, correlating to the huge influx of inflammatory cells. However, in the optic nerve, these genes, upregulated after ONC, showed a lower expression after ONC+IS compared to ONC only. To further clarify these expression data and disentangle the expression profile of the specific inflammatory cell populations, we are currently performing single-cell RNAseq. We also initiated experiments in *Ccr2*$^{-/-}$ mice to specifically define a role for MDMs in axonal regeneration.
Taken together, these data combined with a comprehensive transcriptomics approach will enable us to pinpoint the inflammatory cell populations and processes that are specific to a pro-regenerative response.

Disclosures: L. De Groef: None. **M. Salinas-Navarro:** None. **I. Bollaerts:** None. **K. Movahedi:** None. **L. Moons:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.03/I5

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Paracrine effects of multiple sclerosis donor-derived mesenchymal stem cell-neural progenitors (MSC-NP) on glial cells

Authors: G. CARLING, S. ZANKER, S. A. SADIQ, *V. K. HARRIS
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Abstract: Multiple sclerosis (MS) is an autoimmune-mediated demyelinating disease of the CNS. Patients with progressive MS experience a steady worsening of neurologic function attributed to chronic demyelination and axonal loss. A novel regenerative therapy utilizing autologous mesenchymal stem cell-derived neural progenitors (MSC-NP) is currently under

clinical investigation in patients with progressive MS. Recent results from a phase I trial demonstrated reversal of established disability after repeated intrathecal MSC-NP injections. Pre-clinical studies suggest that the mechanism of action of MSC-NPs occurs through the paracrine release of trophic and immunomodulatory factors. The objective of this study was to investigate the MSC-NP-associated factors that influence glial cell types in the CNS (microglia, astrocytes, and oligodendrocytes), all of which play a role in the pathogenesis and progression of MS. We utilized an *in vitro*co-culture approach with MS patient bone marrow-derived MSC-NPs (n=5) and the following: (1) M1-polarized BV-2 mouse microglial cells, (2) activated mouse primary astrocytes, and (3) spontaneously differentiated rat neural stem cells (rNSC). The degree of activation or differentiation was determined by quantitative PCR and/or by immunofluorescence, and secreted protein levels were determined by ELISA. We observed a dose-dependent decrease in M1 (Nos2) and increase in M2-specific markers (Arg1) in microglial cells in response to co-culture with MSC-NPs, which correlated with increased release of IL-10 and decreased CCL2. Primary astroglial cells co-cultured with MSC-NPs demonstrated reduced expression of activation markers including GFAP and TNF-alpha. Finally, we found a significant increase in the degree of spontaneous oligodendrocyte differentiation from rNSCs in the presence of MSC-NPs, which correlated with an increase in mature oligodendrocyte markers including PLP, along with an increase in pro-myelinating factors. These results suggest that MSC-NPs promote a beneficial shift in activation and differentiation of glial cells with relevance to MS. These studies form the basis of cell-based potency assays that may be used to better predict the therapeutic efficacy of individual batches of autologous MSC-NPs administered to patients during clinical trials.

Disclosures: G. Carling: None. **S. Zanker:** None. **S.A. Sadiq:** None. **V.K. Harris:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.04/I6

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIMH Grant MH091424

Title: An estradiol mediated sensitive period in cerebellar development is disrupted by Poly I:C induced inflammation

Authors: *A. HOLLEY[1], M. M. MCCARTHY[2]
[1]Physiol., Univ. of Maryland Baltimore, Baltimore, MD; [2]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Although the cerebellum is one of the first brain regions to develop, it is one of the last to fully mature. Purkinje neurons, the primary cells of the cerebellum, are present at birth and mature over the first three postnatal weeks, with the second week being particularly important for cytoarchitectural changes. Over the course of their maturation, Purkinje neurons are particularly susceptible and their developmental trajectory can be easily altered by perturbation. Previous work in our lab identified the second postnatal week in the rat as a sensitive period during which there is a natural increase in aromatase expression and estradiol production. Prostaglandin E2 (PGE2) stimulates aromatase expression in the cerebellum and the high levels induced during inflammation results in excessive estradiol within the cerebellum which then stunts Purkinje neuron growth. Both the naturally occurring sensitive period and the magnitude of perturbation are the same in males and females, however enduring consequences are observed in males in the form of a a disruption in social play during the juvenile period, some three weeks later (add reference). We hypothesize that inflammation induced in the second postnatal week is sustained within the cerebellum for several weeks following the initial insult in males and thereby disrupts social play. To test this hypothesis we are treating 2-week old rat pups with poly I:C , a viral mimetic, and measuring the impact on the PGE2-E2 pathway as well as Purkinje neuronal morphology. We used 3 different doses of poly I:C and analyzed both, peripheral and central inflammation at short and long term time points and results will be reported.

Disclosures: A. Holley: None. **M.M. McCarthy:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.05/I7

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: This project was supported by the Dedicated Health Research Funds from the University of New Mexico School of Medicine

Title: Inflammatory cytokines contribute to dysregulation of Nrf1 and Nrf2 in astrocytes

Authors: K. L. SHANLEY, C. HU, *A. S. GARDINER, O. A. BIZZOZERO
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Abstract: Dysregulation of the cellular antioxidant response has recently been implicated in inflammatory disorders like multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE). This pathway is regulated by a family of transcription factors that include the nuclear factor erythroid like-2 proteins (Nrf). One of these factors (Nrf2)

binds to antioxidant response elements (AREs) increasing the transcription of antioxidant and phase II xenobiotic genes, while Nrf1 antagonizes the effects of Nrf2. This study was designed to investigate the role of pro-inflammatory cytokines, present in MS and EAE, on Nrf1 and Nrf2 signaling in an astrocyte cell line. Incubation of astrocytes for 24 h with a combination of interferon γ, interleukin-1β and tumor necrosis factor α (CIII) reduces Nrf2 protein levels by 28% and increases the amount Nrf1 protein by 14%, suggesting an impaired antioxidant response. Indeed, expression of target genes, p62 and GCLc, is diminished in CIII-treated cells, indicating that the decrease in the Nrf2/Nrf1 ratio has functional consequences. We then sought to investigate whether low levels of Nrf2 are caused by increased protein degradation, reduced mRNA translation and/or low transcription. We found that the half-life of Nrf2 protein, measured upon exposure to cycloheximide, in control and CIII-treated cells are identical (~20 min). This is supported by the finding that Nrf2 degradation by the proteasome via Keap1-dependent and GSK-3β dependent mechanisms is not altered in CIII-treated cells. Moreover, in both conditions, Nrf2 protein is localized to the nucleus, with only small amounts present in the cytoplasm. The rate of mRNA translation, determined in the presence of proteasome inhibitors, is unaltered by the cytokine treatment. Furthermore, the proportion of the total Nrf2 mRNA that is bound to polysomes as well as the level of phosphorylated eIF2α, which is involved in cap-independent translation, are unaffected in CIII-treated cells. Finally, we discovered that the relative amount of Nrf2 mRNA, determined by RT-qPCR, is decreased in the CIII condition. Altogether, these studies indicate that the cytokine-induced Nrf2 depletion in astrocytes may result from dysregulation at the transcriptional or post-transcriptional level. Studies are underway to distinguish between these two possibilities. The identification of how cytokines affect the Nrf1/Nrf2 balance in inflammatory conditions is essential for understanding the pathophysiological processes that underlie tissue damage in neuroinflammatory disorders.

Disclosures: K.L. Shanley: None. **C. Hu:** None. **A.S. Gardiner:** None. **O.A. Bizzozero:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.06/I8

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Neuroprotective effect of sirt1 in EAE

Authors: *M. I. ARVAS, F. MUBARIZ, A. KATURI, S. ANDHAVARAPU, C. BEVER, Jr., T. MAKAR
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Abstract: Multiple sclerosis (MS) is a chronic and inflammatory, demyelinating disease associated with axonal loss and gliosis of the central nervous system (CNS). This study aimed to investigate the effect of Sirt1, a NAD linked enzyme on mice with experimental autoimmune encephalomyelitis (EAE), a widely used MS model, and its potential mechanism underlying the action of anti-oxidative stress. Female C57BL/6 Sirt1 neuron specific overexpressing mice were injected with MOG35-55 peptide to set up the EAE model, and to detect the effect of Sirt1 on the progression of EAE. A total of 24 female C57Bl/6 mice were randomized to a control group (N = 6) or EAE (N = 10) and Sirt1 overexpressing EAE mice (N=10). All the mice were sacrificed 30 days (endpoint) after EAE induction. EAE severity score was observed using EAE scale and myelin content was assessed by immunostaining for MBP and Luxol fast blue, lymphocyte and monocyte infiltration and Sirt1 expression. Furthermore, through the immunohistochemical approaches; the potential molecular mechanism of Sirt1 on EAE was evaluated as the levels of oxidative stress and the expression of (nuclear factor erythroid 2-related factor 2) Nrf2 /HO-1 (heme oxygenase-1) signal pathway. Nrf2 regulates HO-1 gene in several cell types. HO-1, a member of inducible cytoprotective protein family regulated by Keap1/Nrf2/ARE pathway, and small molecular antioxidants such as glutathione provide the cell powerful means to counteract oxidative stress. Our experiments showed a change of oxidative stress and Nrf2/HO-1 pathway expression in normal control group, only EAE group and Sirt1 over expressing EAE group, demonstrating that oxidative stress is associated with the pathophysiology of EAE and Sirt1 suppresses oxidative stress by upregulating Nrf2/HO-1 signaling. The overexpression of Sirt1 exerts neuroprotective effects against EAE, notably in suppressing the progression of EAE and pathological changes, cellular infiltration, inflammation, neuronal loss and demyelination. Furthermore, the effect of Sirt1 was probably related to decrease of the levels of oxidative stress, by activation of Nrf2 and increased levels of anti-oxidant enzymes HO-1 and Catalase expression. Therefore, the present study suggests that Sirt1 possesses significant protective effects against in vivo oxidative stress in EAE. So, Sirt1 may be a promising target for developing MS drugs.

Disclosures: M.I. Arvas: None. **F. mubariz:** None. **A. katuri:** None. **S. andhavarapu:** None. **C. Bever:** None. **T. makar:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.07/I9

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF-2017R1A5A2015061
NRF-2017R1D1A1B03035125

KHIDI-HI17C1510

Title: Inhibition of autophagosome-lysosome fusion by ginsenoside Rk1 induces apoptosis in neuroblastoma cells

Authors: *J. OH[1], S. CHUN[2]
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Abstract: Autophagy can results in cellular adaptation, as well as cell survival or cell death. Modulation of autophagy has been increasing regared as a promising cancer therapeutic approach. In this study, we screenced several ginsenosides extracted from *Panax ginseng* and showed that Rk1 inhibit late stage autophagy (autophagosome and lysosome), possibly through changes in autophagy regulator protein expression. Rk1 treatment dose dependently increased the p62 protein expression and GFP-LC3. Also, Autophagy flux inhibitor, chloroquine treatment further enhanced the effect of Rk1 induced apoptosis. These results suggest that minor ginsenosides Rk1 is a novel autophagy inhibitor and could function as a potent anti-cancer agent, and that combination therapy with classical chemotherapeutic drugs might be promising compounds to have therapeutic effect on neuroblastoma cell lines.

Disclosures: J. Oh: None. **S. Chun:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.08/I10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF-2017R1D1A1B03035125
NRF-2017R1A5A2015061
KHIDI-HI17C1510

Title: Adult neurogenesis in the dentate gyrus induced by minor ginsenoside compound K

Authors: *S.-H. YU[1,2], S. CHUN[1]
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Abstract: Adult neurogenesis, a process of generation of new neurons in adulthood, occurs in both subventricular zone and dentate gyrus. Adding new neurons into the dentate gyrus has been linked to learning and memory, and it can be influenced by stress, exercise, and others. It is reported to be reduced in several neurodegenerative disorders including Alzheimer's disease

(AD). Therefore, it might be great value to identify positive regulator of adult neurogenesis. Ginsenosides, one of natural products, have been known to have anti-cancer, anti-metastatic, anti-inflammatory and neuroprotective effects without cell toxicity. In addition, major ginsenoside Rb1 have been known to promote neurogenesis in DG, but the effect of minor ginsenosides is still unknown. Here, we injected Compound K (CK), one of minor ginsenosides, to aged mice intraperitoneally during 5 days (30mg/kg per day). Then mice were injected with BrdU (50mg/kg per day) during 3 days and sacrificed. Through the immunohistochemistry (IHC) method, we examined whether CK enhance the proliferation of newly generated progenitor cells in the granular zone (SGZ) of the adult hippocampus or not. As a result, we found that the number of $BrdU^+/GFAP^+$, $BrdU^+/Ki\text{-}67^+$, $BrdU^+/DCX^+$ cells in the SGZ was increased compared to control. These findings suggest that ginsenoside CK can be used as a positive regulator of adult neurogenesis in the SGZ and this effect could be used as one of tolerable agents underlying its anti-aging actions.

Disclosures: S. Yu: None. **S. Chun:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.09/I11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF-2017R1D1A1B03035125
KHIDI-HI17C1510
NRF-2017R1A5A2015061

Title: Minor ginsenoside promotes strongly progenitor cell proliferation in the dentate gyrus of hippocampus

Authors: *B. KIM[1,2], S. CHUN[1]
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Abstract: Adult neurogenesis is the adding process of new neurons from neural stem cells at the dentate gyrus in hippocampus and the subventricular zone in striatum. It consists of several stages including stem cell proliferation, differentiation, migration and integration. During aging, newly generated neurons continuously decline in most mammals, and it causes the weakness of neuronal plasticity and loss of cognitive memory. Recent studies suggest that promoting neurogenesis in adult mammalian brain might provide a therapeutic way to treat aging-induced neurodegenerative disease such as Alzheimer's disease. Therefore, it might be great value to

identify positive regulators which can increase the proliferation of new neurons in the hippocampus. There are some natural products regarded as regulators for adult neurogenesis. Among them we investigated the effect of minor ginsenoside Rh2, extracted from Korean ginseng, on proliferation of neural progenitor cells. Intraperitoneal Rh2 (30mg/kg) administration to aged mice (4-month old) led to enhance newly generated progenitor cells in the dentate gyrus. Rh2 injection (3 times a week) resulted in increased the number of $BrdU^+/GFAP^+$, $BrdU^+/PCNA^+$, $BrdU^+/DCX^+$ cells compared to control mice. These findings suggest that ginsenoside Rh2 can be used as a regulator of progenitor cells proliferation in the hippocampus and this effect could be used as one of non-toxic methods underlying its anti-aging actions.

Disclosures: B. Kim: None. **S. Chun:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.10/I12

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Modulating microglial phenotypes via colony stimulating factor-1 receptor (CSF1R) inhibition for therapeutic benefit in multiple sclerosis

Authors: *N. A. HAGAN, L. WOODWORTH, A. MAHAN, M. ZELIC, D. OFENGEIM
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Abstract: Microglia serve as the innate immune cells of the central nervous system (CNS) by providing continuous surveillance of the CNS microenvironment and initiating defense mechanisms to protect CNS tissue. Upon injury, microglia transition into an activated state altering their transcriptional profile, transforming their cell morphology, and producing pro-inflammatory cytokines. These activated microglia initially serve a beneficial role, but their continued activation drives neuroinflammation and neurodegeneration. Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the CNS and activated microglia and macrophages play a significant role in mediating disease pathophysiology and progression. We hypothesize that modulating microglia and infiltrating macrophages through the inhibition of a class III receptor tyrosine kinase, colony stimulating factor-1 receptor (CSF1R) will attenuate deleterious CNS inflammation and inhibit subsequent demyelination and neurodegeneration. In our human credentialing experiments, we observed an increase in CSF1R signaling components in CNS tissue derived from MS patients. This finding provided sufficient rationale to generate a novel CSF1R inhibitor for preclinical testing. *In vitro* assays utilizing primary microglia and macrophages demonstrated that our CSF1R inhibitor successfully blocked receptor phosphorylation and downstream signaling and ultimately altered cytokine production. *In vivo*,

our CSF1R inhibitor decidedly improved the neurological impairments observed in the MOG peptide-induced experimental autoimmune encephalomyelitis model of secondary progressive MS (NOD-EAE). Together, these data suggest that CSF1R inhibition should be explored further as a strategy to modulate microglia phenotypes in the context of neuroinflammation and for therapeutic use in MS.

Disclosures: N.A. Hagan: A. Employment/Salary (full or part-time):; Sanofi. **L. Woodworth:** A. Employment/Salary (full or part-time):; Sanofi. **A. Mahan:** A. Employment/Salary (full or part-time):; Sanofi. **M. Zelic:** A. Employment/Salary (full or part-time):; Sanofi. **D. Ofengeim:** A. Employment/Salary (full or part-time):; Sanofi.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.11/I13

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Epigenetics of Cpt1, the key gene in the new paradigm of multiple sclerosis pathogenesis

Authors: *J. LICHOTA, K. JØNSSON, J. D. NIELAND
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Abstract: Multiple Sclerosis (MS) is a disabling and multifactorial disease of the central nervous system (CNS), characterized by degradation of the myelin sheath surrounding the axons, followed by neurological symptoms, such as vertigo, loss of vision, tremor, weakness, and spasms. MS is characterized as an autoimmune disease that is affected by both molecular mimicry and bystander activation. In MS, the blood brain barrier (BBB) is disrupted. One of the major factors contributing to MS symptom development is demyelination of neurons. Demyelination of neurons might be caused by an autoimmune reaction, although it is tempting to speculate in a link between fatty acid metabolism and demyelination or remyelination of neurons. One of the major players in fatty acid metabolism is the rate limiting enzyme carnitine palmitoyl transferase 1 (CPT1). It was demonstrated, that blockage of CPT1 reduces both disease severity and demyelination in a mouse EAE model. Hence, CPT1 and fatty acid metabolism might play a role in the development of the disease. The regulation of Cpt1 genes and the mechanism behind the possible increased expression of Cpt1 in MS patients is yet unknown. We present the evidence that Cpt1 a and c genes are epigenetically regulated, thus presenting an attractive target for pharmaceutical intervention.

Disclosures: J. Lichota: None. **K. Jønsson:** None. **J.D. Nieland:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.12/I14

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Supported by Tisch MS Research Center of New York (private funds)

Title: Novel molecular marker DJ-1 indicates role in cognitive dysfunction in multiple sclerosis

Authors: N. FAVRET, *A. IACOANGELI, S. A. SADIQ
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Abstract: DJ-1 is a key protein associated with mitophagy processes and mitochondrial dysfunctions. Acting as a redox-sensitive protein, DJ-1 promotes neuroprotection by translocating to the mitochondrial membrane upon oxidative stress. DJ-1 has been linked to early onset and progression of Parkinson's disease, a neurodegenerative disease that frequently leads to cognitive impairment. Patients with multiple sclerosis (MS) will also often experience cognitive impairment including deficits in attention, memory and executive functions. We hypothesized that dysregulation of DJ-1 led to cognitive impairment in MS, and specifically examined whether this protein was involved in severe cognitive dysfunction (SCD) of MS patients. We also investigated whether neuronal DJ-1 was translocated to the mitochondrial membrane after a stress paradigm, using cerebrospinal fluid (CSF) from MS patients. With immunoblotting and ELISA techniques, we quantified levels of DJ-1 in CSF samples of (1) subjects with no MS, (2) MS patients without cognitive dysfunction, and (3) MS patients with SCD. Moreover, using a xenogeneic approach, we applied CSF samples from these three groups to hippocampal neurons in culture and assessed DJ-1 relocalization to the outer membrane of mitochondria. Our results revealed that, compared to the unaffected control group, the levels of DJ-1 were significantly reduced in CSF samples of MS patients with SCD. There was no significant reduction of DJ-1 levels between CSF from MS patients without SCD and CSF from the control group. At the cellular level, we observed that in neurons, after oxidative stress-inducing treatments with CSF of MS patients, DJ-1 was localized to the mitochondrial membrane.These results identified DJ-1 as a promising molecular marker of cognitive impairment in MS patients. Furthermore, the translocation of DJ-1 to the mitochondrial membrane occurring in neuronal cultures exposed to CSF from MS patients suggested that this protein may play a role in mitochondrial homeostasis and dysfunction in neurons of MS patients.

Disclosures: N. Favret: None. **A. Iacoangeli:** None. **S.A. Sadiq:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.13/I15

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 3RO1EY020496-S1
NIH Grant P30EY08126-28
Research to Prevent Blindness inc Career Development Award
Vanderbilt Eye Institute

Title: β-Chemokine Ccl5 deficiency preserves retinal ganglion cells in a murine model of optic neuropathy

Authors: *R. L. WEINER[1], W. M. MCLAUGHLIN[2], M. G. DUBNER[2], C. R. FORMICHELLA[2], R. M. SAPPINGTON[1,2,3]
[1]Dept. of Pharmacol., Vanderbilt Univ., Nashville, TN; [2]Vanderbilt Eye Inst., [3]Dept. of Ophthalmology and Visual Sci., Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: Proinflammatory chemokine, CCL5 (C-C motif ligand 5), is constitutively expressed in retina and is associated with inflammatory and neuroprotective responses in diseases such as HIV-dementia, Alzheimer's Disease and Multiple Sclerosis. As in other neurodegenerative disorders, CCL5 and its receptors are differentially expressed in a murine model of chronic retinal ganglion cell (RGC) degeneration. Here we examined the role of CCL5 signaling in an inducible model of RGC degeneration. We induced unilateral glaucoma for 6 weeks in age-matched, 8 month old C57Bl/6 (WT) and ccl5-/- mice, using the Microbead Occlusion Model. Intraocular pressure (IOP) was monitored by tonometry. To assess RGC degeneration, we quantified axon transport facility and RGC soma and axon structure in both the retina and optic nerve. To determine how Ccl5 signaling may impact these disease outcomes, we quantified expression of Ccl5 and its high affinity receptors CCR3 and CCR5 as well as key elements of the downstream signal transduction pathways. We found that Ccl5 deficiency did not alter baseline or experimental IOP (22-25% increase), as compared to WT mice ($p>0.05$ for both). In WT mice, elevated IOP resulted in a 28-36% decrease in Brn3a+ RGCs in the retina and RGC axons in the optic nerve ($p<0.05$), as compared to saline-injected controls. In ccl5-/- mice, the density of RGC soma and axons did not differ between saline- and microbead-injected eyes ($p>0.05$). Immunohistochemistry also revealed reorganization of beta-tubulin+ processes in the inner plexiform layer of microbead-injected WT mice. This reorganization was significantly less appreciable in microbead-injected ccl5-/- mice. Anterograde transport of the neural tracer cholera toxin beta subunit (CTB) to the superior colliculus decreased by almost 40% in WT mice six

weeks after IOP elevation ($p<0.01$), but not in ccl5-/- mice ($p>0.05$). IOP-dependent pathology noted in WT mice was accompanied by increased expression of pro-apoptotic mediators FADD and Bax and downregulation of anti-apoptotic mediator Bcl-2. CCR5 anti-apoptotic activities are linked to AKT/PI3K and MEK/ERK activity. This is also downregulated in the Microbead Occlusion Model with the exception of MEK which is significantly increased ($p<0.01$) as determined by RNAseq mRNA expression. These data implicate Ccl5 as a potential therapeutic target in retinal ganglion cell degeneration. However, further analysis of downstream signaling and differential receptor activity is needed to understand Ccl5/CCR5 and Ccl5/CCR3 roles in inflammation and neurodegeneration.

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Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.14/I16

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: GABAergic synapses and tonic inhibition are upregulated by the HIV protein gp120 via pathways that diverge downstream of the interleukin-1 receptor

Authors: *M. GREEN[1], X. ZHANG[2], S. A. THAYER[3]
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Abstract: Inhibitory signaling is altered in many neurodegenerative diseases and is upregulated in models of neuroinflammation. Here, we show how the inflammatory HIV envelope protein gp120 upregulates two types of GABAergic inhibition in neurons via microglial release of interleukin-1beta (IL-1β). Tonic inhibition produced by extrasynaptic GABARs was measured using patch clamp electrophysiology to record bicuculline-sensitive shifts in holding current. The number of inhibitory synapses were quantified by counting fluorescent puncta labelled with a GFP-tagged intrabody targeting the scaffolding protein gephyrin. Both types of inhibition increased following exposure to gp120 by a mechanism dependent on microglial release of IL-1β and activation of the IL-1 receptor (IL-1R) on neurons. However downstream of the IL-1R the mechanisms diverged. The increase in the number of inhibitory synapses was dependent on Src activation of GluN2A-containing NMDARs and protein synthesis. This is likely an activity dependent change in synaptic function that may be a homeostatic scaling response to compensate for the excitotoxic effects of gp120. However, the increase in tonic inhibition during exposure to gp120 was not dependent on these signaling steps and resulted from the activation of p38. Thus,

these two types of inhibition were regulated through a mechanism that was dependent on microglial activation and diverged downstream of the IL-1R. The viral protein HIV gp120 likely plays a role in HIV-associated neurocognitive disorders (HAND) which affect nearly half of the 37 million patients with HIV. There is no current treatment available. Importantly, we found that the increase in the extrasynaptic tonic current following exposure to gp120 was due to α5-containing GABARs selectively. Drugs that target the α5-containing GABARs are well tolerated and have been shown to rescue cognitive function in other models of neurodegenerative disease. Additionally, these drugs do not affect synaptic inhibition, thus sparing what may be a homeostatic response. Understanding the mechanisms that regulate distinct populations of GABARs following neuroinflammation may guide selective therapeutic agents to better target the inhibitory system. Extrasynaptic GABARs may be a promising target for the treatment of HAND.

Disclosures: M. Green: None. **X. Zhang:** None. **S.A. Thayer:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.15/I17

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: WVU Senate
WVU PSCOR
T32 AG052375 from the National Institute on Aging

Title: Antiviral acute phase response induces neuronal generation of the chemokine CXCL10 in the hippocampus and cortex

Authors: *T. J. PETRISKO, G. W. KONAT
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Abstract: Peripheral viral infections are known co-morbid factors for major neuropathological conditions. Additionally, peripheral infections can exacerbate these conditions. We are able to mimic this comorbidity in a murine model by inducing an antiviral acute phase response (APR) to the dsRNA viral mimetic polyinosinic: polycytidylic acid (PIC) via injection into the intraperitoneal (i.p.) cavity of mice. Previous analysis demonstrated that PIC renders the brain hypersusceptible to kainic acid (KA)- induced seizures and increases basal synaptic transmission in the hippocampus, the ictal site of KA-induced seizures. At the molecular level, PIC challenge results in hippocampal production of the chemokine CXCL10, a known modulator of neuronal activity. The aim of the current study was to identify cells that generate CXCL10 in response to a

peripheral PIC challenge. Eight-week-old female C57BL/6 mice were i.p. injected with 12 mg/kg of PIC and the brains were analyzed with confocal microscopy using CXCL10 and cell specific antibodies to label neurons (NeuN), astrocytes (GFAP), and microglia (Iba1). 24 hours after PIC injection, intense CXCL10 staining was was observed in the neuronal perikarya of the hippocampus. A strong CXCL10 stain was also evident in some, but not all, astrocytes. Microglia did not express CXCL10, but did undergo hypertrophy. Additionally, the number of microglia cell bodies present in the visual field was increased at 24 compared to control brains, indicating migration of microglia toward neuronal cell bodies. The same features were observed in the cortex. Based on these results, we hypothesize that CXCL10 secreted from neurons in response to an antiviral APR recruits microglia to home onto neuronal cell bodies and uncouple inhibitory axosomatic synapses, leading to hyperexcitability of the neuronal circuitry. The augmentation of neuronal hyperexcitability by the APR may underlie the observed exacerbations in neuropathologies accompanied by peripheral infections.

Disclosures: T.J. Petrisko: None. **G.W. Konat:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

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Program #/Poster #: 562.16/J1

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: The University of Southern Mississippi Lucas Endowment for Faculty Excellence award (F.B.)
National Institute of Allergy and Infectious Diseases of the National Institutes of Health R15AI113706 (F.B).

Title: Neurobehavioral and immunohistochemical alterations in immunocompetent mice with congenital zika virus infection

Authors: *P. J. VIG[1], A. PAUL[2], M. LOPEZ[1], B. NEUPANE[3], F. BAI[3]
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Abstract: Zika virus (ZIKV) infection during pregnancy can result in a spectrum of neurodevelopmental defects including vision and hearing loss, seizures, and microcephaly. Recent reports indicate that congenital ZIKV infection could manifest as more subtle cognitive and/or behavioral effects in children who were seemingly unaffected at infancy. However, it may take years before such effects could be well-documented in humans. In the present study we used an immunocompetent model of congenital ZIKV infection in which pregnant wild-type mice are

infected (ip) with ZIKV. To determine if these postnatal deficits were associated with neuron function, we assessed various neurodevelopmental behaviors in ZIKV- and mock-pups. These assessments included balance, motor coordination, forelimb strength, and cognitive development. The results showed that the ZIKV-pups exhibited poor balancing skills and weak fore-limb strength, along with a significant increase in passivity, decrease in locomotion, and cognitive memory decline compared to mock-pups at selected time points. In addition, immunostaining of midsagittal brain sections showed cellular disarrangement and a thinner cortical layer 1 in the brains of ZIKV-pups at D19, a feature associated with microcephaly in human babies. Apart from cortical layer 1 thinness in ZIKV-pups at D40, the density of neurons within L1-6 of the cortex were also reduced. Furthermore, midsagittal brain sections were immunostained with GFAP to identify astrogliosis, a hallmark feature of human and mouse newborns with microcephaly. The GFAP immunostaining exhibited progressive astrogliosis near CA1 neurons of the hippocampus and within the white matter of the cerebellum of ZIKV-pups at D12, D19 and D60, along with enlarged astrocytes surrounding motor neurons within cervical spinal cords, while only minimal reactivity to GFAP was detected in these regions in mock-pups. Reactive astrogliosis in the hippocampus and cerebellum has been linked to defective memory and motor coordination in mice, which were observed in our behavioral studies of ZIKV-pups. Collectively, these observations suggest that a transient and mild ZIKV infection in immunocompetent mice during pregnancy could cause postnatal brain developmental deficits, providing a novel animal model to study congenital ZIKV syndrome.

Disclosures: P.J. Vig: None. **A. Paul:** None. **M. Lopez:** None. **B. Neupane:** None. **F. Bai:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.17/J2

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Multiple Sclerosis Society of Canada Doctoral Studentship

Title: Trigeminal root entry zone pathology in experimental autoimmune encephalomyelitis

Authors: *K. C. THORBURN[1], J. W. PAYLOR[2], I. R. WINSHIP[2], B. J. KERR[3]
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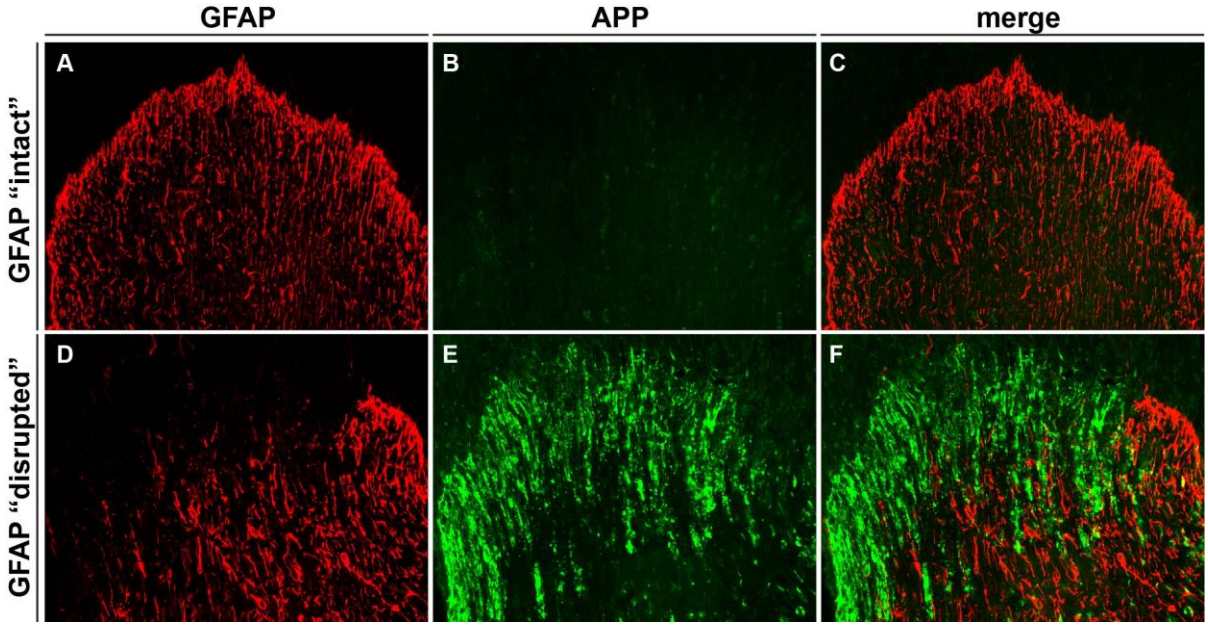
Abstract: Trigeminal lesions, dysfunction and sensory disturbances (e.g. hypoesthesia, pain) are well-recognized, but poorly understood, complications of Multiple Sclerosis (MS). We have previously shown that the animal model experimental autoimmune encephalomyelitis (EAE)

could be a useful tool for understanding MS-related trigeminal pathology and dysfunction. In particular, we found that mice with EAE exhibit facial hypersensitivity as well as inflammation and demyelination at several points along the trigeminal primary afferent pathway. The objective of this study was to further investigate EAE-related changes to the trigeminal primary afferent pathway. We show here that in mice with EAE, immunoreactivity (IR) for amyloid precursor protein (APP), a marker of axonal injury, increases significantly at the trigeminal root entry zone (TREZ) (B, E in Image 1). Interestingly, APP-IR is entirely restricted to the central nervous system (CNS) aspect of the TREZ. Additionally, APP-IR only appears in sections where glial fibrilary acidic protein (GFAP)-IR is disrupted (A, D in Image 1). Preliminary comparisons between sexes suggest that GFAP disruption and APP-IR is significantly greater in female mice. Preliminary data also suggest that APP-IR at the TREZ is not related to the presence of reactive microglia/macrophages or T cells in both sexes. In contrast to previous studies that have used APP as a marker for axonal injury, we do not find that APP co-localizes with the pan-axonal markers SMI-311 or SMI-312. We do however find that APP colocalizes with damaged myelin as well as oligodendrocytes. We are currently examining how GFAP disruption and APP-IR at the TREZ relates to facial sensitivity in mice with EAE. We are also assessing the relationship between APP and GFAP in the brainstem regions involved in trigeminal processing. In summary, we have extended our previous findings to show that disruption of GFAP at the TREZ is associated with the presence of APP in mice with EAE. Taken together, our data suggest that TREZ astrocytes are protective in an inflammatory demyelinating environment.



Disclosures: K.C. Thorburn: None. **J.W. Paylor:** None. **I.R. Winship:** None. **B.J. Kerr:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.18/J3

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: TSGH-C107-016

Title: GP91 deficiency ameliorates oxidative stress associated neural damage and dysfunction in an experimental autoimmune encephalomyelitis mouse model

Authors: *C.-F. HU[1], S.-J. CHEN, Senior[2], J.-S. HONG[3]
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Abstract: The roles of reactive oxygen species (ROS) contributing to the pathogenesis of experimental autoimmune encephalomyelitis (EAE) is not yet totally understood. Recent strategies of multiple sclerosis treatment center on T cell based interventions that work successfully on a subset of patients. In this study, we focus roles of innate immunity in the pathogenesis of EAE. We hypothesized that dysregulated ROS production by both macrophage and microglial NADPH oxidase (NOX2) contributes to neural inflammation, damage and demyelination after EAE induction.
We found that *Nox2* deficient mice are more resistant to EAE induced neural damage compared with control mice (C57). *Nox2* deficiency results in reduced disease severity scores, less body weight loss, less leukocytes infiltration, lower grades of demyelination, decreased oxidative stress markers 3-NT , and lower levels of genes encoded for proinflammatory cytokines IL-1beta, IL-4, IL-6, IL- 17α, IFNɣ and TNFα as well as T cell regulatory cytokines and T cell regulatory factors IL-10 and TGFβ in the spinal cords in comparison with control. Our findings suggest that NOX2-mediated ROS in macrophage and microglia plays an important role in EAE-induced neuronal damage. Roles of NOX2 in macrophage and microglia in the pathogenesis of EAE are being investigated using a various of NOX2 inhibitors. Further studies on the function of dendritic cells and macrophages in *Nox2* deficient mice after EAE induction.

Disclosures: C. Hu: None. **S. Chen:** None. **J. Hong:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.19/J4

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: SERB/PDF/2016/000060

Title: Potential role of estrogen in maintaining the proNGF/NGF and Bax/Bcl2 ratio in hippocampus of aged female rat

Authors: *P. KUMAR[1], P. KAUSHAL[2], P. DHAR[3]
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Abstract: Immunosenescence contributes to the cascade of events leading to neurodegeneration in old age. Immune system undergoes a dynamic change induced by declining levels of estrogen in women during age progression. The dynamic changes in different brain areas including hippocampus might be associated with the progressive decline of estrogen in females as decreased levels of estrogen are reported to alter nerve growth factor status in the hippocampus. ProNGF contributes in activating inflammatory pathway and increasing the production of inflammatory cytokines (Minnone G. et al., 2018). Our aim was to study the role of estrogen on proNGF/NGF and Bax/Bcl2 ratio, expression of the complement system and the status of pro and anti-inflammatory cytokines in female rat hippocampus during age progression. Immunohistochemistry and Western blot techniques were used for proteomic analysis. The observations revealed altered levels of ERs, proNGF/NGF and Bax/Bcl2 ratio, microglial and astrocytes activation, expression of complement proteins and pro- and anti-inflammatory cytokines, decreased synaptic activity, in the hippocampus of middle-aged and aged female rats. Long-term estrogen (E2, 17β estradiol) and tamoxifen (TAM) therapy to these animals could maintain synaptic plasticity (synaptophysin) and regulate microglial and astrocytes activity, nerve growth factor and Bax/Bcl2, complement proteins and pro-inflammatory cytokines. Taken together, these data indicate that estrogen and SERM act as potent modulators in maintaining proNGF/NGF and Bax/Bcl2 balance in the hippocampus of the aging rat, thereby highlighting the multi-faceted regulatory effects of exogenous estrogen and SERM.
(National Postdoctoral Fellow award (PDF/2016/000060) from Department of Science and Technology, Government of India.
Our study add a new perspective to the neuroprotective and neuromodulatory effects of estrogen based on its role in complement system and pro-inflammatory cytokines on one hand and modulation of apoptosis associated proteins on the other.

Disclosures: P. Kumar: None. **P. Kaushal:** None. **P. Dhar:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.20/J5

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Agencia Nacional de Promocion Científica y Tecnologica [PICT 1563]
The University of Buenos Aires [20020130100564
Consejo Nacional de Investigaciones Científicas y Tecnicas [PIP 0707]

Title: Environmental enrichment benefit on visual pathway damage induced by neurinflammation of the optic nerve

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Abstract: Optic neuritis (ON) is an inflammatory, demyelinating, neurodegenerative, and presently untreatable condition of the optic nerve which might induce blindness. We analyzed the effect of environmental enrichment (EE) on visual pathway damage provoked by experimental ON induced by a microinjection of bacterial lipopolysaccharide (LPS) into the optic nerve. For this purpose, LPS was microinjected into the optic nerve from male Wistar rats. After injection, one group of animals was submitted to EE, and another group remained in standard environment (SE) for 21 days. EE prevented the decrease in pupil light reflex (PLR), visual evoked potentials, retinal anterograde transport, phosphorylated neurofilament immunoreactivity, myelination (luxol fast blue staining), and axon (toluidine blue staining) and retinal ganglion cell (Brn3a-immunoreactivity) number. EE also prevented microglial/macrophage reactivity (Iba-1- and ED1-immunoreactivity), and astrocytosis (glial fibrillary acidic protein-immunostaining) induced by experimental ON. LPS-injected optic nerves displayed oxidative damage and increased inducible nitric oxide synthase, cyclooxygenase-2, and interleukin-1b and TNFa mRNA levels which were prevented by EE at 1 day pos-injection. EE increased optic nerve brain-derived neurotrophic factor levels. When EE started at 4 (but not 7) days post-injection of LPS, a preservation of the PLR was observed at 21 days post-LPS, which was blocked by the daily administration of ANA-12 (a Trk-b antagonist) from day 4 to day 7 post-LPS. Moreover, EE from day 4 to day 7 post-LPS significantly

preserved the PLR at 21 days post-injection. Taken together, our data suggest that EE preserved visual functions and reduced neuroinflammation of the optic nerve.

Disclosures: M.L. Aranda: None. **M.F. Gonzalez Fleitas:** None. **P.H. Sande:** None. **D. Dorfman:** None. **R.E. Rosenstein:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

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Program #/Poster #: 562.21/J6

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: IHU (Instituts Hospitalo-Universitaires) Grant - “Investissements d’Avenir” ANR-10-IAIHU-06
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Fondation OCIRP (l’Organisme commun des institutions de rente et de prévoyance) Grant

Title: Multiple sclerosis patient macrophages' transcriptomic signature unveils genetic networks behind their altered pro-regenerative capacity

Authors: *J. FRANSSON[1], C. BACHELIN[1], F. DEKNUYDT[2], L. GUILLOT-NOËL[1], M. EL BEHI[1], A. TENENHAUS[1], H. ABDI[3], V. GUILLEMOT[4], G. BASSIGNANA[1], F. DE VICO FALLANI[1], O. COLIOT[1], C. LOUAPRE[1,5], B. FONTAINE[1,6,5], V. ZUJOVIC[1]
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Abstract: Multiple sclerosis (MS) is a neurological disease in which immune cells invade the central nervous system and destroy myelin. A regenerative process, called remyelination, can occur. The extent and efficacy of remyelination is highly variable among patients but efficient remyelination is critical for a favorable disease evolution. Macrophages contribute to demyelination but also orchestrate the remyelination process, and their role in each process depends on their phenotype, or “activation state”, achieved in response to external signals. We hypothesize that a failure in remyelination in patients could be caused by a dysregulated macrophage function, possibly due to intrinsic capacities of macrophages to correctly respond to

signals.
To test this hypothesis, CD14+ monocytes from MS patients and healthy controls (HC) were differentiated into macrophages. After applying for 24h or 72h pro-inflammatory (LPS and IFNγ) or pro-regenerative (IL-4 or IFNβ) stimuli, we evaluated macrophage (dys-) functionality in different steps essential for efficient myelin repair: their phagocytic capacities when exposed to human myelin and their effect on oligodendrocyte precursor cells (OPC) proliferation and differentiation. In parallel, we established their transcriptomic (RNA sequencing) and secretory profile (Luminex assays).
Our preliminary results provide evidence that among MS patients, there are deficits in macrophages function that appear to be patient specific. For example, macrophages of some patients exhibited a deficit in phagocytic activity in response to pro-regenerative stimuli. At the transcriptomic level, principal component analysis separated transcriptomes of patient samples from controls across all activation states. Among genes differentially expressed between patients and controls, a significant number are involved in molecular pathways important for the pro-regenerative activation state. We next plan to correlate macrophages functionality to specific transcriptomic signatures and identify which molecular pathways to correct in order to steer macrophages biological networks into a specific regenerative state.
Our results highlight a dysfunction in MS patient monocytes, before encountering the inflammatory environment of an MS lesion that might affect their capacity to instruct the remyelination process. In a translational step, we will also include in our analysis patients genotype and clinical data in order to create a full model describing how different defects in macrophage activation can influence the disease course.

Disclosures: J. Fransson: None. **C. Bachelin:** None. **F. Deknuydt:** None. **L. Guillot-Noël:** None. **M. El Behi:** None. **A. Tenenhaus:** None. **H. Abdi:** None. **V. Guillemot:** None. **G. Bassignana:** None. **F. De Vico Fallani:** None. **O. Coliot:** None. **C. Louapre:** None. **B. Fontaine:** None. **V. Zujovic:** None.

Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.22/J7

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: ANR 15-CE16-0009-01
H2020 MSCA fellowship 704514
ARSEP

Title: Effect of anti-VEGF treatment on the innate immune response in a mouse model of multiple sclerosis

Authors: *C. CARAVAGNA[1,2,3,4], A. JAOUËN[1,2,3,4], G. ROUGON[1,2,3,4], F. DEBARBIEUX[1,2,3,4]
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Abstract: Multiple sclerosis is an inflammatory disease, characterized by infiltration of immune cells into the central nervous system leading to myelin and axonal damages. Although this disease was previously thought to be led by adaptive immune cells, e.g. T cells, recent findings lead us to focus on innate immune cells, such as monocytes, neutrophils and microglial cells. We used Thy1-CFP/LysM-EGFP/CD11c-EYFP triple transgenic mice induced for EAE. In these mice Thy1-CFP is expressed by axons, LysM-EGFP by peripheral innate immune cells, CD11c-EYFP by activated microglial cells and both LysM-EGFP and CD11c-EYFP by monocyte-derived dendritic cells. These markers allowed us to precisely study the spatial and temporal recruitment of these cells in the spinal cord of EAE mice, in relation with axonal loss and clinical signs. First on day 10 after induction, EGFP neutrophils and monocytes invade the meninges, then (day 13) they enter into the spinal cord parenchyma through the meninges, rather than by extravasion. Axonal losses occur in the white matter concomitantly with immune cell infiltration. Once in the parenchyma, monocytes mature into EGFP/EYFP monocyte-derived dendritic cells whose density is maximal on day 17 when the axonal degradation and clinical signs stabilize. Meanwhile, microglia is progressively activated in the entire spinal cord and subsequently recruited to plaques. As a direct effect of VEGF on immune cell has been demonstrated in other diseases, we examined the effects of VEGF blockade (with bevacizumab) on the innate immune response in triple fluorescent EAE mice. In animals treated with bevacizumab from disease onset, we found no significant difference in the numbers of fluorescent cells recruited to the spinal cord, compared to controls. We are now focussing on the phenotypes and movements of these cells.

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Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

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Program #/Poster #: 562.23/J8

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: FAPESP Grant 2014/06892- 3

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CAPES
CNPq

Title: Granulocyte-macrophage colony-stimulating factor (GM-CSF) improves mouse peripheral nerve regeneration following axotomy

Authors: *A. L. BOMBEIRO, B. T. N. PEREIRA, A. L. R. OLIVEIRA
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Abstract: Peripheral nerve injuries severely impair the life quality of the patient since full recovery is seldom achieved. Upon axonal disruption, distal nerve stump undergoes fragmentation and myelin breaks down, being regeneration progression dependent on cell debris removal. Besides tissue clearance, macrophages release angiogenic and neurotrophic factors that contribute to axon growth. Based on the importance of macrophages for nerve regeneration, especially during the initial response to injury, we stimulated the proliferation and infiltration of those cells in the sciatic nerve by treating male mice with granulocyte-macrophage colony-stimulating factor (GM-CSF, 50 μg/Kg) or PBS (control) at zero, 24h and 48h following nerve crushing. Sciatic nerves were histologically analyzed (3, 7, 14 and 28 days; n=5/group/day) regarding the presence of macrophages and the regenerative stage, being intact nerves (n=5) used as the baseline control. Functional recovery was followed up by an automated walking track test (CatWalk system, Noldus). According to our data, GM-CSF potentiated early axon growth, as evidenced by the enhanced expression of growth-associated protein (GAP-43) at 7 days post-injury (83% more than PBS, $P < 0.05$). Inducible nitric oxide synthase (iNOS) expression increased in the beginning and at the end of the regenerative process for both PBS and GM-CSF groups, suggesting nitric oxide is involved in axon growth and pruning. As expected, GM-CSF treatment stimulated macrophage infiltration, which was increased at 7 days (60% more than PBS, $P < 0.0001$) and 14 days (23% more than PBS, $P < 0.01$). Curiously, myelin quantification revealed no difference between PBS and GM-CSF groups at any of the analyzed time points. However, GM-CSF anticipated in one week the brain-derived neurotrophic factor (BDNF) production peak, which occurred at 7 days (56% more than PBS, $P < 0.0001$). Ambulation recovery pattern was not improved by GM-CSF treatment. Overall, the present results indicate that GM-CSF has beneficial effects on early axon regeneration, and its use brings new perspectives regarding PNS regeneration.

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Poster

562. Neurotoxicity, Inflammation, and Neuroprotection: Cellular Stress and Death Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 562.24/J9

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: UT BRI RSP I-126377-01
AHA 15SDG25700054
NIH R01MH113986

Title: The regulatory effect of a multiple sclerosis drug candidate on macrophages

Authors: B. LIN, B. KOFFMAN, *J. DU
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Abstract: Multiple sclerosis (MS) is a central nervous system (CNS) disease in which the myelin sheath in brain and spinal cord is damaged. This damage disrupts the communications network resulting in physical, mental, and/or psychiatric problems. Pathogenesis of MS involves autoreactive T cells and pro-inflammatory macrophages (e.g. monocyte-derived macrophages and microglia). We previously reported that BBR3378, a novel aza-anthrapyrazole structurally similar to an FDA-approved MS drug mitoxantrone, can ameliorate experimental autoimmune encephalomyelitis (EAE), a clinically relevant mouse model of MS, without cardiotoxic effects often associated with this family of drugs (i.e. anthracyclines). BBR3378 inhibited production of the pro-inflammatory cytokine IFN-γ both in recently activated T cell blasts and established Th1 effectors via suppressing T-bet regulated gene expression, while sparing the activities of IL-13-producing Th2 cells. In this study, the effect of BBR3378 on macrophages was investigated. BBR3378 inhibited cell proliferation of human brain microglia HMC3 in a concentration-dependent manner (IC_{50}: 429.5 ng/ml), demonstrated by MTT cell viability assays, and 8h treatment of BBR3378 (100 ng/ml) reduced expression of CD68, a typical marker of activated macrophage, as quantified by qRT PCR. Compared to mouse fibroblast L292 (IC_{50}>1000 ng/ml), mouse macrophage RAW 264.7 (IC_{50}: 181.1 ng/ml) was shown more susceptible to BBR3378-mediated inhibition of cell proliferation. LPS-induced inflammation was measured by qRT PCR analysis of expression of TNFα and IL-1α and by using a luciferase reporter construct with NF-κB promoter to assess NF-κB activation. Pre-treating RAW 264.7 cells with BBR3378 (100 ng/ml) did not affect the early response cytokine TNFα expression after 1h LPS stimulation (100 ng/ml) but significantly accelerated decay of TNFα mRNA after 8h. In contrast, the peak IL-1α mRNA level appeared after 8h LPS stimulation but was significantly down-regulated by BBR3378. In addition, BBR3378 inhibited activation of NF-κB, the major cellular event leading to inflammatory cytokine production in response to LPS. Furthermore, BBR3378 blocked COX-

2, an enzyme catalyzing the production of prostaglandin, another critical inflammatory mediator. These preliminary observations together have implicated BBR3378's regulatory activity on macrophages which can potentially contribute to its therapeutic effect on EAE and MS.

Disclosures: B. Lin: None. **B. Koffman:** None. **J. Du:** None.

Poster

563. Ischemia IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 563.01/J10

Topic: C.08. Ischemia

Support: NIH grant NS104117

Title: Novel combinatory treatment for experimental ischemic stroke

Authors: *L. S. BELAYEV[1], S. HONG[1], L. KHOUTOROVA[1], A. OBENAUS[2], N. A. PETASIS[3], N. G. BAZAN[1]
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Abstract: Acute ischemic stroke triggers complex neurovascular, neuroinflammatory, and synaptic alterations. Our study aimed to test the prediction that blocking pro-inflammatory platelet-activating factor-receptors (PAF-Rs) plus administering selected docosanoids after middle cerebral artery occlusion (MCAo) would lead to sustained neurological recovery. Two different types of bioactive small molecules were investigated. The first was LAU-0901, an antagonist of PAF-R that blocks activated pro-inflammatory signaling and has been shown to have promising efficacy in a stroke model. The second, products of DHA, a novel synthetic docosanoid (Aspirin-triggered neuroprotectin D1 methyl-ester; AT-NPD1-ME), which activates cell-survival pathways and possesses potent anti-inflammatory and neuroprotective activity in the brain. Sprague-Dawley rats were anesthetized with isoflurane/nitrous oxide and received 2h MCAo by intraluminal suture. Neurological status was evaluated at 3h and 4h, and on days 1, 2, and 3; a grading scale of 0-12 was employed. Animals were treated with LAU-0901 (i.p. 60mg/kg, 2h after onset of stroke), AT-NPD1-ME (i.v. 333mg/kg, 3h after onset of stroke) and vehicles (cyclodextran and saline). There were four groups: LAU-0901+AT-NPD1; LAU-0901+saline; Cyclodextran+AT-NPD1; and cyclodextran+saline. On day 3, *ex vivo* MRI of the brain was conducted using 11.7 T MRI. LAU-0901 and AT-NPD1 treatments alone improved behavioral scores compared to vehicle groups by 22-32%. The neuroprotective effect was enhanced using the LAU-0901+AT-NPD1, which resulted in improved behavioral scores up to 50% on day 3. Total lesion volumes, which were computed using T2WI, were significantly reduced by 80% with LAU-0901+AT-NPD1 treatment compared to vehicle-treated groups. We

concluded that combination treatment of the PAF-R antagonist, LAU-0901, plus AT-NPD1-ME affords synergistic neuroprotection in the post-ischemic brain and might provide the basis for future therapeutics in patients suffering from ischemic stroke. We are currently exploring the molecular mechanisms involved.

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Poster

563. Ischemia IV

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Program #/Poster #: 563.02/J11

Topic: C.08. Ischemia

Support: NIH Grant R01HL104173
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NIH IDDRC grant U54 HD090257

Title: Mesenchymal stem/stromal cell delivery through cardiopulmonary bypass modulates systemic inflammation and reduces microglia activation in a juvenile porcine model

Authors: T. MAEDA[1], K. SARKISLALI[1], C. LEONETTI[1], F. A. SOMAA[1], G. R. STINETT[1], Z. DHARI[1], B. K. LEWIS[5], M. M. NUSZKOWSKI[2], K. PANCHAPAKESAN[3], K. GRECCO[4], P. VYAS[4], P. J. HANLEY[6], R. ULREY[6], J. A. FRANK[5], R. A. JONAS[1], *N. ISHIBASHI[1]
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Abstract: Neurodevelopmental impairment is emerging as one the most important current challenges for survivors after pediatric cardiac surgery. Cardiopulmonary bypass (CPB) can cause substantial systemic inflammation and trigger prolonged microglial activation in the brain. Mesenchymal stem/stromal cells (MSCs) have significant immunomodulatory properties and regulate microglia activation. We hypothesize that intra-arterial MSC delivery through CPB is neuroprotective by modulating systemic and neuro-inflammatory responses. Two-week old piglets (n=16 total) were randomly assigned to one of 3 groups: (1) Control, (2) Deep hypothermic circulatory arrest (DHCA), (3) DHCA followed by MSC administration. In group 3, ^{18}F-FDG or superparamagnetic iron oxide (SPIO)-labeled MSCs ($10x10^6$ per kg) were delivered through CPB during the rewarming period. Positron emission tomography (PET) was performed 1hr after MSC delivery to determine the whole body distribution of cells with ^{18}F-FDG. Animals

were sacrificed 3hrs after CPB for analysis with magnetic resonance imaging (MRI) and immunohistochemistry. Plasma cytokine/chemokine levels were determined by multiplex immunoassay. Clinically-relevant physiological biomarkers determined the effect of MSC delivery on multi-organ function. It has been well demonstrated that intra-venous injection of MSCs results in high accumulation of cells primarily in the lungs. In contrast our PET study showed that intra-arterial delivery through CPB uniformly distributed MSCs to most of the organs analyzed including brain, heart, and kidney. The lungs and intestine showed lower uptake. T2* weighted brain MRI showed diffuse distribution of hypointense voxels (SPIO particles) throughout the entire brain with large clusters along the lateral and third ventricles. Immunohistochemistry revealed an even distribution of SPIOs within the cortex and white matter. We have previously demonstrated an increase in permeability of the blood-brain barrier after DHCA. Consistent with this we identified SPIOs located in the extra-vascular space. MSC delivery through CPB modulated plasma cytokine/chemokine expression following surgery. In the brain MSC treatment reduced microglia expansion/activation and inhibited caspase activation resulting from CPB. Various physiological biomarkers after MSC delivery did not differ compared with CPB group. No evidence of either embolic events or microstrokes was observed by MRI and histology. MSC delivery during CPB has the translational potential to minimize systemic inflammation and reduce microglial expansion and caspase activation in children undergoing CPB.

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Poster

563. Ischemia IV

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Program #/Poster #: 563.03/J12

Topic: C.08. Ischemia

Support: The Heart and Stroke Foundation of Canada
Natural Sciences and Engineering Research Council
Saskatchewan Health Research Foundation
Canadian Foundation for Innovation
University of Saskatchewan College of Medicine

Title: Gamma burst oscillations (gbos) using low field magnetic stimulation (lfms) improves post-stroke cognitive and psychiatric deficits in an animal stroke model

Authors: *H. KIM[1], M. ZAKI[2], J. STOCKWELL[2], Y. ZHANG[2], F. S. CAYABYAB[3]
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Abstract: Stroke survivors often suffer from disability, including motor, psychiatric, and cognitive deficits. Early therapeutic intervention with the clot-busting agent TPA can indeed be effective, but the few who receive this treatment still suffer from neurological deficits. We hypothesize that neuroprotective adjunct therapy is required to reduce post-stroke adult disability. The potential non-invasive stroke treatment involving low field magnetic stimulation (LFMS) is under investigation, and we are delineating the cellular mechanisms involved in the therapeutic benefits of this novel treatment in our animal model of post-stroke depression. Using an established focal cortical pial vessel disruption (PVD) stroke model in Sprague-Dawley rats, we studied the efficacy of gamma burst stimulation or LFMS (40 Hz, <0.1 Tesla) in reducing hippocampal neuronal damage and associated behavioural deficits. Levels of anxiety, depressive and cognitive behavioural deficits were measured using the open field test (OFT), forced swim test (FST) and Y-maze, respectively. In vitro electrophysiological recordings were performed to correlate cognitive deficits with changes in hippocampal synaptic plasticity. Various tissue staining methods followed by confocal microscopy were employed to visualize the effects of PVD and LFMS on hippocampal protein and cell expression. Western blotting was then used to quantify these expressions. PVD treatments produced hippocampal-dependent spatial memory deficits, which were associated with decreased long-term potentiation in hippocampal brain slices. Increased anxiety and depressive behaviours were observed in PVD-treated animals but not in sham animals (similar surgical procedures but with pial vessels left intact). Increased neuronal damage was confirmed using propidium iodide and Fluro-jade C labeling followed by confocal imaging. In contrast, all animals that received daily LFMS (20min, 3d) showed significant improvements in their depression, anxiety, and spatial memory impairments. The results showed that restoring gamma oscillations with LFMS counters the damaging effects of pro-neurotoxicity pathways after stroke. Clinical implications of this non-invasive therapy include potential treatments of post-stroke depression and dementia, and other neurodegenerative diseases.

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Poster

563. Ischemia IV

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Program #/Poster #: 563.04/J13

Topic: C.08. Ischemia

Title: The effect of sodium ozagrel, edaravone, or heparin on the development of infarcted lesions in our three-vessel occlusion (3-VO) model

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Abstract: Ischemic stroke, which may seriously affect the quality of life for a prolonged period, involved 10.3 million patients worldwide in 2013. Sodium Ozagrel (SO) is a thromboxane A_2 (TXA_2) synthase inhibitor developed as an antiplatelet drug, which has been used for the treatment of neurological deficits (motor functions) associated with cerebral thrombosis, since Mar. 1998, or for the prevention of cerebral vasospasm (delayed ischemic symptoms) after subarachnoid hemorrhage (SAH), since Jun. 1995. Edaravone is a free radical scavenger developed as a neuro/vascular protectant, which has been approved for the treatment of ischemic stroke in Japan (for this particular purpose), since Apr. 2001. Here, we examined the effect of SO, Edaravone, or heparin (a potent anti-coagulant that has been used clinically) on the development of cerebral infarction, using our original three-vessel occlusion (3-VO) model (Yang et al., Eur Neurol, 2014). Male C57BL/6J mice received either SO; 10 mg/kg, Edaravone; 3 mg/kg, heparin; 600 U/kg, or saline (as the vehicle control), i.v., at 5 min and 3 h (twice) after the induction of ischemia (N= 8 in each treated group, or 12 in the control). Infarcted lesion volumes and neurological deficits were analyzed 24 h after ischemia, using the TTC stain or the tail suspension test, respectively. Regional cerebral blood flow (rCBF) was monitored before, during and after ischemia, using laser Doppler flowmetry. In the results, SO or Edaravone, significantly reduced the infarcted lesion volumes (12.4 $mm^3 \pm 4.5$, or 13.7 $mm^3 \pm 6.5$, respectively), compared to the control (20.2 $mm^3 \pm 5.1$). The treatment with SO or Edaravone did not affect the levels of rCBF during ischemia, indicating that reduced lesion sizes were not due to increased rCBF (i.e. there was no reduction in the ischemic stress applied by the 3-VO method). The anti-platelet agent, SO may protect the brain after focal ischemia. The treatment with heparin did not improve the rCBF or infarcted lesion volumes, but induced hemorrhagic transformation as a side effect in some cases. The neurological deficits were relatively less in the SO or Edarabone groups compared to the control, but without a significant difference. It was possible to reduce the anatomical lesion sizes using the anti-platelet agent, or the neuro/vascular protectant, but these methods failed to improve neurological deficits after the induction of 3-VO. Although there is a possibility of type II error in the present functional assessment, it is mandatory to find an agent that can reduce infarcted lesion volumes and improve neurological functions, by acute administrations after the onset of ischemic stroke.

Disclosures: K. Yamato: None. **Y. Nakajo:** None. **J.C. Takahashi:** None. **H. Yanamoto:** None.

Poster

563. Ischemia IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 563.05/J14

Topic: C.08. Ischemia

Support: NIH Grant DK102912

Title: Injury site-targeted complement inhibition improves motor & cognitive recovery after murine ischemic stroke

Authors: *A. TOUTONJI[1], S. TOMLINSON[2]
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Abstract: Introduction: Ischemia induces the expression of surface epitopes (Danger Associated Molecular Patterns), which upon return of blood flow (reperfusion), bind circulating self-reactive natural IgM antibodies These bound IgM antibodies activate complement and drive secondary ischemia reperfusion injury. From an IgM mAb hybridoma that recognizes a post-ischemic stroke neoepitope, we derived a single chain antibody (scFv) for use as a targeting vehicle to deliver a complement inhibitor specifically to the post-ischemic brain.
Methods: We linked the murine complement inhibitor, Crry, to a scFv that specifically binds to neoepitopes (a subset of phospholipids) expressed in the post-ischemic brain. The construct, termed C2scFv-Crry, was administered intravenously 90 minutes after ischemia in a 1-hour middle cerebral artery occlusion (MCAO) mouse stroke model. Mice were then tested for recovery over 21 days using neurological severity score (NSS) for symmetry, corner test for forelimb laterality, and passive avoidance task for fear memory. All personnel involved in conduct & analysis of experiments were blinded, and behavior was scored separately by two researchers.
Results: Compared to control mice (vehicle treated), mice that received C2-Crry performed significantly better on motor and cognitive tasks. C2-Crry treated mice had significantly lower scores on NSS both acutely and at 21 days (using non-parametric t-tests), lower magnitudes of laterality on corner test, and longer latency to enter the dark room on passive avoidance task (using student t-tests & two-way ANOVA to compare groups at single & at multiple time points, respectively).
Conclusion: Targeted-complement inhibition using C2-Crry improves motor and cognitive recovery after 1-hour MCAO in mice. This can be attributed to neuroprotection by reducing inflammation and cellular death, investigation of which is currently underway and which will be reported on.

Disclosures: A. Toutonji: None. **S. Tomlinson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder.

Poster

563. Ischemia IV

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Program #/Poster #: 563.06/K1

Topic: C.08. Ischemia

Support: NIH grant 1R01NS096225-01A1
American Heart Association 17GRNT33660336
American Heart Association 13SDG1395001413

Title: Neuroprotective effects of palmitic acid methyl ester against cerebral ischemia

Authors: *A. DO COUTO E SILVA[1], R. H.-C. LEE[2], C. Y.-C. WU[2], H. POSSOIT[2], C. T. CITADIN[1], P.-Y. CHEN[2], T.-H. HSIEH[2], R. AZIZBAYEVA[3], J. T. NEUMANN[3], H. W. LIN[2]
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Abstract: Cardiopulmonary arrest (CA) affects more than 350,000 people each year in the USA. Disruption of cerebral blood flow (CBF), more specifically CA-induced hypoperfusion (decrease in CBF), results in severe and selective brain damage contributing to neuronal cell death, which leads to cognitive impairment. Functional neuroprotective therapies remain few and ineffective. Our goal is to identify novel neuroprotective therapies which could modulate CBF and provide neuroprotection after ischemia. Previously, we discovered a saturated fatty acid, palmitic acid methyl ester (PAME), as a novel vasodilator/neuroprotective agent. PAME is released from the superior cervical ganglion (sympathetic nervous system), which innervates major cerebral arteries and is enhanced in the presence of arginine derivatives. Furthermore, arginine is a substrate for protein arginine methyltransferases (PRMTs). PRMTs can methylate various biological targets causing pre or post-transcriptional/translational modifications. Therefore, our hypothesis is that methylation of palmitic acid (PA) to form PAME via PRMTs is essential to enable PAME's therapeutic actions against ischemia, providing enhancements in CBF, neuroprotection, and functional recovery. To characterize the therapeutic properties of PA vs PAME, we subjected organotypic hippocampal slices to oxygen glucose deprivation (OGD) and visualized total cell death via propidium iodide staining. Our results suggest that treatment with PAME after OGD, but not PA, enhances neuroprotection in the CA1 region of the hippocampus [vehicle OGD (0.538 ±0.022) and PAME OGD (0.232 ±0.055)]. To investigate if the methylation of PA is needed for CBF enhancement after ischemia, we utilized a global model of cerebral ischemia [asphyxial CA (ACA, 6min)]. The rat skull was thinned to visualize red blood

cell speed (an indicative measure of CBF) in microvessels of the neocortex via intra-vital two-photon laser scanning microscopy. Our results suggest that PAME enhances cortical CBF while maintaining systemic blood pressure *in vivo.* Additionally, histopathological analyses suggest that treatment with PAME, but not PA enhances neuronal survival after ACA. Functional cognitive outcomes were tested post-ACA via spontaneous alternation test (T-maze). Treatment 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 results suggest that methylation of PA to form PAME, enhances CBF, neuroprotection, and functional outcomes after CA.

Disclosures: A. Do Couto E Silva: None. **R.H. Lee:** None. **C.Y. Wu:** None. **H. Possoit:** None. **C.T. Citadin:** None. **P. Chen:** None. **T. Hsieh:** None. **R. Azizbayeva:** None. **J.T. Neumann:** None. **H.W. Lin:** None.

Poster

563. Ischemia IV

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Program #/Poster #: 563.07/K2

Topic: C.08. Ischemia

Support: National Natural Science Foundation of China 81271307

Title: Outcomes in mild acute ischemic stroke treated with intravenous thrombolysis

Authors: *R. ZHANG, H. WEI, X. QIN
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Abstract: Background
Stroke is a leading cause of death and disability in the world. Minor stroke, a subtype of stroke, accounts for about two-thirds of stroke approximately. Intravenous thrombolysis by recombinant tissue plasminogen activator (rt-PA) has been widely confirmed to be an effective and safe therapy for minor stroke patients. However, the cost of rtPA is too expensive to afford for many poor patients. At this time, urokinase, another drug for intravenous thrombolysis becomes their choice because of its cheap price. In fact, urokinase is being widely used in poor areas of China to treat poor minor stroke patients as a substitute of rtPA. However, the use of urokinase is lack of clinical study, and the safety and efficacy of it remains unclear. Therefore, it is of high significance to explore the safety and efficacy of urokinase treatment for acute minor stroke patients.
Methods
Minor stroke was defined as the baseline NIHSS score is ≤ 5. Of 610 acute ischemic stroke patients underwent urokinase thrombolysis ≤ 6 hours we collected in 22 hospitals at Chongqing

and Sichuan Province of China, a total number of 126 minor stroke patients was included into this study. Outcomes were the 3-months favorable functional outcome (modified Rankin Scale score 0-1), mortality and bleeding. Results were compared among different thrombolysis time window groups (< 3 hours, 3-4.5 hours, 4.5-6 hours). SAS9.2 was used for data analysis. Values of $p < 0.05$ were regarded as statistically significant.
Results
Of the 126 minor stroke patients, 4 cases (4/126, 3.2%) died during 3 months. 1 case (1/126, 0.7%) had symptomatic cerebral hemorrhage. 2 cases (2/126, 1.6%) had asymptomatic hemorrhaging. 12 cases (12/126, 9.5%) have oral
mucosal bleeding. The MRS scores of 105 cases (105/126, 83.3%) were 0 or 1 at 3 months. Furthermore, when grouped the patients by thrombolysis time window, the death and bleeding rates of the different groups were low and were not statistically significant ($p > 0.05$). The favorable functional outcomes in each time window groups were as follows respectively: 37 cases (37/41, 90.24%), 35 cases (35/42, 83.33%) and 33 cases (33/43, 76.74%), while there is no significance among them ($p > 0.05$). But when using logistic regression analysis, we found that for the 3 thrombolytic time window level, the poor prognosis risk is 2.486 times higher when the time window adds a level.
Conclusions
For thrombolysis of minor stroke, it is safe and effective to use urokinase within 6 hours of the disease onset. But the longer the time of treatment start, the higher rate of poor prognosis is.

Disclosures: R. Zhang: None. **H. Wei:** None. **X. Qin:** None.

Poster

563. Ischemia IV

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Program #/Poster #: 563.08/K3

Topic: C.08. Ischemia

Support: American Heart Association 15GRNT25700284

Title: Protective and restorative effects of stem cell factor and granulocyte-colony stimulating factor on brain repair through VEGF-mediated angiogenesis in a mouse model of CADASIL

Authors: *S. PING
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Abstract: Cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy (CADASIL), a NOTCH3 gene mutation-induced cerebral small vascular disease, is characterized by progressive degeneration of vascular smooth muscle cells (VSMCs) in cerebral small arteries. Our earlier studies have revealed increases in angiogenesis and

improvements in spatial learning and memory by stem cell factor and granulocyte colony-stimulating factor (SCF+G-CSF) treatment in a transgenic moue model of CADASIL (TgNotch3R90C). This study aimed to determine how SCF+G-CSF promotes angiogenesis and whether SCF+G-CSF-enhanced angiogenesis is a key mechanism involved in brain repair and cognitive improvement in TgNotch3R90C mice. Avastin, a FDA-approved drug for preventing angiogenesis by neutralizing VEGF's biologic activity, was used for blocking SCF+G-CSF-increased angiogenesis. SCF+G-CSF, SCF+G-CSF-Avastin, Avastin or an equal volume of vehicle solution were injected (s.c.) at 9 and 10 months of age in TgNotch3R90C mice. Age-matched C57BL/6J mice served as wild type (WT) control. After neurobehavioral testing mice were sacrificed at 15 months of age for pathological examination. Our data showed that SCF+G-CSF-improved spatial learning and memory was eliminated by Avastin. Decreased blood vessel density and impaired VSMC structure in the brains of TgNotch3R90C mice was restored by SCF+G-CSF treatment, whereas the SCF+G-CSF-enhanced angiogenesis and VSMC protection were completely abolished by Avastin. Avastin also blocked the SCF+G-CSF-enhanced neural network remodeling (by MAP2 and SMI312 immunostaining), neurogenesis (by Doublecortin), and synaptogenesis (by Synaptophysin) in the brains of TgNotch3R90C mice. VEGF expression was decreased in the cerebral VSMCs and the whole brain of TgNotch3R90C mice. SCF+G-CSF treatment significantly increased VEGF expression in both cultured cerebral VSMCs and brain tissue of TgNotch3R90C mice. These findings suggest that SCF+G-CSF-increased angiogenesis via VEGF plays a key role on brain repair in a mouse model of CADASIL. This study provides novel insights into how hematopoietic growth factors restrict CADASIL pathology.
This study was supported by American Heart Association (15GRNT25700284).

Disclosures: S. Ping: None.

Poster

563. Ischemia IV

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Program #/Poster #: 563.09/K4

Topic: C.08. Ischemia

Support: Sancilio & Company, Inc
1R01NS096225-01A1 NIH/NINDS
17GRNT33660336 AHA

Title: SC411 improves cerebral blood cell flow after ischemia in the Townes mouse model of sickle cell disease

Authors: *C. Y.-C. WU[1], A. DAAK[2], M. A. LOPEZ-TOLEDANO[2], A. L. W. RABINOWICZ, 33137[2], H. LIN[1]

[1]Neurol., LSU Hlth. Sci. Ctr. Shreveport, Shreveport, LA; [2]Sancilio & Company, Inc, Riviera Beach, FL

Abstract: Background: Sickle cell disease (SCD) is an inherited blood disorder caused by a gene mutation that results in abnormal hemoglobin S (HbS). Under low oxygen tension, HbS polymerizes to form rigid/deformed red blood cell (RBC). RBC of SCD patients are characterized by enhanced expression of adhesion molecules and increased propensity to adhere to the endothelial wall causing episodic vaso-occlusion. Ischemic stroke is one of the major complications, leading to physical and/or cognitive impairments. SCD patients present a deficit in docosahexanoic acid (DHA). DHA is known to have potent anti-inflammatory, anti-adhesion, and anti-oxidant properties. DHA treatment may reduce RBC adhesion and enhance cerebral blood cell flow (CBF). The effect of SC411, a novel highly purified DHA ethyl ester formulation with a proprietary delivery platform (Advanced Lipid Technology® (ALT®), on CBF was investigated.
Objective: To investigate the effect of two oral doses of SC411 on reversing DHA deficiency and to improve cerebral blood cell in the HbSS-Townes SCD mouse model.
Methods: Transgenic sickle cell mice (HbSS-Townes) were fed with two doses of SC411 (36 mg DHA /kg/day or 180 mg DHA /kg/day) or control (soybean oil) for 56 days and subjected to 3 hrs of hypoxia (10% O_2) at days 28 and 56. RBC flow was measured in real-time using two-photon laser scanning microscopy at 0, 28, 56 days.
Results: HbSS-Townes mice presented with lower DHA and EPA levels as compared to healthy HbAA controls. DHA levels were higher in Townes mice as compared to controls in SC411-treated groups after 4 weeks of intervention. RBC flow was also enhanced in HbSS-Townes mice treated with 180 mg DHA/kg/day, challenged with repeated bouts of hypoxia, on both 28 and 56 days.
Conclusions: Preliminary findings from this ongoing study suggest that treatment with SC411 improves RBC flow in the HbSS-Townes SCD mouse model.

Disclosures: C.Y. Wu: 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.; SCI and LSU. **A. Daak:** None. **M.A. Lopez-Toledano:** None. **A.L.W. Rabinowicz:** None. **H. Lin:** None.

Poster

563. Ischemia IV

Location: SDCC Halls B-H

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Program #/Poster #: 563.10/K5

Topic: C.08. Ischemia

Title: Impact of therapeutic hypothermia on cerebral autoregulation and neuroglial protection in an asymmetric ischemia-reperfusion model

Authors: *E. CHOI[1], G. PARK[1], H. SHIN[1], S.-J. LEE[2], M. CHOI[2], J. HONG[2]
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Abstract: BACKGROUND: Cerebral autoregulation (CA) is the ability to maintain sufficient and stable cerebral blood flow (CBF) despite changes in cerebral perfusion pressure (CPP). It is a biological marker of cerebrovascular reserve and its impairment can lead to subsequent brain damage in the setting of stroke, traumatic brain injury, and global cerebral ischemia. Therapeutic hypothermia (TH) is a promising neuroprotection strategy in numerous experimental and clinical situations. Therefore, we are to investigate CA function as a biological change of cerebrovascular reserve after TH in an asymmetric ischemia-reperfusion model. **METHODS:** We made asymmetric ischemia-reperfusion model "chimeric model" with different ischemic mode in each hemisphere for mimicking global ischemia and for obtaining asymmetric ischemia-reperfusion contrast between two hemispheres. This model was established in male Sprague-Dawley rats through combination with transient middle cerebral artery occlusion (tMCAO) in for 30 minutes the right side and transient four-vessel occlusion (4-VO) for 8 minutes. Temperature management [hypothermia (33℃), normothermia (37℃), and hyperthermia (39℃)] was maintained in a temperature controller for 120 minutes after 4-VO. We sequentially measured cerebrovascular reserve capacity (CVRC) with acetazolamide (ACZ, 50mg/kg), modified neurological severe score (mNSS), cell death, endothelial cell and mitochondrial functions at 1, 2, 3, 5, and 7 days after procedure. We also analyzed histological and molecular characteristics amongst different temperature conditions. **RESULTS:** (vs. contralateral CVRC), ipsilateral CVRC was significantly decreased until 3 days and gradually recovered to normal level from 5 days after injury. (vs. normothermia or hyperthermia), hypothermia showed the restoration of CVRC with enhancement of endothelial functions (eNOS and tight junction proteins) at 3 days after injury. They also showed neuroprotective effects by decreasing inflammatory responses, oxidative stress, and pro-apoptosis (Bax and cytochrome C) and by increasing anti-apoptotic protein (Bcl-2) at 3 days after injury. Consequentially, they led to a decrease in infarct volume, neurological deficit scores, blood brain barrier (BBB) damage at 7 days after injury. **CONCLUSIONS:** Our data indicate that TH alleviates CA failure and subsequent cerebral damage in asymmetric ischemia-reperfusion model as restoring endothelial function. Rapid monitoring and management of CA would be a therapeutic target in certain ischemia-reperfusion injuries such as cardiac arrest and embolic stroke.

Disclosures: E. Choi: None. **G. Park:** None. **H. Shin:** None. **S. Lee:** None. **M. Choi:** None. **J. Hong:** None.

Poster

563. Ischemia IV

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Program #/Poster #: 563.11/K6

Topic: C.08. Ischemia

Title: Backward directional arteriogenesis by cranial burr hole and erythropoietin pretreatment in ischemic rat model with cerebral perfusion impairment

Authors: *G. PARK[1,2], E. CHOI[1,2], H. SHIN[1,2], K.-E. LEE[1], M. CHOI[1], S.-J. LEE[1], J. HONG[1]
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Abstract: BACKGROUND: Cranial burr hole procedure can be a potential revascularization strategy even in acute stroke patients with perfusion impairment, however, it cannot guarantee stable revascularization from the extracranium. Erythropoietin (EPO) is an attractive candidate with an angiogenic potential with proven safety in high-dose administration to promote successful revascularization. We investigated the efficacy and safety of a combined treatment by cranial burr hole with erythropoietin (EPO) pretreatment in severe ischemic rat model with cerebral perfusion impairment. We also evaluated its cellular mechanism. **METHODS:** Severe ischemia with cerebral hypoperfusion was established in male Sprague-Dawley rats (250 to 270g) through permanent bilateral internal carotid artery ligation (bICAL) and transient middle cerebral artery occlusion (tMCAO) for 30 minutes. Experimental models received intraperitoneal injection of recombinant human erythropoietin (EPO, 5,000 U/kg) or saline for 3 consecutive days at 7 days after ischemic injury. Cranial burr hole and cracking dura mater as a mechanical barrier disruption (MBD) from extracranium to intracranium were performed on the right hemisphere. We sequentially evaluated modified neurological severe score (mNSS), infarct volume, revascularization take rate, hemodynamics, BBB breakdown, histology and molecular analysis up to 2 months. **RESULTS**: Our modified tMCAO model with bICAL (vs. original tMCAO model) showed a prolonged reduction of CBF and increased expression of hypoxia-related factors in ipsilateral hemisphere. Hemisphere with MBD (vs. hemisphere without MBD) had inflammatory responses such as glial activation and upregulations of pro-inflammatory factors, while EPO-combination group suppressed inflammatory responses. Combination treatment group (vs. no treatment group or MBD-only group) had a successful transdural anastomosis with the upregulation of pro-angiogenic factors, vessel maturation (PDGF-beta, TIE-2, alpha-SMA), and endothelial cell proliferation (BrdU). They also showed the induction of cell survival pathways (pAkt and eNOS) through activation of erythropoietin receptor. Finally, transdural revascularization and neuroprotection in combination group made reduction of the infarct volume and improvement of the neurological outcome. **CONCLUSIONS:** Our finding indicate that a combination therapy of MBD and systemic EPO pretreatment resulted in a

successful revascularization as well as neuroglial protection in ischemia rat model with cerebral hypoperfusion.

Disclosures: G. Park: None. **E. Choi:** None. **H. Shin:** None. **K. Lee:** None. **M. Choi:** None. **S. Lee:** None. **J. Hong:** None.

Poster

563. Ischemia IV

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Program #/Poster #: 563.12/K7

Topic: C.08. Ischemia

Support: CalciGenix

Title: The calcium binding protein apoaequorin alters cytokine expression following direct hippocampal brain infusion in a rat model

Authors: *C. W. SMIES, J. R. MOYER, Jr
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Abstract: As the aging population continues to grow, the number of individuals that experience a stroke will continue to rise. Thus, it is important to study the cellular and molecular mechanisms by which the negative effects of stroke can be ameliorated. Ischemic strokes are the most prevalent type of stroke and induce excitotoxicity via calcium dysregulation (Choi, 1999). Several molecules exist that are able to assist in calcium buffering and sequestration, including the calcium binding protein apoaequorin (AQ; Toma et al., 2005). Using an in vitro stoke model, an intra-hippocampal infusion of AQ is neuroprotective for up to 1 or 2 days later, whereas changes in cytokine mRNA expression (e.g. IL-10, TNF-α) are observed as early as 1 h following an AQ infusion (Detert et al., 2013). This disconnect between when neuroprotection is observed and when cytokine mRNA is expressed demonstrates the need to further investigate the cellular and molecular neuroprotective mechanisms of AQ. Ischemic preconditioning is a small insult that does not cause damage on its own, but proves to protect against major insults that occur at a later time. In addition to the neuroprotection gained from preconditioning, ischemic preconditioning has also been shown to alter anti-inflammatory cytokine expression, such as TNF-α (Wang et al., 2000), thus RT-qPCR is used to explore if AQ-induced cytokine mRNA expression is similar to that of ischemic preconditioning. To further elucidate the connection between AQ and cytokine expression it is important to know if and when cytokine mRNA is translated. Given that AQ infusion induces cytokine mRNA expression 1 h later, but requires a delay of 1 d to produce a neuroprotective effect (Detert et al., 2013), the timing of cytokine translation may be contributing to the delay of neuroprotection observed following an infusion of AQ. A combination of RT-qPCR and Western blotting techniques are used to determine if and

when cytokines induced by AQ are translated. This research will further explore the cellular mechanisms and temporal relationship between AQ-induced neuroprotection and ischemia.

Disclosures: C.W. Smies: None. **J.R. Moyer:** None.

Poster

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Topic: C.08. Ischemia

Support: NIH EY10343
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Bright Focus Foundation

Title: Mesenchymal stem cell-derived extracellular vesicles and retinal ischemia-reperfusion

Authors: *B. MATHEW[1], S. RAVINDRAN[2], L. A. TORRES[3], C.-C. HUANG[4], M. CHINNAKESAVALU[4], J. LOPEZ[4], M. SHARMA[4], X. LIU[6], S. ROTH[5]
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Abstract: Retinal ischemia is a major cause of vision impairment/loss and a common underlying mechanism associated with diseases such as glaucoma, diabetic retinopathy, and central retinal artery occlusion. The regenerative capacity of the diseased human retina is very limited (Jorstad et. al 2017). Our previous studies have shown the neuroprotective effects of intravitreal injection of mesenchymal stem cells (MSC) and MSC conditioned media in retinal ischemia (Mathew et. al 2017, Roth et. al 2016). We hypothesize that the neuroprotective effects of MSCs are largely mediated by extracellular vesicles (EVs), approximately 30nm -150nm in size secreted by most cells and involved in cell-to-cell communication. EVs were isolated from MSC conditioned media using a centrifugation and precipitation process. EVs were characterized using immunoblotting (CD63, CD9, HSP70), Nanosight analysis, and Transmission Electron Microscopy. MSC derived EVs were tested in our in-vitro oxygen-glucose deprivation (OGD) model of retinal ischemia in the R28 cell line, a well-characterized retinal precursor line consisting of neuronal and glial cells. We found that pre-treatment of R28 cells with MSC derived Evs 24 hours prior to OGD significantly reduced cell death, apoptosis and increased cell proliferation compared to the OGD control condition. Further, we studied the uptake of extracellular vesicles in our R28 cell line using fluorescent labelled EVs. Our results indicate that EV uptake is dependent on Heparin Sulfate Proteoglycans (HSPGs), and immuno-localization

studies indicated that EV uptake in retina depends on the caveolar endocytic pathway. Using our in vivo rat model of retinal ischemia, we have demonstrated EV induced functional recovery from retinal ischemia (ERG), increased neuroprotection as evidenced by decreased retinal inflammation(IL-6,TNF-a and Il-1b), apoptosis (TUNEL), and increased RGC count (IHC and flat mount).Taken together, MSC derived EVs play a key role in neuro-protection and offer potential in treating retinal ischemic injury.

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Poster

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Title: Autophagy and retinal ischemic post-conditioning

Authors: *M. CHENNAKESAVALU, B. MATHEW, C. STELMAN, M. SHARMA, L. TORRES, S. ROTH
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Abstract: Retinal ischemia is a major cause of vision impairment and a common underlying pathology associated with diseases such as glaucoma, diabetic retinopathy, and central retinal artery occlusion that currently affect millions worldwide. Our previous studies have demonstrated the potent neuroprotection conferred in retina by post-conditioning (post-C), a brief period of ischemia given 24 hours following prolonged and damaging initial ischemia in rat. Currently, the mechanisms guiding the remarkable protection induced by post-C are largely uncharacterized. Based on ischemic preconditioning studies in heart and brain, we hypothesize that autophagy, an intracellular catabolic “recycling” system, plays a key role in facilitating post-C induced neuroprotection in retina. Using our rat in-vivo model of retinal ischemic injury, we observed significant increases in the expression of autophagy proteins LC3-II and Beclin-1 and a

significant decrease in the expression of P62 in the post-C group vs. sham post-C group. Similar results in LC3-II, P62, and Beclin-1 were observed in the in-vitro model of retinal ischemia. Changes in expression of LC3-II, P62, and Beclin-1 in our in-vivo and in-vitro models were consistent with increased levels of autophagy in post-C. To further study the involvement of autophagy in post-C, we blocked two key proteins involved in autophagosome formation (Atg5 and Atg7) using small interfering RNA (siRNA). Blockade of Atg5/7 attenuated the functional protective effect of post-C (measured by electroretinography) and increased histological damage compared to treatment with non-silencing siRNA in-vivo. Blockade of Atg5 in-vitro similarly attenuated post-C induced protection measured by cell proliferation and viability. Utilizing tandem RFP-GFP-LC3B in-vitro, we visualized greater levels of autophagic flux with post-C. Further, induction of autophagy via TAT-Beclin attenuated cell death and increased cell proliferation in retinal neurons subjected to oxygen-glucose deprivation, suggesting the key role of autophagy in protection from retinal ischemic injury. Taken as a whole, our results suggest that autophagy is a key underlying mechanism in the post-C induced neuroprotection in retina, and that the supplementation of autophagy offers promise in the treatment of retinal ischemic injury.

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Poster

563. Ischemia IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 563.15/K10

Topic: C.08. Ischemia

Support: Alzheimer's Research UK Grant Grant ARUK-SRF-2013-4

Title: Neuroprotective effects of astrocyte-specific overexpression of Nrf2 in a mouse model of stroke

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Abstract: Cerebrovascular pathology such as that caused by stroke increases risk of cognitive decline and dementia by mechanisms including inflammation and oxidative stress. Nrf2 is a transcription factor and master regulator of a battery of antioxidant and anti-inflammatory genes. Nrf2 activation in astrocytes has previously been shown to confer protection in models of neurodegeneration. We hypothesised that overexpression of Nrf2, specifically in astrocytes,

would attenuate neuronal damage following stroke by reducing oxidative stress and inflammation. GFAP-Nrf2 male mice (2 to 3 fold increase of Nrf2 expression in GFAP-astrocytes) and C57Bl/6J (wild-type; WT) littermates controls (3-4 months old) underwent ischaemia (transient middle cerebral artery occlusion for 60 mins) (n=7-15/group) or control sham surgery and brains were collected at 24 hours. Indices of cellular neuroinflammation (Iba1, microglia; GFAP, reactive astrocytes) and oxidative stress (3-Nitrotyrosine) were quantified in the peri-infarct area using immunohistochemistry. Neuronal damage was assessed histologically. Gene expression for Nrf2 and Nrf2-related genes *hmox1*, *nqo1* and *slc7a11* were analysed by qRT-PCR (n=7-11/group). All surgeries and analyses were carried out blinded to experimental groups. There was a significant reduction in neuronal damage in GFAP-Nrf2 mice compared with WT mice following ischaemia (p=0.048). 3-Nitrotyrosine immunostaining was increased after ischaemia but there was a marked reduction in GFAP-Nrf2 compared with WT mice after ischaemia (p=0.003). Microgliosis was increased following ischaemia but not altered between WT and GFAP-Nrf2 mice. Reactive astrocytes were increased in the peri-infarct area after ischaemia, and furthermore were significantly increased in GFAP-Nrf2 compared with WT stroke mice (p=0.0007). Nrf2-related gene expression was unchanged after ischaemia in WT mice compared to shams but significantly increased in GFAP-Nrf2 mice following ischaemia. We have shown that the overexpression of Nrf2 in astrocytes reduces neuronal damage and oxidative stress following stroke and this was paralleled by increased reactive astrocytosis and expression of Nrf2-related genes. We are currently investigating if boosting Nrf2-signalling in astrocytes exerts long-term protection after stroke.

Disclosures: J.H. Fowler: None. **M. Aimable:** None. **L. Hegarty:** None. **K. Nagassima:** None. **J.A. Johnson:** None. **G.E. Hardingham:** None. **K. Horsburgh:** None.

Poster

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Topic: C.08. Ischemia

Support: NINDS Grant 5R01NS097511-03

Title: Dendrimer N-acetyl-cysteine to enhance glial restricted precursor transplantation and recovery following neonatal white matter injury in mice

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Abstract: Between 1.5 and 2 percent of live births in the United States are classified as very preterm with 5 to 20 percent of these infants diagnosed with spastic cerebral palsy. Neonatal white matter injury (NWMI) remains the predominant cause of brain injury in this population. During injury, early inflammatory cascades result in cell death and maturation arrest in oligodendrocytic lineage cells, and previous work demonstrates that transplanted glial restricted precursors (GRP) can facilitate short term recovery though long-term survival of these exogenous cells is low. Hydroxyl polyamidoamine dendrimer therapy successfully targets injured and activated cell types in the brain with low toxicity and high specificity. Acute administration of dendrimer conjugated to N-Acetyl-Cysteine (D-NAC) has been successful in other animal models. Mitigation of the inflammatory response using D-NAC following NWMI in conjunction with GRP transplantation may result in the improved survival of both endogenous and transplanted glial cells, resulting in healthy oligodendrocytes capable of typical myelination patterns in the long term. GRPs were obtained from embryonic day 13.5 mouse embryos. To establish the dynamics of dendrimer with GRPs, cells were exposed to dendrimer conjugated CY5 (D-CY5) *in vitro*, then analyzed to determine uptake. Preterm equivalent CD-1 mice underwent right common carotid artery ligations or a sham surgery at post-natal day (P)5. At P10, a treatment group received a single dose of D-NAC intraperitoneally. Those mice went on to receive either vehicle or an intracallosal injection of 100,000 green fluorescent protein (GFP) expressing GRPs at P22. A second group received a vehicle injection at P10, followed by GFP GRP intracallosal injection at P22. To interrogate the effect of administration time and localized delivery on transplanted cell survival, cells were loaded with D-NAC prior to transplantation. The D-NAC treated GRPs were then transplanted at P22 into ligated animals not previously exposed to D-NAC. D-CY5 successfully colocalizes to GRPs *in vitro* suggesting that transplanted GRPs pretreated with D-NAC may be efficacious *in vivo*. Furthermore, preliminary results at 4 and 8-week post-transplant indicate behavioral recovery to be both treatment and sex dependent. These results show, for the first time, that GRPs incorporate dendrimer conjugated therapies. Dendrimer-drug uptake *in vivo* may allow for a combined effect, reducing NWMI related inflammation while enhancing survival of GRPs for long term recovery. This multi-tiered approach can result in long term efficacy, expanding upon the acute successes each treatment has seen individually.

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Poster

563. Ischemia IV

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Topic: C.08. Ischemia

Support: NIH Grant R01NS081936
NIH Grant R56NS100088

Title: Using structural equation modeling to investigate predisposition of regional tetrahydrobiopterin for hypertonia following antenatal hypoxia-ischemia

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Abstract: Background: Tetrahydrobiopterin (BH4) levels in brain are low in prematurity. Without any insult, the rabbit fetuses mature and develop normally. Our previous hypothesis was that normally low BH4 levels in prematurity would fall below a threshold defining injury during hypoxia-ischemia (H-I) to cause critical brain injury. We have previously shown that MRI biomarkers can predict which fetuses will develop postnatal hypertonia following antenatal H-I.Objective: Our new hypothesis was that there is a combination of regional BH4 levels that determine which fetuses are predisposed to developing hypertonia following antenatal H-I.Methods: In vivo global HI of fetuses was induced in pregnant New Zealand white rabbits at 25 days gestation with a 4F Fogarty balloon catheter in a 3T magnet. Using MRI patterns of change in brain apparent diffusion coefficient (ADC, four patterns), we categorized fetuses predicted to get hypertonia based on criteria of ADC decrease below a nadir of 80% and presence of evidence of reperfusion-reoxygenation injury (patterns III and IV) and compared to those that were Non-hypertonia (Patterns I and II). Enough animals were enrolled to have a power of 80%. BH4 concentrations were assayed using HPLC-electrochemical detection. Statistical analyses used were ANOVA, t-test, logistic regression, correlation, structural equation modeling and path analysis. Results: Thalamus and cerebellum BH4 levels immediately after H-I were decreased in Hypertonia group vs Non-hypertonia (t-test) as well as different between patterns of MRI and sham (ANOVA). No differences were found for the cerebral cortex or basal ganglia alone using t-test and ANOVA. The interaction of all four regions was found to be significantly influencing Hypertonia on ANOVA and logistic regression. Interactions of two regions showed Cortex and Cerebellum correlations and Cortex and Basal ganglia correlations to be different between Hypertonia and Non-hypertonia. Additional analyses using structural equation modeling indicate that all four brain regions contribute to causing hypertonia. The best model obtained with path analysis shows thalamus to affect hypertonia the most. The error of the path to Hypertonia in this

model is 0.15. Conclusions: Only some fetuses will become hypertonic with a certain fetal insult. There is a combination of regional brain BH4 that predisposes to hypertonia following antenatal H-I. Our findings may explain prematurity by itself could be a risk factor for cerebral palsy in other studies.

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Poster

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Topic: C.08. Ischemia

Support: FAPESP 2016/17329-3
FAPESP 2014/16711-6
CNPq

Title: Neonatal anoxia in rats: Protein levels of hippocampal interneurons and spatial memory in adult rats

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Abstract: The neonatal anoxia is one of the most common causes of morbidity and mortality in neonates. This injury in early life corresponds to 23% of neonatal deaths in all the world being an important issue for public health. The longlasting sequelae include motor deficits, behavioral and sensory and /or cognitive impairments, such as memory and learning deficits. The hippocampus is a vulnerable structure to oxygen deprivation due its high metabolic demand and capacity for synaptic plasticy. The presente model of neonatal anoxia has showed, in previous work, cell death in hippocampus 24 hours after oxygen deprivation, and in adult life, it was observed decrease of neurogenesis and impairments in spatial and working memory (Takada et al. 2015; Takada el al. 2016). In this work, we hypothesize that important interneurons population related to memory consolidation and electrophysiologic modulation can be altered by oxygen deprivation in neonatal rats. In this way, the aim of this study was to performe memory task and characterize the population of distinct hippocampal interneurons, such as parvalbumin, calretinin and calbindin, and proteins related to synapsis, synapsin I and synaptophysin of adult rats submitted to neonatal anoxia. P1/P2 neonates Wistar rats (Rattus norvegicus) were divided in anoxia and sham groups. Anoxia was performed according to described system of neonatal anoxia, composed by 25 minutes of 100% nitrogen gas exposure at 37°C (Takada et al., 2011).

At P60, the rats were euthanized and the hippocampi removed for western blotting analysis. In spatial reference memory and working memory task, the Barnes maze apparatus was used. Our preview results showed an increase of synapsin I protein levels in anoxia group ($p<0.05$). However, we detected no difference in protein levels to parvalbumin, calretinin, calbindin and synaptophysin. In spatial memory task, we observed that anoxia groups presented a higher escape latency compared to control group in spatial reference memory task. The Barnes Maze protocol used to test working memory was not sensible to detect alterations. These data suggest that neonatal anoxia can cause alterations in hippocampus of adult rats in synaptic proteins, but not in protein levels of interneurons, and also causes an impairment in spatial memory task.

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Poster

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Program #/Poster #: 563.19/K14

Topic: C.08. Ischemia

Support: FAPESP Grant 2016/16892-6

Title: Neonatal anoxia in rats: Decrease of parvalbumin hippocampal interneurons during development of rats

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Abstract: Neonatal anoxia is an important public health concern worldwide, once it may lead to hypoxic-ischemic encephalopathy, composed by serious permanent sequels (memory and learning deficits, cerebral palsy, hyperactivity, hearing deficiencies and others). The brain is one of the most susceptible organs to oxygen deprivation, since it demands high energy rate. Among the brain structures, the hippocampus (HPC), important to mnemonic processes, is one of the most sensitive areas to anoxia and it is characterized by a well-known morphology and neuronal circuits. Its cell variety is largely composed by interneurons, inhibitory cells that form local circuits through synapses with the main cells of the HPC, thereby promoting harmonization to hippocampal oscillations, such as theta and gamma, and controlling the activity and local rhythmicity. Anoxia results in numerous events leading to injury and neuronal death in the HPC and can alter the connectivity and hippocampal function. However, the impact of anoxia in the balance of excitatory and inhibitory neurons is not well known. Thus, the aim of this study was investigate the parvalbumin (PV)-positive population of cells, an important class of interneurons

with a well-defined fast-spiking electrophysiological signature. These interneurons have a crucial role in the process of learning and memory during development of rats submitted to neonatal oxygen deprivation. Briefly, P1-P2 neonate Wistar male rats (Rattus novergicus) were maintained in a semi hermetic chamber for 25 minutes, at 37°C, saturated by 100% nitrogen gas (Takada et al., 2011); control groups were exposed to room air. In P30, the rats were transcardial perfused and their brains were processed for PV immunohistochemistry. Hippocampal PV-positive cells were count using stereological analysis. Results revealed PV population decrease in hippocampus of rats from anoxia group (P=0.007), specifically in CA3 region (P=0.007), and a tendency in CA1 region (P=0.072). It suggests that neonatal anoxia reflects in PV population long term susceptibility. The decrease of this population was identified in CA3, which is involved in process of integration of hippocampal information; and CA1, principal output pathway of the hippocampal information. This important finding might reflect in primordial control of the excitability and oscillations that could contribute to the better understanding of the pathophysiology of neonatal anoxia sequelae and provide subsidies for future therapeutic approaches related to cognitive and learning deficits caused by neonatal anoxia.

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Poster

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Topic: C.08. Ischemia

Title: Molecular mechanism of action of galantamine in reducing hyperoxia-induced brain injury in neonatal mice

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Abstract: Background: Hyperoxia affects brain development in premature infants leading to excess free radical production with subsequent inflammation, astrogliosis, microgliosis and apoptosis. Galantamine, an acetyl cholinesterase inhibitor, showed a protective role in hypoxic brain injury by its anti-inflammatory effects. **Objective:** To explore the mechanism by which galantamine reduces hyperoxic brain injury in neonatal mice. **Design/Methods:** WT mouse pups were housed in a hyperoxia chamber (FiO2 95%) for 7 days. Half the control as well the test group, were injected daily with galantamine intraperitoneally (IP) (5mg/kg/dose) and the other half were injected with saline. After exposure, brain tissue was studied for : IF staining for ChAT, NeuN, Iba-1, CD68, CNPase and GFAP; multiplex ELISA for Pro-inflammatory markers

and HMGB1; western immunoblot for NF-κB activity; fluorometric assay for Caspase 3, ROS assay and acetyl cholinesterase activity. MicroRNA profile panel was studied using a custom designed microarray plate. All results were compared to control group housed in room air (RA). **Results:** IF staining showed a significant increase of ChAT expression accompanied by a significant reduction in acetylcholinesterase activity in the hyperoxia groups treated with galantamine vs. saline group. In galantamine treated hyperoxia group, oligodendrocytes were preserved and thus the myelination. Also, CD68 was decreased, indicating less microglial activation which leads to a reduction of neuronal apoptosis (caspase 3). Both inflammatory markers (HMGB1, IL 12p70, IL6, KC GRO and IL10, pP65), and ROS, showed a significant decrease among hyperoxia galantamine treated group compared to the saline group. MicroRNA profile showed a significant >3 fold increases of the following: mir181a-3p; mir185-3p and mir146a-5p; and a significant reduction by >5 fold decrease for both mir21a-3p and mir494-5p among saline treated hyperoxia group in comparison to control RA. All these findings were reversed in galantamine hyperoxia treated group: mir181a-3p; mir185-3p and mir146a-5p were significantly downregulated and mir21a-3p and mir494-5p were upregulated as compared to saline group. **Conclusion(s):** Galantamine shows a potent anti-inflammatory and antioxidant activity in hyperoxia-induced brain injury in neonatal mice. Hyperoxia exposure has a specific impact on microRNA profile expression in neonatal brain tissue. Treating neonatal animals exposed to hyperoxia with galantamine leads to a specific modification of microRNA expression which could be a new therapeutic target.

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Poster

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Topic: C.08. Ischemia

Support: CIHR, CGS M
VHRN, Graduate Student Performance Award
RI-MUHC, Desjardins Studentship in Child Health Research

Title: Impact of sildenafil on vasculature, gliosis and inflammatory cytokine expression on retinal injury secondary to hypoxia-ischemia

Authors: *P. BALIAN[1,2], A. YAZDANI[2], A. BÉLANGER[2], V. BLEAU[2], Z. KHOJA[2], P. WINTERMARK[2]
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Abstract: Background: Birth asphyxia often leads to long-term neurological consequences including visual impairments. These impairments are associated to hypoxic-ischemic injuries to visual pathways in the brain, but also injuries to the neuroretina itself. Treatment with sildenafil has been shown to ameliorate retinal function in a rat model of term neonatal asphyxia, however, the underlying cellular mechanisms explaining this amelioration remain to be elucidated. Our goal is to determine the impact of sildenafil on retinal neurons, inflammation and vasculature following hypoxia-ischemia (HI).
Methods: Neonatal HI was induced in rat pups at P10 by left common carotid ligation followed by 2-hour exposure to 8% oxygen. Subsequently, animals were randomly administered a vehicle solution or 50 mg/kg of sildenafil for 7 days. At P12 and P17, ELISA was performed to measure inflammatory cytokine IL1B levels. At P30, immunohistochemistry on radial sections of the retina was performed to examine retinal ganglion cells (Brn3a), bipolar cells (Chx10), astrocytes (GFAP), as well as activated (Nestin) and non-activated (GS) Muller glia. Immunohistochemistry on flatmount preparations of the retina was performed to assess retinal vasculature (Lectin).
Results: At P12, IL1B levels increased with HI compared to sham, but normalized by P17. At P30, HI caused a decrease in the number of retinal ganglion cells and bipolar cells, as well as persistent inflammation marked by an increase in the number of astrocytes and an increase in the number of activated Muller glia. HI also induced a loss of blood vessels in deeper layers of retinal vasculature. Sildenafil administration restored the number of retinal neurons, reduced inflammation by regulating gliosis and IL1B expression at P12, and improved retinal vascularization by increasing vascular branching in deep layers.
Conclusion: Sildenafil seems to improve retinal injuries by reestablishing retinal neuron numbers back to sham levels, modulating inflammation following HI and improving retinal vasculature.

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Poster

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Topic: C.08. Ischemia

Title: The effects of sildenafil on the suppression of RNF213(a susceptibility gene of Moyamoya disease) under hypoxia

Authors: *H.-S. SHIN[1], G. PARK[2], E. CHOI[2], M. CHOI[3], S. LEE[2], J. HONG[4]
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Abstract: BACKGROUND: Moyamoya disease (MMD) is a rare and progressive occlusive disorder of cerebral vasculature around the circle of Willis with abnormal compensatory collateral vessels so called ‘moyamoya’. Ring-finger protein 213 (RNF213, a specific susceptibility gene of MMD) was recently identified through genome-wide linkage analyses. Even if allelic variations of RNF213 are associated with the risk of MMD and intracranial stenosis in Asians, its role under hypoxic condition has still been unclear. Meanwhile, phosphodiesterase (PDE) 5 inhibitors including sildenafil increase the level of nitric oxide (NO) with the activation of cyclic guanosine monophosphate (cGMP) through intracellular signal processes. Therefore, we are to investigate that sildenafil can restore the endothelial function under hypoxia or normoxia with a suppression of RNF213 in a cell line. **METHODS:** We incubated Human Umbilical Vein Endothelial Cells (HUVECs) with chemical inducer of hypoxia [$CoCl_2$ (200 μM/ml)] for 1 hour. A cell line was treated with Sildenafil in a dose-dependent manner (1uM, 5uM, 10uM, 20uM). For physiological hypoxic insult, HUVECs transferred to an anaerobic chamber. The culture medium was replaced with the saturated with N_2 gas. After the incubation of the cells in hypoxic chamber, HUVECs were transferred to normoxic chamber and treated with Sildenafil in a dose-dependent manner. **RESULTS**: mRNA and protein levels of RNF213 expressed about 5-fold under $CoCl_2$-induced hypoxia. In addition, HUVECs treated with $CoCl_2$ were significantly decreased the angiogenic ability via the induction of RNF213. The administration of sildenafil mitigates a fragile endothelial tube formation with a downregulation of RNF213 and hypoxia-inducible factor-1 alpha (HIF-1α) in a dose-dependent manner. **CONCLUSIONS:** Our results suggest that endothelial function is closely related to a hypoxic stress condition under a suppression of RNF213 with the moyamoya susceptibility. In our data, sildenafil restored impaired endothelial function through angiogenic transduction pathway along with an attenuated RNF213.

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Poster

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BMBF 01ED1510A

Title: The effects of a one-year extensive exercise program on the progression of mild cognitive impairment

Authors: *S. SCHNEIDER[1], M. OLDE RIKKERT[2], B. LAWLOR[3]
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Abstract: A lack of physical exercise plays a major role in the pathophysiology of vascular, metabolic, and metastatic diseases. Regular physical exercise has been successfully proven to counteract this deconditioning. Human and animal studies have demonstrated that regular physical activity targets brain function by increasing cognitive reserve. There is also evidence of structural changes caused by exercise in preventing or delaying the genesis of neurodegeneration. A considerable number of studies have targeted the effects of physical activity on functional and structural brain changes in patients at greater risk for Alzheimer's disease (AD). Epidemiological studies have shown that leisure-time physical activity at midlife is associated with a decreased risk of dementia and AD later in life. Although initial studies indicate enhanced behavioural performance in dementia patients after three months of exercise, little is known about the effect of an extensive, controlled and regular exercise regimen on the progressive neuropathology of cognitive impairment with and without dementia. This study aimed to determine the effects of an extensive exercise program in the prodromal phase of AD, known as mild cognitive impairment (MCI) with respect to the progression of the disease. 225 previously sedentary patients with diagnosed MCI underwent a standardized one-year extensive aerobic exercise intervention (3 units à 30-60min / week, according to WHO recommendations). Changes in the progression of cognitive impairment were monitored by means of cognitive testing (Cogstate, MoCA) and self-rated quality of Life (DemQOL) in comparison to a matched sedentary group as well as a group of subjects undergoing stretching and toning exercises. First results show an increase in physical fitness ($p < .05$) for participants attending sessions at least twice / week, which is mirrored by an increase in cognitive performance ($p < .05$) and self-rated quality of life ($p < .001$). No changes where obtained for participants attending only one session per week. Data of the sedentary control group revealed an ongoing decrease of physical fitness and cognitive performance in the course of the year. It is concluded that an extensive exercise program is able to decelerate further cognitive decline in MCI patients, even if they were following a sedentary life-style so far. Data emphasizes the importance of an active lifestyle and regular physical exercise for brain health in the context of an increasing sedentary society and is of high relevance for socio-economic and health-political decisions related to the increasing number of neurodegenerative diseases.

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Poster

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Support: NIH Trailblazer R21 grant (1R21EB024793-01) to Y.A.
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Title: Multimodal detection of spreading depolarization and repolarization during cardiac arrest and resuscitation: An ultra-early biomarker of neurological outcome

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Abstract: Intro: Spreading depolarizations (SD) are known to occur during acute and ongoing brain injury, such as ischemia and trauma. Most studies suggests that SD contribute to and exacerbate ongoing brain injury. Recent data from humans demonstrates SD occurring during cardiac arrest (CA), though the significance of SD is unknown. Using a rodent model of cardiac arrest and resuscitation, we have found that the timing of occurrence of SD during cardiac arrest is an early biomarker of neurological outcome.
Methods: Wistar rats (n=26) underwent asphyxial cardiac arrest followed by cardiopulmonary resuscitation (CPR) while monitoring electrocorticography (ECoG), cerebral blood flow (CBF), and brain metabolism. Neurological recovery was measured at various post-resuscitation time points with quantitative ECoG parameters and behavioral tests (i.e. Neurological Deficit Score; NDS). SD during CA and repolarization post-CPR was detected with ECoG after applying a 1-Hz low-pass filter, and onset was determined visually and confirmed algorithmically. Laser speckle imaging (LSI) and spatial frequency domain imaging (SFDI) were used to measure CBF, tissue oxygenation, and cerebral metabolic rate of oxygen (CMRO2).
Results: SD was captured directly by DC potential measurement and indirectly by ECoG. Earlier onset of SD during cardiac arrest is closely associated with better neurological recovery, with r value ranging 0.65-0.80 ($p<0.001$). During the SD period, we found a wave of decreasing CBF beginning 2.28 +/- 0.34 min post-asphyxia that lasted 1.05 +/- 0.29 min. Simultaneous spatio-temporal propagation of changes in tissue scattering were also detected and preceded by an inflection point in the CMRO2, suggesting a transient increase in cerebral metabolic activity. Post-CPR, a spreading repolarization (SR) was captured by DC potential measurement, ECoG, LSI, and SFDI, suggesting a mirror image phenomenon of SD during the CA phase. Earlier onset of SR was associated with better neurological outcome ($r= 0.5-0.6$, $p<0.01$). SR always preceded the initial EEG burst during the recovery phase.
Conclusion: Our findings demonstrate the first evidence for a unifying model of SD and SR occurring during CA and post-CPR, respectively, with earlier onset of SD and SR associated with better neurological outcome. Metabolic mechanisms underlying these findings are being explored. These data have important implications for prognostication, as they may be the earliest reported biomarker of outcome, and invoke various novel therapeutic interventions during CA.

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Poster

563. Ischemia IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 563.25/L2

Topic: C.08. Ischemia

Support: SfN-IBRO award

Title: Roflumilast, a phosphodiesterase 4 inhibitor, prevents memory impairments and increases hippocampal neurogenesis after transient global cerebral ischemia in rats

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Abstract: Background: Brain ischemic processes, such as the transient global cerebral ischemia (TGCI), is an immediate and severe outcome of reversible cardiac arrest. TGCI compromise the blood supply to the brain and causes neuropsychological, emotional, cognitive and physical deficiencies associated with neurodegeneration, reducing patients' quality of life. Phosphodiesterase inhibitors (PDE-I) may represent a novel therapeutic strategy for the treatment of cerebral ischemia sequelae. Rolipram, a PDE4-I, improved emotional and cognitive outcomes of TGCI. However, rolipram presents severe emetic effects which make impossible its clinical use. Roflumilast, a PDE4-I with less emetic effects than rolipram, has been approved for the treatment of chronic obstructive pulmonary disease. However, there are no reports of the effects of roflumilast on the sequelae of cerebral ischemia. The objective of this study was to evaluate the effects of roflumilast in rats with TGCI. **Methods**: Thus, Wistar rats (ethical committee approval 5529100517) underwent 4-vessel occlusion model of TGCI. Roflumilast (0.003 or 0.01 mg/Kg) or vehicle was administered for 21 days after ischemia. On day 7,14 and 21 the rats were tested in the aversive radial maze (AvRM), to evaluated retrograde memory. The parameters analyzed were: latency time and the number of reference and operational erros. After behavioral testing, on day 21, the rat brains were examined for hippocampal neurogenesis using immunohistochemistry for doublecortin (DCX). Neuronal specific nuclear protein (NeuN), brain derived neurotrophic factor (BDNF), growth associated protein 43 (GAP43), microtubule-2 associated protein (MAP-2), synaptophysin and post-synaptic density protein 95 (PSD95) were also evaluated using Western blotting. **Results**: TGCI caused persistent retrograde amnesia in this effect was prevented by roflumilast. Ischemic animals treated with roflumilast (0.003 and

0.01 mg/Kg) presented a decrease in the latency time ($F_{3,120}$=21.14, p<0.0001), number of reference errors ($F_{3,120}$=17.34, p<0.0001) and operational errors ($F_{3,120}$=13.68, p<0.0001) compared to controls. Roflumilast increased the number of DCX ($F_{3,21}$=13.68, p<0.0001) but did not change the hippocampal levels of NeuN BDNF, MAP-2, GAP-43, PSD-95 and synaptophysin, in ischemic animals compared to controls (p>0.05). **Conclusions**: Roflumilast prevented memory impairments and increased hippocampal neurogenesis in TGCI animals. The protective effects of roflumilast seem to be independent of increasing synaptic plasticity.

Disclosures: J.M. Bonato: A. Employment/Salary (full or part-time):; Capes. 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.; SfN-IBRO award. **E. Meyer:** None. **H. Milani:** None. **J. Prickaerts:** None. **R.M.M.W. de Oliveira:** None.

Poster

563. Ischemia IV

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 563.26/L3

Topic: C.08. Ischemia

Support: NIH Grant 1R21NS097899

Title: Aberrant network activities in neural cultures from patients with chronic mountain sickness

Authors: *H. YAO[1], H. W. ZHAO[1], W. WU[1], J. WANG[1], P. D. NEGRAES[2], A. R. MUOTRI[2], G. G. HADDAD[1,3,4]
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Abstract: Chronic mountain sickness (CMS) is manifested by neurological symptoms such as migraine headache, dizziness, and cognitive deficits. The underlying pathological mechanisms are not well understood. Since our previous work has shown alterations in Na^+ or K^+ currents in neural cells derived from CMS patients, we hypothesize that the neural network activities could also be altered in such cells derived from CMS patients. Skin biopsies were obtained from both CMS patients and healthy highlanders (non-CMS) who live in the Peruvian Andes (~14000 ft). Fibroblasts were grown and reprogramed into induced pluripotent stem cells (iPSCs) which then were differentiated into neurons in a 3D system. Cultures were characterized by typical neuronal markers, MAP2 or Tuj1, and showed positive staining to VGLUT2, suggesting that most of the cells are glutamatergic neurons although our patch clamp recordings have identified a small portion of glial cells. Using a multielectrode array system (Axion Biosystems), we studied the

neural network activity of these neural cells. Our results show that the network activity in non-CMS (normoxia) cultures increases overtime. The number of spikes (Ns) within a 5-min recording increased from 79±5 (n=6) at 5 weeks in culture (WIC) to 1125±58 (n=6, $p<0.05$) at 9 WIC, and plateaued thereafter. Under chronic hypoxia (5% O_2), the Ns increased from 102±6 (n=6) at 5 WIC to 478±19 (n=6, $p<0.05$) at 9 WIC. The difference between hypoxia and normoxia at 9 WIC was significant, suggesting that hypoxia hindered neuronal maturation in non-CMS cultures. In CMS neuronal cultures, the Ns changed from 849±54 (n=6) at 5 WIC to 1182±98 (n=6) at 9 WIC and, under hypoxia, the Ns increased from 923±65 (n=6) at 5 WIC to 3089±324 (n=6) at 9 WIC. Hence, hypoxia significantly enhanced the firing rate at 9 WIC in CMS neural networks. Furthermore, the Ns was significantly higher in CMS (849±54, n=6, $p<0.05$) than in non-CMS (79±5, n=6) at 5 WIC under normoxia. Our findings show that: a), under normoxia, CMS neural network exhibits a higher excitability than non-CMS counterparts; b), chronic hypoxia decreased the Ns in non-CMS neurons but increased the Ns in CMS neurons. Compared with CMS, non-CMS neural network show reduced activity under hypoxia which may suggest that an adaptation mechanism exists in the brain of healthy highlanders.

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Poster

563. Ischemia IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 563.27/L4

Topic: C.08. Ischemia

Title: A role of aryl hydrocarbon receptor in vasogenic brain edema

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Abstract: Vasogenic edema, a type of brain edema is a severe complication that accompanies ischemic stroke. We reported that neuroinflammation after ischemic stroke resulted in the aggravation of vasogenic edema. The aryl hydrocarbon receptor (AhR) is a cytosolic transcription factor which is involved in the metabolism of xenobiotic substances. Recently, it has been reported that AhR activation can regulate the inflammatory response. However, little is known about the effect of AhR on vasogenic edema. To that end, we investigated that a role of AhR in a mouse model of ischemic stroke.ICR mice underwent permanent middle cerebral artery

occlusion (pMCAO). Microglial activity was assessed by double immunostaining of Iba1-CD68 until 24 h after occlusion. MRI and TTC staining were conducted to visualize the vasogenic edema and infarct area, respectively, at the same time points with immunostaining. Minocycline, a tetracycline derivative with microglial activation inhibitor and CH223191, a potent and specific AhR antagonist, were intraperitoneally administrated before surgery.We clarified the non-ischemic area and ischemic area after pMCAO using 3-hydroxymethyl PROXYL during the MRI scans. Microglia were activated in the ischemic area 3 hours after ischemia and time-dependently activated in the non-ischemic area. Vasogenic edema subsequently occurred in the ischemic area and progressed to the non-ischemic area. This progression of vasogenic edema was prevented by inhibition of microglial activation. These results suggested that activated microglia after ischemia enhanced vasogenic edema. Next, we examined the involvement of AhR in edema progression. The expression of AhR mRNA increased 3 hours after ischemia in the striatum and cerebral cortex. The increase in the AhR expression was suppressed by the administration of Minocycline. Administration of CH223191 suppressed vasogenic edema progression and decreased the infarct size. These results indicate that AhR in activated microglia after ischemic stroke aggravate vasogenic edema.We revealed that AhR, which is upregulated in microglia after ischemia, can exacerbate vasogenic edema and increase the infarct size. We will also present the interaction of activated microglia and BBB using *in vitro* BBB model.

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Poster

563. Ischemia IV

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Program #/Poster #: 563.28/L5

Topic: C.08. Ischemia

Support: NS10690
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Title: Regional heterogeneity in consequences of spreading depolarization in metabolically compromised tissues

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Abstract: Slowly-propagating waves of neuronal and glial depolarization (spreading depolarization; SD) can contribute to the progression of stroke and traumatic brain injuries. We are interested in cellular determinants that confer SD vulnerability in order to develop interventions that can limit glutamate-mediated excitotoxicity during SD. We used complementary *in vitro* and *in vivo* mouse models of metabolic compromise to assess differences in glutamate and Ca^{2+} signaling. Two photon imaging was used *in vivo* to examine neuronal intracellular Ca^{2+} (GCaMP) and extracellular glutamate with viral transfection of iGluSnFr. SDs were initiated with focal KCl microinjection and, in some animals, focal ischemia generated with distal middle cerebral artery occlusion or photothrombosis. After stroke, SD-associated glutamate and Ca^{2+} signals were prolonged in penumbral versus remote regions with better perfusion. In non-injured animals, we observed microheterogeneity in neuronal recovery after SD, related to vascular proximity. Thus, decay of Ca^{2+} signals was significantly delayed in regions more distant from penetrating arterioles. Consistent with metabolic microheterogeneity, NADH autofluorescence after SD increased with distance from arterioles. Complementary studies were conducted in brain slices, to model penumbral conditions. Moderate restriction of metabolic substrates did not alone cause damage, but rendered slices vulnerable to SD. The adenosine A1 receptor antagonist DPCPX increased EPSP amplitude and normalized paired pulse ratio, consistent with metabolic compromise and accumulation of extracellular adenosine. KCl-evoked SDs still propagated but resulted in prolonged inhibition of functional recovery. EPSP suppression after SD was partially due to A1R activation, but DPCPX did not fully restore EPSP amplitude, implying persistent dysfunction or injury. Glutamate and neuronal Ca^{2+} transients were significantly longer, consistent with a role of these mediators in extended dysfunction after SD in vulnerable tissues. The glutamate receptor antagonists memantine (100µM) and ketamine (30µM) did not prevent SD but reduced the duration of Ca^{2+} loading and significantly improved recovery after SD in vulnerable slices. These results demonstrate that penumbral regions have greater glutamate and neuronal Ca^{2+} loading after SD and also raise the possibility that subtle damage resulting from SD may occur first in neurons distant from arterioles. While improved vascular supply is expected to be helpful for SD recovery, results with the slice model indicate that targeting glutamate-mediated excitotoxicity can significantly improve recovery.

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Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 564.01/L6

Topic: C.10. Brain Injury and Trauma

Support: National Institutes of Health (NIH)

Title: Acrolein involvement in the aberrant presentation of alpha-synuclein post-mild blast traumatic brain injury

Authors: *S. HERR[1], G. G. ACOSTA[2], N. RACE[1], R. SHI[3]
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Abstract: Survivors of blast-induced traumatic brain injury (bTBI) have increased susceptibility to Parkinson's disease (PD), characterized by α-synuclein aggregation and the progressive degeneration of nigrostriatal dopaminergic neurons. Using an established blast-induced traumatic brain injury (bTBI) rat model, we evaluated the changes of α-synuclein and tyrosine hydroxylase (TH), known hallmarks of PD, and acrolein, a reactive aldehyde and marker of oxidative stress, aiming to reveal key pathways leading to PD post bTBI. Indicated in both animal models of PD and TBI, acrolein is likely a point of pathogenic convergence. Here we show that after a single mild bTBI, acrolein is elevated up to a week, systemically in urine, and in whole brain tissue,specifically the substantia nigra and striatum. Acrolein elevation is accompanied by heightened α- synuclein oligomerization, dopaminergic dysregulation, and acrolein/α-synuclein interaction in the same brain regions. We further show that acrolein can directly modify and oligomerize α- synuclein *in vitro*. Taken together, our data suggests acrolein likely plays an important role in inducing PD pathology following bTBI by encouraging α-synuclein aggregation. These results are expected to advance our understanding of the long-term post-bTBI pathological changes leading to the development of PD, and suggest intervention targets to curtail such pathology.

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Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 564.02/L7

Topic: C.10. Brain Injury and Trauma

Support: National Institutes of Health (NIH)

Title: Mechanisms of secondary injury and auditory deficits following mild blast induced trauma

Authors: *J. FERNANDEZ[1], E. X. HAN[2], N. RACE[2], J. LAI[3], E. L. BARTLETT[2], R. SHI[4]
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Abstract: Some of the most commonly reported functional deficits after (blast induced) traumatic brain injury (bTBI) in patients are auditory in nature. Although injury to the peripheral auditory system after bTBI has been thoroughly investigated, few studies have examined the effects of bTBI on the central auditory system. In particular, the underlying structural deficits within the auditory system and the effects of blast injury on the different auditory areas within the central auditory system are still poorly understood. Oxidative stress, along with inflammation, have been suggested as key players in secondary molecular damage in other models of CNS injury, including TBI. However, the role of this secondary pathway of damage on the auditory system following bTBI has yet to be examined thoroughly. Potentially, both mechanical and secondary oxidative injury to the central auditory system contribute to deficits in communication, memory, and learning seen among veterans, and thus warrants examination. Here, we present data showing increased levels of oxidative stress, combined with other biochemical changes, following bTBI in rats, suggesting a potential secondary mechanism for injury within the auditory system. In addition, an array of audiometric tests (DPOAE, ABR, EFR, IRN) were used to assess auditory deficits after mild primary bTBI. We show potential deficits in the auditory nerve/brain stem region, as well as temporal processing impairments, suggesting potential damage to the auditory nerve and/or inferior colliculus. Taken together, these data suggest key mechanisms of molecular damage that may play an important role in injury to the central auditory system following bTBI and subsequently in the behavioral and functional deficits commonly seen after blast-induced trauma.

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Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

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Program #/Poster #: 564.03/L8

Topic: C.10. Brain Injury and Trauma

Support: Moody Project for Translational Traumatic Brain Injury

Title: Chronic epigenetic changes in hippocampal neural stem cells in a rat fluid percussion injury model of traumatic brain injury

Authors: *E. BISHOP, D. R. BOONE, I. BOLDING, M. PARSLEY, D. DEWITT, D. PROUGH, M.-A. MICCI
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Abstract: Traumatic brain injury (TBI) results in a range of cognitive dysfunctions that significantly affect the quality of life for post-TBI survivors. Using a rat fluid percussion injury

(FPI) model, we have previously shown that, two weeks following injury, increased gliogenesis is coupled with reduced maturation and integration of new neurons in the hippocampus dentate gyrus (DG). The aim of this work was to build on our short-term studies and determine the chronic effect of TBI on the genetic and epigenetic regulation of neurogenesis in the rat FPI model, and in neural stem cells harvested and cultured from injured and control rats.
Adult male Sprague-Dawley rats were randomized to receive FPI or sham surgery. The brains were collected 6 months later and the DGs were collected by laser capture for gene expression analysis (Neurogenesis PCR array, Qiagen) and qRT-PCR analysis of miRNAs known to regulate neurogenesis. Additionally, neural stem cells (NSCs) were isolated from the hippocampus 5 weeks after FPI or Sham injury and cultured for one month under proliferating conditions.
We found that genes known to regulate neuronal differentiation, migration and survival were significantly altered in the hippocampus DG up to 6 months after FPI as compared to sham rats. Additionally, the expression of miRNAs known to regulate neurogenesis (miR9, miR24, miR124, miR132, miR134, miR184) was significantly increased. Interestingly, the same miRNAs were increased in NSCs isolated from the DG of FPI rats and cultured for an additional 30 days as compared to cultured NSC isolated from control rats. Our data strongly suggest that FPI produces chronic genetic and epigenetic changes in hippocampal NSCs. Furthermore, our results show that persistent epigenetic changes in hippocampal NSC are independent of the microenvironment as they are maintained even after the cells are removed from the brain and cultured under standard conditions. Understanding the mechanisms underlying impaired neurogenesis after TBI will aid the development of novel therapeutic interventions for the treatment of TBI survivors.

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Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

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Program #/Poster #: 564.04/L9

Topic: C.10. Brain Injury and Trauma

Support: The Moody Project for Translational Traumatic Brain Injury Research

Title: Iron deposition and microglia activation in a rat model of chronic traumatic brain injury

Authors: *J. GUPTARAK, A. C. GRANT, M. O. PARSLEY, K. M. JOHNSON, I. J. BOLDING, D. S. DEWITT, D. S. PROUGH, S. L. SELL, M. A. MICCI
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Abstract: Activated microglia and iron accumulation have been shown to play a role in neurodegeneration and demyelination after traumatic brain injury (TBI) and in neurodegenerative disorders such as Parkinson's and Alzheimer's disease. Using a rat model of TBI our group has previously reported persistent behavioral deficits in working memory up to one year after injury. Such deficits could be associated with the presence of activated microglia and increased iron deposition, known to stimulate the generation of reactive oxygen species (ROS), in those brain areas involved in cognitive function (cortex and hippocampus). In this study we used the fluid percussion injury model (FPI) to investigate the distribution of activated microglia, iron deposition and demyelination in the rat brain at 6 and 12 months after injury. Adult male Sprague-Dawley rats were anesthetized and randomized to receive FPI or SHAM surgery (N=5-6/group). Microglia was identified by immunofluorescence analysis using a specific antibody against CD68 (a marker of activated microglia). Iron accumulation and myelin were identified using specific histological stains (Perl' Prussian blue and Weil respectively). A total of 6 sections/brain (bregma levels -1.92 mm to - 7.08 mm) were analyzed. An Investigator who was blinded to the experimental groups performed volume analysis and histological stain quantifications using Image J. Statistical analysis between two groups was performed using unpaired, two-tailed t-test. Cortical and hippocampal atrophy was observed at 6 and 12 months after FPI. Activated microglia (CD68 positive cells) and iron deposition were observed in the cortex (motor, sensory and auditory cortex) corpus callosum/deep cerebral white matter/external capsule, thalamic nuclei and hippocampus at 6 and 12 months after FPI. Myelin thickness was significantly reduced in the corpus callosum in the ipsilateral site to the injury as compared to both the contralateral site and uninjured SHAM brains at 6 months and 12 months after surgery. This study strongly suggests that TBI results in chronic microglia activation, demyelination and iron deposition leading to a progressive degenerative process possibly mediated by iron-induced oxidative damage in those brain areas involved in cognitive functions such as working memory.

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Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

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Support: FP7-HEALTH project 602102 (EPITARGET)
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2014/15/N/NZ4/04561
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Title: Traumatic brain injury causes chronic down-regulation of miR-124 in dentate gyrus

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Abstract: Traumatic brain injury (TBI) induces molecular and cellular changes that can in time lead to the development of post-traumatic comorbidities such as hippocampus-related memory decline.
We hypothesize that TBI causes chronic changes in a hippocampal network that are regulated by microRNAs.
TBI was induced to adult rats with the lateral fluid-percussion method. Changes in gene expression were detected from the dentate gyrus at 3 months post-TBI using microarray. The data obtained was used to investigate molecular networks that could contribute to the development of post-TBI comorbidities. Differential expression of key molecules was validated with PCR, *in situ* hybridization and immunohistochemistry.
Ingenuity Pathway Analysis of microarray data indicated that TBI causes upregulation of 30 targets of microRNA-124-3p suggesting the downregulation of this microRNA. Text mining and bioinformatics analysis connected the miR-124 targeted networks with inflammation and proliferation. Both droplet digital PCR and *in situ* hybridization confirmed the chronic downregulation of miR-124 ($p<0.05$) in the dentate gyrus. The upregulation two targets of miR-124, *Plp2*, and *Stat3*, was validated with quantitative PCR ($p<0.05$). Immunohistochemical analysis of STAT3 revealed that the upregulation of *Stat3* extents also to protein level.
Our findings indicate that miR-124 is a chronic regulator of molecular networks relevant to post-traumatic hippocampal pathologies.

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Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

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Topic: C.10. Brain Injury and Trauma

Support: European Social Fund and European Regional Development Fund - Project MAGNET (No. CZ.02.1.01/0.0/0.0/15_003/0000492

Title: Mechanisms underlying axonal swelling formation

Authors: *V. M. POZO DEVOTO, V. LACOVICH, M. NOVAKOVA, M. FEOLE, K. TEXLOVA, G. B. STOKIN
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Abstract: Axonal swellings (AxS) are focal enlargements of axons found post-mortem in a range of biological and pathological settings including traumatic brain injury, Alzheimer's disease and multiple sclerosis. Despite their description in a range of settings, the mechanisms involved in the formation of AxS remain poorly understood. Here we developed a novel *in vitro* experimental paradigm to test for mechanisms underlying AxS formation. Human neuronal progenitor cells were seeded into microfluidic chambers and terminally differentiated to neurons. In these chambers axons grow into microchannels, which are crossed by a perpendicular channel to which we connected a syringe pump. Syringe pump generates force, which subjects axons to bending stress. After full characterization of the channel fluid dynamics, we tested how axons respond to the stress. Detailed analysis of the kinetics by time-lapse imaging showed a significant increase in the number and size of axonal enlargements during and after stress as visualised by transducing neuronal culture with a membrane targeted Cherry. We have first studied these axonal enlargements by scanning electron microscopy to reveal and distinguish physiological versus pathological enlargements following axonal injury. We next performed super-resolution microscopy to demonstrate loss of Spectrin BII periodicity of the sub-axolemmal cytoskeleton. We then investigated whether enlargements form as a result of membrane leakage in response to shear stress. We incubated axons with fluorescent dextrans of different sizes at the time of injury and learned that enlargements do not form as a result of membrane leakage. Considering many reports found increased Ca^{2+} following axonal injury, we then asked whether Ca^{2+}concentrations increase in axonal enlargements. We found a significant increase in Ca^{2+} within the enlargements as visualised by the Fluo 4AM Ca^{2+}sensor. To confirm this finding further we also used the ratiometric Fura 2AM Ca^{2+} sensor in addition to imaging $Na^{+}$and K^{+}concentrations prior, during and after axonal injury with the aim of confirming the role and specificity of Ca^{2+} in axonal enlargements during injury. Furthermore, to understand the role of Ca^{2+}in enlarged axons following injury we next depleted Ca^{2+} from media and blocked different axonal membrane and ER/mitochondrial Ca^{2+}channels. In summary, we created a unique cell culture paradigm to study the response of axons to physical injury and provide novel insight into mechanisms responsible for the formation of axonal swellings.

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Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 564.07/L12

Topic: C.10. Brain Injury and Trauma

Title: pH change-induced zinc release causes cell and tissue injury

Authors: *Z. WANG, Y. V. LI
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Abstract: Intracellular pH (pH_i) is stringently regulated and varies greatly among different organelles. For example, the pH in lysosome, nucleus, and mitochondrial matrix are 4.7, 7.2 and 8, respectively. The pH in the cytosol is around 7.2. A stable pH is critical for normal neuronal function. However, pathological conditions, such as ischemic stroke, traumatic brain injury, and epileptic seizure are accompanied by a marked reduction of pH_i. The following reperfusion process brings pH_i back to physiological level. PH change affects metal ions homeostasis, such as zinc homeostasis. As an important trace element, zinc is required for normal cellular structures and functions. The concentration of zinc is tightly regulated in the cells. However, a number of studies have shown that a lowering of pH_i can interrupt zinc homeostasis by causing zinc release from loosely bound proteins and subcellular organelles. In the present study, we investigated the effect of zinc during pH_i change in both cell and animal model. We proposed a strategy to save cells and tissues by reducing intracellular zinc concentration. Sodium dithionite (DT) was used to induce intracellular acidification, and the following reperfusion with ACSF was used to bring pH_i back to normal. In the animal model, the traumatic brain injury (TBI) caused a fall in local pH in the beginning, followed by latterly pH recovery through reperfusion with ACSF. Zinc chelator was applied to reduce zinc concentration of cells and tissues. The specific zinc fluorescent indicator was used to detect zinc concentration change in both cells and tissues. TTC staining was used to measure brain infarction levels. Results showed that DT-induced intracellular acidification caused zinc release, which led to cell morphological changes, resulting in cell injury. The following reperfusion-induced pH_i recovery caused more damage than that of intracellular acidification. This injury was attenuated by the application of zinc chelator, suggesting the importance of zinc homeostasis in cell protection against pH recovery-induced cell injury. The similar results were also observed in TBI. TBI caused zinc release, resulting in tissue damage. Zinc chelation exhibited a neuroprotective effect on TBI-induced brain damage through reducing brain infarct volumes.

Disclosures: Z. Wang: None. **Y.V. Li:** None.

Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 564.08/L13

Topic: C.10. Brain Injury and Trauma

Support: NIAID IAA

Title: Delayed effects of acute radiation exposure in BBT-059 treated survivors

Authors: *N. K. SHARMA[1], S. BISWAS[1], S. STONE[1], C. FAM[2], G. COX[2], V. KUMAR[1], S. GHOSH[1]
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Abstract: Introduction: BBT-059, developed by Bolder Biotechnology (BBT), is a long acting PEGylated IL-11 analog. Previously, we demonstrated that BBT-059 is effective as a radiation countermeasure in CD2F1 male mice when a single dose was administered either at -24 h pre- or 24 h post-total body irradiation (TBI). In this study, we show that surviving animals remain healthy up to 6 months post-TBI. Methods: Twelve to fourteen week old CD2F1/ male mice used in these studies. BBT-059 was prepared in formulation buffer (10mM sodium phosphate, 4% mannitol, 1% sucrose, pH 6.2) at the specific doses used in studies. Formulation buffer and saline (9%) were used as controls. Drug and controls were injected as a single dose (0.1 mL) subcutaneously (SC) at the nape of the neck. The experimental animals received a single exposure of ^{60}Co gamma TBI at an estimated dose rate of 0.6 Gy/min in the AFRRI radiation facility. Survived animals post-30 day after radiation were monitored up to 6 months. Blood and bone marrow analyzed for CBC counts, serum chemistry, and colony forming units (CFU) to understand the longterm effects of the survivors at 1 and 6 months post-TBI. Histopathological and immunohistochemical analysis of Brain and other major organs were performed. Results: Mortality was monitored up to 6 months post-TBI. There was an increase in the CBC counts and CFU in the 6 months post-TBI survivors compared to one month group. Mitochondrial damage in brain was seen in survivor of mice treated with higher radiation dose. Increased Glomerular with messangium and lung fibrosis was observed at 6 months post-TBI. In heart, after 6 months some vessels had evidence of smooth muscle hypertrophy of the arterial/ arteriolar wall as compared to naïve. Immunohistochemistry revealed an increase in the B- Catenin expression after 1 and 6 months in brain and kidney. Mice with higher radiation doses developed cataract after 6 Months. However, after six months serum biochemistry for blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase did not show any difference between naïve, untreated and treated groups. The results indicate that significant delayed effects of acute radiation exposure occur in Brain, lung, heart, and kidney in survivor animals. Conclusion: We have shown that BBT-059 treated animals survived up to 6 months post-TBI. Significant survival benefit with BBT-059 and as well as its long term effect suggests that the drug could be developed as a novel radiation countermeasure for civilians exposed in a fall out field for use after radiation in the aftermath of a radiation event.

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Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 564.09/L14

Topic: C.10. Brain Injury and Trauma

Support: NIGMS 1P20GM109089-01

Title: Cerebral blood flow and cognitive deficits following a single and multiple mild traumatic brain injuries

Authors: *R. A. MORTON[1], J. M. PACHECO[1], H. ZHANG[1], J. GUERIN[2], J. L. BRIGMAN[3]
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Abstract: It is estimated that there are approximately 3.8 million sports related concussions every year (Langlios, 2006). Many concussions do not result in emergency room visits but can result in a myriad of cognitive and behavioral deficits including: the inability to concentrate, feeling "foggy", headaches, and depression (Brent, 2017), and are referred to as post-concussion syndrome. The mechanisms of these symptoms remain unclear, however, cerebral blood flow has been implicated in mTBI. The objective of these studies is to identify the time course of CBF hypoperfusion immediately following a single impact and identify cortical mediated behavior deficits following a single mild traumatic brain injury (mTBI) versus multiple mTBIs. To achieve these objectives, we have used a closed skull impact model in wild type C57/Bl6 mice utilizing both male and female animals between the ages of 8 - 12 weeks. Mice were impacted at a speed of 4 m/s with a 5mm head deflection. These mTBIs did not result in significant tissue damage, hemorrhaging, or cell death. We used Laser Speckle Contrast Imaging to monitor CBF immediately following a single mTBI. Our preliminary data indicate that immediately following an impact the CBF is reduced to 46.87% baseline levels and remains significantly reduced after 90 minutes (69.45%; $p<0.0001$). To assess for cortical/striatal mediated behavioral deficits we have utilized a discrimination/reversal touchscreen-based task. Animals that received a single mTBI showed no significant differences in discrimination or reversal learning. However, animals that received a mTBI every day for four days had significantly more incorrect trials (Sham=112; mTBI=485.75, $p<0.0001$), correction trials (Sham=206; mTBI=1000; $p=0.0009$) and did reach the criteria of 85% correct within the 40 day cut-off. Overall, these data suggest that a single mTBI results in long-term reductions in CBF but does not impair cortical/striatal mediated cognitive behavior. However, multiple mTBIs results in significant impairment in discrimination learning that is largely mediated by the medial prefrontal cortex.

Disclosures: R.A. Morton: None. **J.M. Pacheco:** None. **H. Zhang:** None. **J. Guerin:** None. **J.L. Brigman:** None.

Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 564.10/L15

Topic: C.10. Brain Injury and Trauma

Title: In-vitro assessments of brain injuries and different neuronal network topologies on development of epilepsy

Authors: *S. GHIASVAND, Y. BERDICHEVSKY
Lehigh Univ., Bethlehem, PA

Abstract: Introduction: Post-traumatic epilepsy(PTE) is the most common form of acquired epilepsy. Following the injury, a cascade of molecular and cellular alterations leads to the epileptogenesis and the onset of seizures. Anti-epileptic drugs(AEDs) are the common treatment for PTE. However, a significant portion of patients are resistant to these drugs. This lack of efficacy cannot be alleviated in part due to an incomplete understanding of the changes that occur at the molecular and cellular levels following traumatic injury. Additionally, it is not clear what neuronal network topologies result in spontaneous epileptiform activity. Therefore, in order to describe the changes associated with various forms of injury we have employed *in-vitro* models of brain injury. Effects of chemically and mechanically induced injuries were investigated through electrical and optical recordings to assess seizure onset and propagation.
Materials and Methods: Organotypic hippocampal cultures(OHCs) were used as an *in-vitro* model of PTE. Two different neuronal network sizes of the hippocampal cultures, including whole hippocampal cultures and small portion of CA3 sub-region were cultured. Secondly, a brief pulse of 0.3% triton solution was used to kill a portion of cells in the OHC, thereby reducing the neuronal network density. Lastly, an *in-vitro* model of mechanical traumatic brain injury (TBI) was employed by dropping a 0.2g mass from a height of 2mm on the various regions of OHCs.
Results and Discussion: Contrary to our initial expectations the significantly smaller neuronal network from CA3 micro-cultures displayed significantly higher seizure rate and duration, suggesting that relative deafferentation might play the critical role in driving epileptogenesis. Reducing the density of the network by chemical injury resulted in significantly suppressed ictal activity. Preliminary results from mechanically induced TBI have demonstrated that we can selectively induce cell death in specific sub-regions of OHCs.
Conclusion: Our results indicate that the proportion of deafferentation can be the driving impetus of epileptogenesis. In future experiments we hope to prevent the consequent alterations of deafferentation at the cellular and network level to validate the centrality of deafferentation in PTE.

Disclosures: S. Ghiasvand: None. **Y. Berdichevsky:** None.

Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 564.11/L16

Topic: C.10. Brain Injury and Trauma

Title: Closed nest pre-weaning environment improves the development of physical characteristics and buffers hippocampal injury in neonatal hypoxic ischemic injury

Authors: *L. ROLLINS[1,3], B. M. MASON[2], T. DONALDSON[4]
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Abstract: Term neonates with hypoxic-ischemic (HI) injury are at risk for devastating neurological sequelae. Maternal care taking behavior has been found to alter the trajectory of normal brain development and may also impact neurodevelopment with exposure to HI injury. Maternal care-taking behavior can be highly influenced by environmental stress and may, therefore, mediate the effects of such stressors on injury and repair for these neurologically high-risk neonates. In the present study, we investigated whether altering early environment for maternal care-taking impacts neurodevelopment and neuroprotection in HI rat offspring. The Rice-Vannucci model was used to induce HI in 26 postnatal day (PND) 7 Long-Evans pups. Dams and litters were randomized to a closed nest (CN) or normal standard housing (SH) condition. Performance on a neurodevelopmental battery and characteristics of physical development were assessed daily from PND8-PND21 to quantify effects of the CN condition on HI injury. Brains were harvested at PND 60 and analyzed for morphological differences. Results indicate that HI injured animals reared in the CN condition showed significantly earlier development of physical characteristics, exhibiting ear unfolding an average of 2.23 days earlier ($p<0.001$), eye opening 1.85 (L) and 1.07 (R) days earlier ($p<0.001$), left ear twitch 1.9 days earlier ($p<0.05$), and audible startle response 1.46 days earlier ($p<0.01$) than those in the SH condition. There was also a trend observed for earlier development of negative geotaxis ($p=.084$) in the CN condition by 2 days. In addition, animals in the CN condition were consistently found to have a significantly higher body weight than those in AF ($p<0.001$) throughout the pre-weaning period. Finally, CN animals had significantly greater hippocampal tissue ($p<0.001$) in the ipsilateral hemisphere than SH animals with no difference in ipsilateral cortical area ($p>0.05$), indicating potential neuroprotection for vulnerable white matter areas. These findings indicate that, in comparison to SH housing, CN housing during the pre-weaning period promotes

maternal care-taking behavior to increase weight gain, improves the development of reflexes, physical characteristics, and supports neuroprotection in pups exposed to neonatal HI.

Disclosures: L. Rollins: None. **B.M. Mason:** None. **T. Donaldson:** None.

Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 564.12/L17

Topic: C.10. Brain Injury and Trauma

Title: Alterations in the deep layer cortex of $SOD1^{G93A}$ rats throughout disease progression and following repetitive mild TBI

Authors: *M. ALKASLASI, N. CHO, N. DHILLON, N. LINAVAL, J. GHOULIAN, A. YANG, G. BARMPARAS, E. LEY, G. M. THOMSEN
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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by a progressive loss of motor neurons leading to paralysis and death within 3-5 years of disease onset. Much of the etiology of ALS is likely rooted in complex interactions between genetic and environmental risk factors. Traumatic brain injury (TBI) is an environmental risk factor often linked to neurodegeneration. Populations prone to repetitive head injury, such as professional athletes and veterans are reported to have a higher incidence of neurodegenerative disease, including ALS. In this study, we assessed changes within layer V of the motor cortex of the $SOD1^{G93A}$ ALS rat model that occur over the time course of disease as well as following repetitive mild TBI.
$SOD1^{G93A}$ rats were administered mild, bilateral, closed-skull, controlled cortical impact (CCI) TBI at post-natal day (p) 60 once weekly for 5 weeks. Uninjured $SOD1^{G93A}$ sham controls were exposed to anesthesia only. Rats were euthanized at acute (p90), short (p165) or long (p235) timepoints, with $SOD1^{G93A}$ rats in the long group euthanized at disease endpoint (p150-235). TBI rats were classified as having “mild” or “severe” injuries based on rotorod performance within 6 days following the final injury. Brains were processed histologically to assess brain atrophy, microglial activation and health of layer V corticospinal motor neurons. RNA was also extracted and analyzed from brain tissue microdissected from layer V/VI of the motor cortex. Transcriptional profiling was performed to examine differentially regulated genes within this cortical region.
Following rTBI, $SOD1^{G93A}$ rats exhibiting initial severe symptoms showed exacerbated functional and pathological disease symptoms. These animals experienced earlier onset of forelimb paralysis and shortened survival relative to their sham counterparts (median onset: 150 vs 169 days, $p<.0001$; median survival: 168 vs 179 days, $p=.038$). Pathologically, $SOD1^{G93A}$ rats

in the severe group displayed increased cortical and corpus callosum atrophy, altered inflammation, and a reduction in large corticospinal motor neurons residing in layer V. RNA sequencing of brain tissue from layer V/VI revealed that $SOD1^{G93A}$ animals show significant alterations in genes associated with astrocyte activation and microglia development. Identifying and understanding changes in critical genes associated with ALS within the motor cortex will be important for developing therapeutic strategies targeting these relevant pathways.

Disclosures: M. Alkaslasi: None. **N. Cho:** None. **N. Dhillon:** None. **N. Linaval:** None. **J. Ghoulian:** None. **A. Yang:** None. **G. Barmparas:** None. **E. Ley:** None. **G.M. Thomsen:** None.

Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 564.13/L18

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant GM119831
NIH Grant R01NS084026

Title: Transcriptional and epigenomic signatures identified via systems-based modeling of hippocampal pathology in a rat model of traumatic brain injury

Authors: *I. ZDILAR, K. TERCOVICH, B. LYETH, G. GURKOFF, A. NORD
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Abstract: Outcome following traumatic brain injury (TBI) is influenced by the initial mechanical insult, combined with secondary effects, including neuroinflammation, apoptosis, and altered neurotransmission. Rather than focusing on a single target for investigation we propose that measures of systems-level changes can provide a readout that allows us to comprehensively assay across processes related to pathology following TBI. Further, we hypothesize that epigenetic changes persist in the brain after initial recovery from TBI. Ipsilateral hippocampus was isolated either 1 or 14 days following lateral fluid percussion or sham injury and processed for RNA sequencing and genome-wide analysis of select histone post-translational modifications. At day 1 post-injury we observed a characteristic increase in expression and epigenetic modifications at genes related to inflammation apoptosis, metabolism, extracellular matrix receptor interactions, and calcium signaling, with neurotransmitter systems not profoundly altered. While still elevated as compared to sham at day 14, there was a reduction in expression of inflammatory genes, apoptosis, and altered metabolism as compared to 24 hours post-injury. Unique to the later two-week time point was a widespread decrease in transcription of neurotransmitter receptors and ion channels, a larger effect than would be explained by cell loss alone. Many of these perturbed molecules also play key roles in learning and memory,

processes highly relevant to the hippocampus and long-term outcome following TBI. Finally, there was an increase in coagulation cascades as well as a reduction in steroid synthesis at the later time point. Our data indicate that transcriptomic and epigenetic alterations persist in the brain long after the acute injury response. This systems-level analysis identified clear modulation of multiple systems over time following injury and demonstrate linked perturbation to gene expression and epigenetic state following TBI.

Disclosures: I. Zdilar: None. **K. Tercovich:** None. **B. Lyeth:** None. **G. Gurkoff:** None. **A. Nord:** None.

Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 564.14/M1

Topic: C.10. Brain Injury and Trauma

Support: NIH Program Project grant 1P01NS082184, Project 3

Title: Down-regulation of wnt/beta-catenin reduces new vessel formation and increases hemorrhage after traumatic brain injury

Authors: *A. SALEHI[1], A. JULLIENNE[2], K. M. WENDEL[4], J. LEE[2], M. HAMER[2], J. TANG[3], J. ZHANG[5], W. J. PEARCE[6], A. OBENAUS[7]
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Abstract: Traumatic brain injury (TBI) results in damage to the cerebral vasculature and is often associated with hypoperfusion, edema, hemorrhage, and cell death. While numerous studies have revealed that the cerebral vasculature is injured after TBI, there are scant studies looking at repair of the vessel network following brain injury. At present there are no studies that have comprehensively examined the molecular mechanism(s) underlying revascularization after TBI. One possible molecular mechanism may be the Wnt/β-catenin pathway, which promotes blood vessel formation during vascular development. We previously reported increased Wnt/β-catenin expression and activation of Wnt target genes in blood vessels after TBI which coincides with revascularization at 7 days post injury (dpi). The objective of this exploratory study is to investigate the role of β-catenin in revascularization after TBI. A controlled cortical impact mouse model was used to induce a moderate TBI in adult male C57BL/6J mice (8 weeks old) which leads to gross injury to cerebral vessels. To assess the role of β-catenin in revascularization, we utilized JW74 (tankyrase inhibitor) to inhibit β-catenin expression. JW74

or vehicle (dimethyl sulfoxide/polyethylene glycol 400) was administered orally for 6 consecutive days and underwent terminal vessel painting to label the entire cerebral vasculature at 7dpi (n=6-8/group). Innovative analysis methods were employed, including fractals to measure complexity along with a classical analysis to measure vessel features. Ex vivo T2-weighted imaging and susceptibility weighted imaging was undertaken to assess edema and hemorrhage. Sex differences were not assessed. All experiments and analyses were performed by investigators blinded to the treatment and subject. We report that JW74 treated mice showed a robust reduction in vessel features including vessel area, branch points, and average vessel length compared to vehicle treated mice. We observed a reduction in number of vessels and vessel complexity in JW74 treated mice. T2 and susceptibility weighted imaging of vessel painted brains at 7 dpi revealed an increase in hemorrhage and edema volumes following JW74 treatment. Our findings suggest that endogenous developmental programs, such as Wnt/β-catenin, likely become activated after TBI to initiate repair. Treatment regimens to enhance activation Wnt/β-catenin may contribute to the vascular repair process after TBI and represents a potential target for future therapeutics.

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Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 564.15/M2

Topic: C.10. Brain Injury and Trauma

Support: R01 NS50465

Title: Gut microbes may decide the fate of brain injury

Authors: *W. Z. AMARAL[1], L. ROYES[4], L. YING[2], I. AHN[5], J. LANG[2], X. YANG[2], A. LUSIS[2], F. GOMEZ-PINILLA[3]
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Abstract: Traumatic brain injury (TBI) accounts for more than 90,000 newly disabled persons annually in the USA with the upsurge in metabolic neuropathies increasingly recognized to worsen outcomes. The sequela of TBI includes acute and chronic effects, with marked alterations in metabolic, inflammatory and enteric function, affecting both brain and periphery. Accordingly, functional gastrointestinal disorder is a notable complication of TBI. Because gut function is greatly disrupted after brain injury, TBI may consequently disrupt the gut microbiota

and its products, contributing to central and peripheral complications in TBI. The microbiome is getting recognition as a key player in the gut-brain axis, exerting powerful effects on host metabolic and inflammatory status, and altering brain function and behavior. After brain injury, functional alterations in the brain-gut axis may also disrupt immune-mediated regulation of gut permeability, increasing the translocation of bacteria to the host. Bacteria may accumulate in host organs, including liver and brain, exacerbating metabolic and inflammatory effects of TBI on both the brain and periphery. We used fluid percussion injury in rats in order to examine neuroenteric alterations, the modulation of inflammatory mediators, and the subsequent disruption of tight junction proteins in the gut barrier. In addition, we tracked the changes in gut microbiota profiles at 24 hours, 6 and 21 days after injury, and assessed the accumulation of bacteria in the liver and brain. The potential role of the gut bacteria in mediating or moderating the bidirectional interactions between gut and brain after TBI presents an opportunity to elucidate underlying gut-brain axis mechanisms and to help develop novel application of probiotics, dietary therapeutics and pharmacological compounds in the prevention or reversal of secondary complications of TBI.

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Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 564.16/M3

Topic: C.10. Brain Injury and Trauma

Support: JFK Neuroscience Institute

Title: Loss of pericyte impairs blood-brain barrier integrity following traumatic brain injury

Authors: S. BHOWMICK[1], V. D'MELLO[1], A. WALLERSTEIN[1], *P. ABDUL-MUNEER[2,1]
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Abstract: Background: Blood-brain barrier (BBB) constitutes a neurovascular unit formed by microvascular endothelial cells, pericytes, and astrocytes. Disruption of BBB is a hallmark of many neurological disorders including traumatic brain injury. Loss of pericyte have been implicated in injury, however, how the crosstalk between pericytes, endothelial cells, and astrocytes ultimately leads to BBB dysfunction and subsequent progression in TBI remains elusive. In this study, we hypothesized that following TBI, loss of pericytes is a consequence of downregulation in the platelet derived growth factor B (PDGF-B)-platelet derived growth factor receptor β (PDGFR-β) signaling pathway that results in the impairment of BBB integrity and

leads to neurovascular dysfunction following TBI. **Method:** In this study, mice were subjected mild (7psi) and moderate (15 psi) fluid percussion injury (FPI) injury and tissue samples collected from the site of injury were analyzed for expression of proteins using western blotting, immunohistochemistry and ELISA approach. The integrity of the vasculature was analyzed by assessing the permittivity of small-molecular-weight sodium fluorescein (Na-Fl) (MW 376) and high-molecular-weight-tracer Evans blue (EB) (MW 961) across the BBB. Further, BBB permeability after FPI was analyzed by detecting peripheral S100β and NSE in blood serum samples. **Results:** Our data provide substantial evidence that expression of various pericyte markers such as PDGFRβ, NG2 and CD 13 reduces significantly following FPI injury with subsequent reduction in the expression of certain proteins such as N-cadherin and Connexin 43 that connect endothelium and pericyte and tight junction proteins such as Occludin, Claudin 5, ZO-1, and JAM-a. Loss of pericytes further results in the permeability of BBB marked by a significant increase in Aquaporin4 in injury groups and increase water leakage as compared to control animals. Similarly, FPI greatly increased the permittivity of small-molecular-weight Na-Fl (MW 376) and high-molecular-weight-tracer EB (MW 961) across the BBB compared with respective controls. Intriguingly, the injury inflicted animals also showed significantly higher levels of S100β and NSE in the blood samples compared with controls. In conclusion, our data provide an insight that brain trauma causes an early loss of pericyte function and results in BBB dysregulation that initiates pathological consequences associated with TBI. Thus, a therapeutic approach targeting restoration of pericyte function could lead to a better outcome in the treatment of TBI.

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Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 564.18/M5

Topic: C.10. Brain Injury and Trauma

Support: EraNet Neuron TRAINS
University Bordeaux VIVA
Laboratory of Excellence TRAIL (ANR-10-LABX-57)

Title: Role of CXCR3 in astrogliosis after mild traumatic brain injury

Authors: M. FOURNIER[1], J. AUSSUDRE[1], M. TORRES-NUPAN[1], F. CASSE[1], C. BILLOTET[2], A. BIKFALVI[2], *J. BADAUT[1]
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Abstract: Traumatic brain injury (TBI) is the first cause of disability among young adults and children. Mild TBI (mTBI) is defined to include no or transient loss of consciousness, no visible alterations on conventional medical imaging and no short-term cognitive deficits. Mild TBI represent most of TBI cases. Even with mTBI, clinical studies report long-term psychological and behavioral consequences. Those dysfunctions are not only caused by the local primary injury but also by the development of a chronic inflammation altering the brain properties. The chemokine receptor CXCR3 has been previously associated to microglia activation after injury but its role has been under-explored in the process of reactive astrocyte and cerebral blood vessels changes after mTBI. We hypothesized that activation of CXCR3 will contribute to promote astrogliosis after mTBI with consequences on blood-brain barrier (BBB) properties, synaptic plasticity and behavior outcomes. The role of CXCR3 after mTBI was investigated using a closed head injury (CHI) model and comparing behavior outcomes, neuroimaging (T2WI), neuronal plasticity, astrocytes and BBB properties between wild-type (WT) and CXCR3 knockout (CXCR3-KO) mice from 1 to 30 days after injury. T2WI did not show any gross brain morphological changes in all experimental groups, suggesting that CHI model mimics mTBI. Increase of foot-faults (230%) was observed 7d after injury compared to sham in the WT-group but not in CXCR3-KO group. In Morris Water Maze test, WT group with mTBI exhibited spatial learning and memory alterations compared to sham 30 days after injury. In contrast, CXCR3-KO group did not show memory alterations. In the WT group, the behavioral dysfunctions were associated to an increase of the GFAP staining in the hippocampus 7 days after injury, whereas no change in GFAP staining were observed in CXCR3-KO group after mTBI. However, no change in the BBB was observed using IgG extravasation staining in all experimental groups. In conclusion, absence of CXCR3 is beneficial for both locomotor and cognitive outcomes, associated to reduced astrogliosis 7 days to 1 month after mTBI. Our preliminary results suggest that CXCR3 pathway is an interesting target to treat memory and sensory motor dysfunctions associated to the inflammation occurring after a mTBI.
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Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 564.19/M6

Topic: C.10. Brain Injury and Trauma

Support: These studies were completed as part of an interdisciplinary research team funded by the Moody Project for Translational TBI Research.

Title: Brain region-specific changes in microRNA expression in chronic traumatic brain injury

Authors: *D. BOONE[1], H. WEISZ[1], H. SPRATT[2], D. PROUGH[1], D. DEWITT[1], H. HELLMICH[1]
[1]Anesthesiol., [2]Preventative Med. and Community Hlth., Univ. of Texas Med. Br. at Galveston, Galveston, TX

Abstract: Presently there are still no approved treatments for traumatic brain injury (TBI). This suggests that we still lack a full understanding of TBI mechanisms. Previously we reported acute changes in small non-coding microRNAs (miRNAs) after experimental fluid percussion brain injury (FPI). Our objective in this study was to examine TBI-induced miRNA changes up to a year after injury. Adult male, Sprague-Dawley rats (300-350 g) were subjected to either TBI or sham injury and survived for 24 hr, 2 wk, 3 mo, 6 mo, and 1 yr. We microdissected the hippocampus and cortex regions and isolated total RNA. cDNA libraries were prepared for miRNA sequencing and sequenced on a NextSeq550 Illumina platform. MicroRNA-sequencing data was analyzed using EdgeR. Ingenuity Pathway Analysis (IPA) was used to identify pathways regulated by significantly altered miRNAs at all post injury intervals. Expression of selected miRNAs was confirmed using digital PCR analysis. Bioinformatics analysis showed the majority of TBI-dysregulated miRNAs (i.e., miR-146a-5p, miR-142-3p, miR-17-5p, and miR-221-5p) are predicted to target genes involved in inflammation and neurodegeneration. Our data suggest that TBI- induced miRNAs may have dual effects on inflammation because some of these miRNAs are known to suppress gene expression. We have previously shown that knocking down individual TBI-induced genes does not result in altered functional outcomes. Because miRNAs coordinately regulate multiple genes in cell signaling pathways affected by TBI, we have identified specific miRNAs that regulate these genes as potential therapeutic targets for brain injury.

Disclosures: D. Boone: None. **H. Weisz:** None. **H. Spratt:** None. **D. Prough:** None. **D. DeWitt:** None. **H. Hellmich:** None.

Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 564.20/M7

Topic: C.10. Brain Injury and Trauma

Support: NEI RO1EY027881 (PAR, LIB)
NEI RO1EY024481 (PAR, LIB)
NINDS RO1NS066019 (PAR)
NIH R21MH104318 (PAR)
2T32EY007145-19 (NH)

IDDRC HD018655

Title: Reversal of glutamate transport contributes to retinal zinc elevation and ganglion cell death after optic nerve injury

Authors: *N. HANOVICE[1], Y. LI[2], N. C. DANBOLT[3], L. I. BENOWITZ[1], P. A. ROSENBERG[1]
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Abstract: Glaucoma, a leading cause of blindness worldwide, is characterized by the progressive and irreversible loss of retinal ganglion cells (RGCs). Though mounting evidence shows that glaucoma initiates with an insult to RGC axons in the optic nerve head/lamina cribrosa, the precise mechanisms linking axonal injury to RGC apoptosis are unclear. Recently, we discovered that retinal interneurons play a critical and previously uncharacterized role in linking optic nerve crush (ONC) to RGC death in mice: namely, that ONC leads to a rapid increase of mobile zinc (Zn^{2+}) in amacrine cell (AC) terminals that is transmitted to RGCs, and that chelation of Zn^{2+} improves RGC survival and axonal regeneration. The signals linking RGC injury to Zn^{2+} accumulation in amacrine cells remain largely unknown. Here, we present evidence that the glutamate transporter GLT-1 expressed in retinal bipolar cells, Muller glia, and/or astrocytes responds to ONC by exporting glutamate, which activates NMDA receptors and culminates in Zn^{2+} elevation in synaptic terminals of amacrine cells and subsequent accumulation in RGCs. We previously showed that Zn^{2+} is liberated from intracellular reserves by nitric oxide (NO) that is generated by NO synthase-1 (NOS1) [Li et al. *SfN 2017. #742.09*]. NOS 1 is commonly activated by Ca^{2+} entry through activated NMDA receptors. To determine whether NMDA receptor activation is required for Zn^{2+} accumulation, we injected the NMDA receptor inhibitor MK801 prior to ONC, and found that this prevented Zn^{2+} elevation. Since NMDA receptor activation is mediated by glutamate, we next investigated the source of extracellular glutamate. Aside from vesicular release at synapses, glutamate can be exported by glutamate transporters operating in reverse. We found that both DL-threo-beta-benzyloxyaspartate, a general glutamate transport blocker, and dihydrokainate, a relatively specific blocker of GLT-1, prevented Zn^{2+} accumulation and enhanced RGC survival. The effect of each inhibitor on Zn^{2+} elevation was overridden by the addition of the NO donor DETA-NONOate, indicating that reversal of transport by GLT-1 is upstream of NMDA receptor activation. Immunohistochemistry using GLT-1 specific antibodies revealed that GLT-1 is expressed in bipolar cells, Muller glia, and astrocytes, but not the soma or dendrites of RGCs. Together, these results establish GLT-1 as a critical mediator of RGC degeneration and shed further light on the signaling events linking ONC to RGC degeneration.

Disclosures: N. Hanovice: None. **Y. Li:** None. **N.C. Danbolt:** None. **L.I. Benowitz:** None. **P.A. Rosenberg:** None.

Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 564.21/M8

Topic: C.10. Brain Injury and Trauma

Support: Bill and Melinda Gates Foundation Cysticercosis Elimination in Peru grants 23981 and 33848 (H.H.G.)
NIH grant 5D43TW006581 (Infectious Diseases Training Program in Peru) (R.H.G.)
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Innovate Perú Nro.135-PNICP-PIAP-2015

Title: Fibrosis genes over-expression rat model for neurocysticercosis

Authors: *D. G. DÁVILA[1], R. P. CARMEN[1], R. H. GILMAN[2], R. CELIZ[1], E. BERNAL[1], A. D. DELGADO[1], C. QUISPE[1], B. J. CONDORI[1], F. ANCAJIMA[1], M. VERASTEGUI[1]
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Abstract: Neurocysticercosis is a brain infectious disease with higher prevalence in developing countries. While neurocysticercosis pathology has been studied few data reports gene expression results which can explain changes seen in this pathology. Neurocysticercosis is characterized to form a fibrotic layer surrounding the parasite followed by an extensive area of gliosis. Fibrosis and gliosis varied depending on the viability of the cyst and its location. This study tried to characterize fibrotic gene response in viable cyst located in parenchymal tissue. In order to explain that, we used a rat model for neurocysticercosis, in which 13 rats of 12 days old were infected with *T. solium* oncospheres. After 6 months of infection, rats were euthanized and the tissue surrounding the cysticercus were dissected to study gene expression of fibronectin1, collagen1a1, collagen 3a1, Matrix metalloproteinase 2 and 9 (Mmp-2,9) by quantitative reverse transcription PCR. Twelve sections from six non-infected rats were used as a control and five housekeeping genes were tested. Lactate dehydrogenase A was the more stable housekeeping gene and was used for normalization. We found overexpression of all the aforementioned genes with exception of Mmp-9 in the tissue surrounding the cyst in compared to the non-infected tissue. The highest fold of change was found for col3a1 followed by Mmp-2, fibronectin, and col1a1 (values of a median of fold expression were 17.19, 8.25, 7.24 and 5.24, respectively. $P<0.01$). This data seems to be congruent with the pathology of neurocysticercosis where viable cysts are encapsulated by a fibrotic tissue. We highlight the importance of Mmp-2 which can be working as a regulatory molecule involved in the fibrotic response in neurocysticercosis.

Disclosures: D.G. Dávila: None. **R.P. Carmen:** None. **R.H. Gilman:** None. **R. Celiz:** None. **E. Bernal:** None. **A.D. Delgado:** None. **C. Quispe:** None. **B.J. Condori:** None. **F. Ancajima:** None. **M. Verastegui:** None.

Poster

564. Brain Injury and Trauma: Cellular and Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 564.22/M9

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R25GM066567

Title: The role of interneuron death in traumatic brain injury

Authors: *A. M. ORTIZ RIVERA[1], J. KOENIG[1], M. ARMBRUSTER[3], D. KONG[2], C. G. DULLA[1]
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Abstract: Over 200,000 cases of traumatic brain injury (TBI) occur annually. Post-TBI pathologies include motor and cognitive dysfunction, as well as post-traumatic epilepsy (PTE). How TBI leads to these pathologies is largely unknown. Using the controlled cortical impact (CCI) model of TBI, we found that inhibitory GABAergic interneurons are lost. Similar losses in GABAergic interneurons are seen following human TBI. Parvalbumin (PV+) interneurons, which powerfully constrain cortical network function, are among those lost. However, it is unknown whether the loss of PV+ interneurons contributes directly to cortical network dysfunction after TBI. Studying interneuron loss post-TBI is challenging because it occurs alongside inflammation, vascular changes, and immune cell infiltration. Determining the role of interneuron loss in the pathophysiology of PTE could allow identification of novel drug targets. To elucidate the link between interneuron loss and PTE, we used viral (AAV5-taCasp3-TEVP) and genetic (cell type-specific Cre) tools to induce apoptosis in interneurons in the absence of traumatic injury. The virus expresses activated Caspase-3 in cells expressing Cre-recombinase to induce programmed cell death. We used both *PV-Cre* and *vGAT-Cre* to target PV+ and GABAergic interneurons, respectively. **We hypothesize that genetic elimination of GABAergic interneurons in the cortex will recapitulate phenotypes seen following TBI.** First, we established the viral approach to ablate GABAergic cells. Using immunohistochemical and genetic labeling strategies, we confirm that a viral approach can ablate interneurons with cell type specificity. Then, we assessed behavioral deficits using Rotarod and other assays. Preliminary findings suggest that there is a trend toward motor dysfunction in Cre+ animals following viral infection. These studies suggest viral ablation of cortical interneurons can lead to TBI-like phenotypes. Future studies will utilize these approaches to examine *in vitro* network

function and the development of seizures following cortical interneuron ablation. If virally-induced interneuron ablation mimics TBI-related phenotypes, we will know that interneuron loss is sufficient to lead to post-TBI deficits.

Disclosures: A.M. Ortiz Rivera: None. **J. Koenig:** None. **M. Armbruster:** None. **D. Kong:** None. **C.G. Dulla:** None.

Poster

565. Brain Injury and Trauma: Brain: Histology and Cellular Markers of Brain Injury

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 565.01/M10

Topic: C.10. Brain Injury and Trauma

Title: Diffuse axonal injury in the rat - a study of post-traumatic axonal injury and oligodendrocyte activity in a rotation injury model

Authors: M. LOSURDO[1,2], *M. K. SKOLD[1,3]
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Abstract: Traumatic brain injury is a major medical problem with 2 million cases annually in Europe. Road traffic accidents is the main cause of TBI in adults[1]. The mechanics of impact is often of acceleration-deceleration where the head rotates in the sagittal plane with the neck acting as pivot. The brain is then accelerated within the rigid skull and it is subjected to shear stress with stretching and tearing of axons. In moderate to severe impacts, this result is diffuse axonal injury with oligodendrocyte and myelin degeneration.In this study we examine the extent and degree of diffuse axonal injury in rats that were exposed to rotational trauma. This new rotational injury model allows for precise quantification of trauma intensity delivered by adjusting the angular acceleration.Brain tissue was collected 24 and 72 hours after injury from animals subjected to acceleration ranging between 1.34 - 1.93 Mrad/s^2. Analyses were carried out by means of immunohistochemistry. Axonal injury was investigated through anti-APP antibody and anti-MBP antibody, while oligodendrocyte death and reactive proliferation through anti-Olig2 antibody, anti-NG2 antibody and anti-A2B5 antibody. Regions investigated were corpus callosum, external capsule, hippocampus, fimbriae and brainstem. Sham-operated and penetration-injury exposed subjects were used as controls. The results show that APP was upregulated predominantly at the borders between the corpus callosum and cortex. MBP downregulation was mainly observed at higher acceleration in the corpus callosum. Slight Olig2 downregulation was observed throughout the regions of interest. NG2 appeared to be upregulated in the hippocampus. We conclude that axonal injury is already present 24 hours after injury, that injury is more extensive in the frontal part of the brain subjected to greater rotational force and that APP is most clearly visible at the interface between white and gray matter where

the shear forces are more pronounced. Instead, in the investigated 24-72 hours post-injury time window, processes of degradation (myelin fragmentation and oligodendrocyte death) and of reactive regeneration (oligodendrocyte progenitors proliferation) may be just starting, thus making it a good time window for intervention.

Disclosures: M. Losurdo: None. **M.K. Skold:** None.

Poster

565. Brain Injury and Trauma: Brain: Histology and Cellular Markers of Brain Injury

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 565.02/M11

Topic: C.10. Brain Injury and Trauma

Support: NIH grant NS077675

Title: Intensity specific repetitive mild traumatic brain injury evokes an exacerbated burden of axonal injury in neocortical parvalbumin interneurons

Authors: *Y. OGINO, M. VASCAK, J. T. POVLISHOCK
Anat. and Neurobio., Virginia Commonwealth Univ., Richmond, VA

Abstract: Mild traumatic brain injury (mTBI) is a major health care issue that can result in significant morbidity, particularly in those individuals who had sustained repetitive mTBI. While multiple factors may be at work in the genesis of this morbidity, recent work in both animals and humans suggest neocortical involvement in this process. Recent studies from our lab have demonstrated, in the case of uncomplicated mTBI, the occurrence of diffuse axonal injury (DAI) in the layer V neurons as well as its interneuronal subpopulation leading to an imbalance of excitation and inhibition, which most likely contributes to disordered brain function. In the current communication, we extend these studies to determine if repetitive mTBI of varying intensity can exacerbate the burden of DAI within the parvalbumin (PV) interneurons that are key regulators of cortical excitatory/inhibitory balance.
Mice were subjected to mild central fluid percussion at the injury magnitude of 1.4 or 1.6 atm, with or without a repetitive insult at a 3 h interval. All animals were physiologically monitored prior to injury and, after injury, their righting reflex time was assessed as a surrogate for the duration of any loss of consciousness. DAI in PV interneurons in the layer V was quantitatively assessed 24h post-injury via immunofluorescent double-labeling of p-c-Jun and PV, with electron-microscopic (EM) analysis.
Through these strategies, we confirmed that mTBI evoked PV interneuronal DAI. Importantly, with repetitive injuries, the number of PV interneurons sustaining DAI increased, with the caveat that this increase was linked to the intensity of the mTBI as these changes where the most conspicuous in the 1.4 vs the 1.6 atm injury group. Irrespective of the injury intensity, however,

the observed DAI and concomitant p-c-Jun expression occurred without any evidence of overt tissue damage or neuronal death. In fact, ultrastructural analysis revealed with the exception of the DAI, normal cortical detail with the preservation of the neuronal soma and their dendritic domains as well as the retention of intact glial and vascular elements.
These studies demonstrate that the PV interneurons are vulnerable to the forces of repetitive mTBI in terms of their axonal projections. These studies join with others ongoing in our lab that have confirmed a comparable exacerbation of DAI within the layer V neurons, mandating the need for future studies probing the subsequent excitatory/inhibitory imbalances and their overall functional consequences. (NIH grant NS077675).

Disclosures: Y. Ogino: None. **M. Vascak:** None. **J.T. Povlishock:** None.

Poster

565. Brain Injury and Trauma: Brain: Histology and Cellular Markers of Brain Injury

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 565.03/M12

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R37HD059288

Title: Time span of neurodegenerations after traumatic brain injury in the mouse, as detected with Neurosilver impregnation, Fluoro-Jade C and APP immunohistochemical staining

Authors: *G. XIONG[1], H. METHENY[1], A. S. COHEN[1,2]
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Abstract: Neurodegeneration is a key pathological finding after traumatic brain injury (TBI), likely contributing to neuronal loss and neurological impairments. A major effort has been made over past decades to develop effective diagnostic techniques and specific sensitive markers to detect degenerations after TBI. However, previous investigations on TBI-induced degenerations usually adopted only one marker and/or focused at only one specific time point. In the present study, we used multiple markers to monitor neurodegenerations including Neurosilver impregnation (NS), Fluoro-Jade C (FJC) and amyloid precursor protein (APP) immunohistochemical staining. Multiple time points and different brain regions were tested after moderate to severe TBI induced by lateral fluid percussion injury (lFPI). Neuronal cell bodies positive for NS and FJC could be identified in the sensory and motor cortices, and hippocampus from beginning at 1 hr (after lFPI). Cell body staining in the hippocampus was dramatically decreased at 48 hr. At 7 d, cell body staining was mainly seen in the cortices and thalamus. Beaded dendritic staining could be detected starting at 6 hr and peaked at 10 hr in the hippocampus, extending from FJC-stained cell bodies. Clusters of spheroids and varicosities

could be detected in white matter tracts including the corpus callosum (cc) and alveus with APP but not FJC or NS. APP-positive spheroids could also be found in gray matter such as the cortex, caudate putamen (CPu) and thalamus. This APP staining appeared as early as 3 hr, reached peak staining at 10 hr and diminished 5 d. Axonal retraction bulbs and Wallerian degeneration could be clearly detected with NS. They appeared beginning at 4 d, peaked at 7 d and dramatically decreased at 1 mo. FJC demonstrated a small number of axonal degeneration among stained astrocytes in adjacent slices to those stained with NS. Diffuse axonal degenerations could be traced from the sensory and motor cortices to a variety of subcortical structures: that were seen passing through cc to enter CPu and globus pallidus, entering the thalamus via the internal capsule, following the cerebral peduncle and pyramidal tract to cross the midline at pyramidal decussation. Wallerian degeneration could also be detected in the hippocampus, spinal trigeminal nucleus and the cerebellum, or traveling the optical tract to enter the superior colliculus. The present study demonstrates that different markers for TBI-induced degenerations may be most effective at different time points. In addition to the well-studied pathology in white matter, there are also specific cortical regions and related subcortical nuclei that exhibit diffuse axonal pathology.

Disclosures: G. Xiong: None. **H. Metheny:** None. **A.S. Cohen:** None.

Poster

565. Brain Injury and Trauma: Brain: Histology and Cellular Markers of Brain Injury

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 565.04/M13

Topic: C.10. Brain Injury and Trauma

Support: NIH UO1 NS086659-02

Title: Astrocytic degeneration in chronic traumatic encephalopathy

Authors: *E. T. HSU[1], M. GANGOLLI[2], S. SU[1], L. HOLLERAN[3], T. D. STEIN[4], V. E. ALVAREZ[5], A. C. MCKEE[5], R. E. SCHMIDT[6], D. L. BRODY[2]
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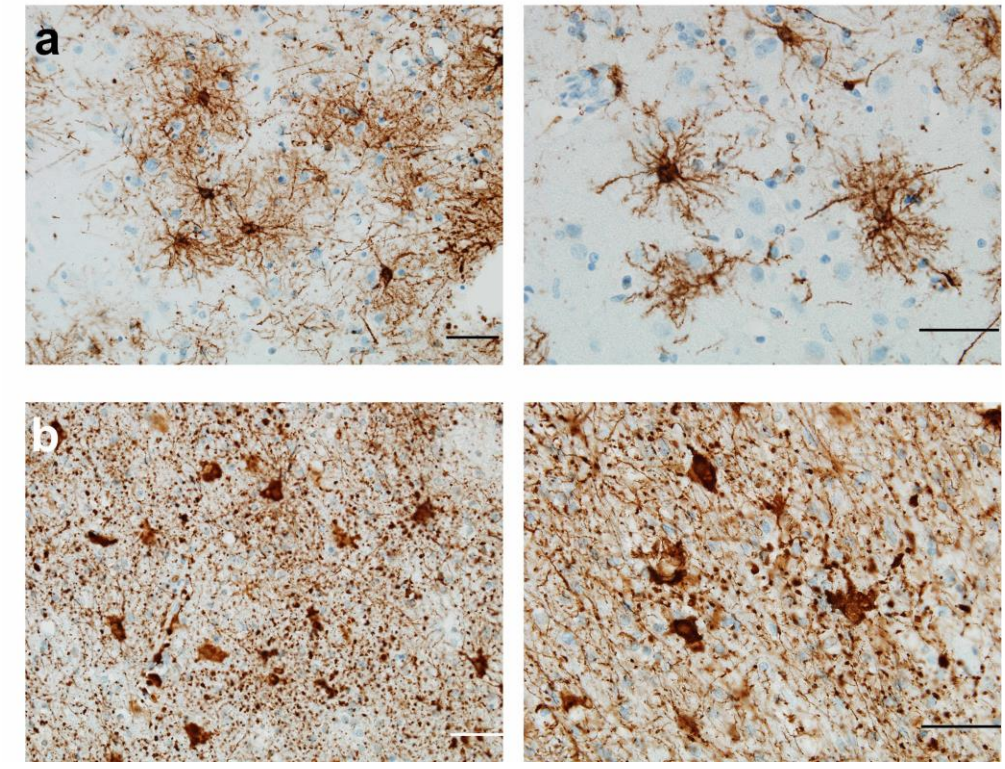
Abstract: Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease associated with repeated head traumas. Using immunohistochemistry for Glial Fibrillary Acidic Protein (GFAP) as a marker, plus automated quantitative analysis, we examined the characteristics and extent of astrogliosis present in stage III CTE and stage IV CTE, along with Alzheimer's Disease (AD), and Frontotemporal dementia (FTD) cases. Surprisingly, overall astrogliosis in CTE patients was more diffuse compared to that of AD and FTD patients, which was concentrated in

the sulcal depths; this localization was the converse of the sulcal predisposition of tau pathology in CTE. Of 13 brains of patients with CTE, 9 exhibited signs of a degenerating astrocyte pathology, characterized by beaded, broken processes. This astrocytic degeneration was typically found to be diffuse throughout the white matter, although two cases demonstrated astrocytic degeneration in the gray matter. The degeneration was also observed in 2 of 3 AD and 2 of 3 FTD brains, with overall similar characteristics across diseases. We found that the extent of the white matter astrocytic degeneration was strongly correlated with the level of overall astrogliosis in both the white and gray mater. However, the astrocytic degeneration was not correlated with the overall extent of tau pathology. Specifically, there was no correlation between the levels of p-tau in the sulcal depths and astrocytic degeneration in the white matter adjacent to the sulcal depths. Thus, astrocytic degeneration and overall astrogliosis appear to represent distinct, though not unique, pathological features of CTE. Further investigation into these astroglial pathologies could provide new insights into the mechanisms underlying CTE and represent a potential target for *in vivo* assessment of CTE as well as other neurodegenerative disorders.



Disclosures: E.T. Hsu: None. **M. Gangolli:** None. **S. Su:** None. **L. Holleran:** None. **T.D. Stein:** None. **V.E. Alvarez:** None. **A.C. McKee:** None. **R.E. Schmidt:** None. **D.L. Brody:** None.

Poster

565. Brain Injury and Trauma: Brain: Histology and Cellular Markers of Brain Injury

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 565.05/M14

Topic: C.10. Brain Injury and Trauma

Title: Morphological changes in prefrontal cortex, dentate gyrus and hippocampus CA1 in the animal model of metabolic syndrome

Authors: *A. CASTRO-MENDEZ[1], J. C. PENAGOS-CORZO[1], R. A. VAZQUEZ, SR[2]
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Abstract: Metabolic Syndrome (MS) is considered a global epidemic. Several studies show that this will have a greater incidence over the years. MS is a set of physiological, biochemical, clinical and metabolic factors associated with obesity, thus increasing the risk of suffering cardiovascular disease. It has been suggested that MS can cause complications in the brain, since chronic hyperglycemia and insulin resistance are risk factors for neuronal outburst, death when inducing a state of oxidative stress and an inflammatory response that affects cognitive processes. However, the neuronal mechanisms that are involved have not been studied deeply. The objective of the present study was to corroborate the presence of morphological changes in rats with a metabolic syndrome model.
Eight obese Zucker Diabetic Fatty rats (OZDF) were used, aged three months, these rats presented an increase in the weight, size, dyslipidemia and metabolic alterations that mimics the metabolic syndrome. In addition, seven Long Evans rats of three months of age were used, which formed the control group. Finally, eight Lean rats were used at three months of age. Ten neurons per animal from each group were analyzed in three regions: layer three of the prefrontal cortex, dentate gyrus and CA1 hippocampus, using Golgi-Cox Stain.
In this study, changes in dendritic morphology were found in the OZDF model, which reflects that this model of Metabolic Syndrome induces alterations at the level of the central nervous system in the brain.
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Disclosures: A. Castro-Mendez: None. **J.C. Penagos-Corzo:** None. **R.A. Vazquez:** None.

Poster

565. Brain Injury and Trauma: Brain: Histology and Cellular Markers of Brain Injury

Location: SDCC Halls B-H

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Program #/Poster #: 565.06/M15

Topic: C.10. Brain Injury and Trauma

Support: Direction Générale de l'Armement
TRAINS EraNet Neuron
CNSAflame EraNet Neuron
Inserm

Title: Spatiotemporal astroglial evolution following juvenile mild traumatic brain injury

Authors: *T. CLÉMENT[1], J. B. LEE[2], A. ICHKOVA[1], M.-L. FOURNIER[1], J. AUSSUDRE[1], M. O. OGIER[3], F. CANINI[3], M. KOEHL[4], N. D. ABROUS[4], A. OBENAUS[2,5], J. BADAUT[1,2]
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Abstract: Traumatic brain injury (TBI) is a leading cause of disability and death among children worldwide. Mild TBI (mTBI) represents around 80% of all pediatric emergency visits and is associated with a higher probability to develop long-term affective and learning disorders. Astrocytes are involved in various physiological homeostatic brain functions including neuronal survival, myelination, regulation of neurotransmission, synaptogenesis and neurogenesis. Astrocytes become reactive after brain insults, a process termed astrogliosis. Thus, it is possible that post-traumatic astrogliosis may impact brain structure and contribute to the long-term affective and cognitive disorders emerging after juvenile mTBI. A Closed-Head Injury with Long-Term Disorder (CHILD) was induced over the left-parietal cortex using an electromagnetic impactor in juvenile C57BL6 wild-type mice and Nestin$xCre^{ert2}$ mice (100 mg/kg Tamoxifen injected 30min after CHILD) on postnatal day 17. Glial Fibrillary Acidic Protein immunolabeling was performed at 1, 7, and 30 days post-injury (dpi) and Nestin immunolabeling was carried out at 7dpi. Semi-automatic skeleton analysis was performed to depict astrocyte morphology in the somatosensory cortex (SSC), dentate gyrus (DG), amygdala and prefrontal cortex (PFC) in both brain hemispheres. Nestin and GFAP positive astrocytes were present in the SSC and PFC in sham mice. The number of double positive cells was not changed after injury for the WT. Nestin$xCre^{ert2}$ mice had increased number of nestin-positive cells in various brain regions, including the DG, but no Nestin-GFAP double labeling was observed after CHILD. There was no change in the total number of GFAP-positive astrocytes after CHILD. The morphology of GFAP-positive cells was altered over time after injury in the SSC, DG, amygdala

and PFC. Astrocytes were hypertrophic (60% size increase in SSC) and abnormally ramified (20% increase in DG) at 7 dpi in WT. However, GFAP-positive astrocytes exhibited similar morphology in sham and TBI mice in all brain regions at 30 dpi.Taken together, our results indicate that juvenile mTBI produces transient changes of astrocyte morphology in remote brain regions (ie. the PFC) in the acute phase post-injury. Thus, GFAP positive astrocytes may contribute to early neuronal network reorganization priming the long-term affective and cognitive disorders after juvenile mTBI.

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Poster

565. Brain Injury and Trauma: Brain: Histology and Cellular Markers of Brain Injury

Location: SDCC Halls B-H

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Program #/Poster #: 565.07/M16

Topic: C.10. Brain Injury and Trauma

Support: MOST 106-2311-B-016 -002-MY3
MND Grant MAB-107-079

Title: Olfactory bulb lesion induces acute cell death in olfactory cortical areas and commissural fibers in rats

Authors: *C.-F. F. CHEN[1], C.-H. LIN[2], H.-T. YANG[1]
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Abstract: Bilateral olfactory bulbectomy (the surgical removal of both olfactory bulbs) is a common procedure to induce a rat model for major depression. The olfactory bulbectomy leads to abnormality in a wide range of behavioral tests and depression-like behaviors in the rats. However, how this brain injury eventually leads to the behavioral deficits remains unclear. In the present study, we focus on revealing acute effects of olfactory bulbectomy on the olfactory cortical circuits. We performed unilateral bulbectomy on 8-week-old Sprague-Dawley rats and examined the brain tissues 24/48/72 hours after this surgical treatment. We used terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay to detect apoptotic cells over brain sections in 30 μm thickness. Our results showed that unilateral bulbectomy led to apoptosis in several olfactory cortical regions, including endopiriform nucleus and piriform cortex, as soon as 24 hours after the surgical lesion. In contrast to previous data, we found that apoptosis in the piriform cortex peaks at 24 hours and gradually decreases until 72 hours after the bulbectomy. To our surprise, however, we observed heavy TUNEL labeling in corpus callosum, with the ipsilateral (lesion side) significantly heavier than the contralateral (unlesion side).

Interestingly, the number of apoptotic cells in corpus callosum increases every 24 hours following the lesion. These findings suggest that olfactory bulb trauma could lead to acute and pervasive cell death in the brain. Mechanisms underlying this lesion-induced cell death may be more complicated than sensory deprivation.

Disclosures: C.F. Chen: None. **C. Lin:** None. **H. Yang:** None.

Poster

565. Brain Injury and Trauma: Brain: Histology and Cellular Markers of Brain Injury

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 565.08/M17

Topic: C.10. Brain Injury and Trauma

Support: United States Army Medical Research and Materiel Command W81XWH-13-1-0017

Title: Temporal analysis of biomarkers of brain damage in ovine survival models of hemorrhage and blast / hemorrhage polytrauma with perfluorocarbon treatment

Authors: *J. PARSONS[1], S. THUMMALA[2], J. MCCARTER[2], C. SWEENEY[2], P. MIDDLETON[2], J. ZHU[1], B. SPIESS[1]
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Abstract: Background: Secondary blast injury (due to airborne shrapnel) can result in severe hemorrhage in the far forward battlefield and can be life threatening with the absence of blood products and significant delay to forward surgical teams. Perfluorocarbon (PFC) oxygen therapeutics are capable of effectively oxygenating sensitive tissue in the absence of red blood cells. Alpha II spectrin breakdown products (markers of neuronal necrosis / apoptosis) and S100B (marker of blood brain barrier breakdown) can be measured in plasma and are associated with outcomes and efficacy of PFC therapy. PFC may improve the "golden hour" during en route care of far forward battlefield polytrauma soldiers.
Methods: Ovine (male, juvenile, 25-30 kg) were subjected to hemorrhagic shock or exposed to blast overpressure (~10-15psi) utilizing an Advanced Blast Simulator just prior to hemorrhagic shock. Mean arterial blood pressure (MAP) was maintained at ~30mmHg for 60 minutes. Sheep were resuscitated with Hespan until MAP was 65 mmHg for 10 minutes then randomized to receive intravenous PFC or saline (both 5 ml/kg). Venous plasma was collected 2 days before injury (baseline) and at 1, 4, and 7 days post injury. Plasma sample proteins were balanced by UV-Vis spectrophotometry, resolved by SDS-PAGE, and transferred to nitrocellulose. Western blots were probed for Alpha-II spectrin breakdown products or S100B using commercially available antibodies, visualized by chemiluminescence, and densitometerically analyzed. Experimental groups (all n=8): Hemorrhage+saline, Hemorrhage+PFC, polytrauma+saline, polytrauma+PFC, and controls.

Results: Total blood loss was 32.3-52.0% (Hemorrhage+saline), 29.6-48.0% (Hemorrhage+PFC), 7.4-45.7% (polytrauma+saline), and 15.2-51.6% (polytrauma+PFC). Lactate levels went from baseline to maximal mmol/L of 0.6-2.5 (Hemorrhage+saline), 0.8-2.4 (Hemorrhage+PFC), 1.5-2.2 (polytrauma+saline), and 0.5-1.4 (polytrauma+PFC). Both alpha II spectrin breakdown products and S100B biomarkers were not observed in any of the groups at any of the time points analyzed.
Conclusion: Neither alpha II spectrin nor S100B biomarkers were observed in the plasma of any of the groups at any time points. It is possible that alpha II spectrin breakdown products were generated, however, since the blood brain barrier remained intact, the breakdown products would only be found in brain tissue. It is also feasible that the level of hemorrhage and polytrauma for these studies was not severe enough, as lactate levels were not greatly increased. Efficacy of PFC as a treatment modality cannot be assessed from this study as changes in biomarkers were not observed.

Disclosures: J. Parsons: None. **S. Thummala:** None. **J. McCarter:** None. **C. Sweeney:** None. **P. Middleton:** None. **J. Zhu:** None. **B. Spiess:** None.

Poster

565. Brain Injury and Trauma: Brain: Histology and Cellular Markers of Brain Injury

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 565.09/M18

Topic: C.10. Brain Injury and Trauma

Support: NINDS U54 NS100064

Title: Temporal evolution of tau hyperphosphorylation in the lateral fluid percussion rat model of severe traumatic brain injury: An EpiBioS4Rx Project 2 Study

Authors: *P. G. SALETTI[1], C. P. LISGARAS[1], W. B. MOWREY[2], Q. LI[1], W. LIU[1], P. M. CASILLAS-ESPINOSA[6], I. ALI[6], R. D. BRADY[6], N. JONES[6], S. R. SHULTZ[6], T. J. O'BRIEN[6], S. L. MOSHÉ[1,3,4,5], A. S. GALANOPOULOU[1,3,5]
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Abstract: Background: The project 2 of EpiBioS4Rx is a multicenter preclinical study that aims to identify targets and new therapies to prevent post-traumatic epilepsy (PTE) using the lateral fluid percussion injury (LFPI) model of severe traumatic brain injury (TBI). Hyperphosphorylated tau (p-tau) is implicated in neurodegenerative processes and has also been linked to TBI and PTE. **Objective:** To determine the temporal evolution of p-tau in the LFPI

model, specifically at 2-days and 1-week post-TBI. **Methods:** Male 11 week old Sprague-Dawley rats were randomized into naïve control (n=6), sham (craniotomy only; n=5/timepoint) or LFPI (n=5/timepoint) rats. Sham and LFPI rats were subjected to a 5 mm left parietal craniotomy; LFPI rats received a pulse of 3.2 (±0.1) atm at the craniotomy preserving the dura. Rats were euthanized at 2-days or 1-week post-craniotomy and perfused brains underwent immunohistochemistry for either p-tau at Ser202/Thr205 (AT8 antibody) or Thr231 (AT180 antibody). Signal densitometry of individual AT8 or AT180 cell somata was done with ImageJ. Results were referred as percentage of right cerebral cortex of the naïve control stained in the same assay. Background regions of interest devoid of cell somata were used to compare non-somatic background staining. Regions of interest were the primary motor (M1), somatosensory (S2a, S2b) and granular insular (GI) cortices. Densitometry and data analyses were done blinded to groups. Statistical analyses included linear mixed model and paired t-test; α was set at 0.05. **Results:** There was an overall trend for increased AT8 somatic immunoreactivity (AT8-ir) at the left (ipsilateral to injury) cortical regions, 2-days post-LFPI (LFPI-2d group). Statistical significances ($p<0.05$) were as follows: left > right LFPI-2d at M1, S2a, S2b, GI; LFPI-2d > controls at GI. Diffuse AT8-ir increase in the background was noted in the LFPI-2d compared to other groups at M1, S2a, S2b, and GI. Somatic AT180-ir was increased at the left M1 region of LFPI-2d ($p=0.01$) and Sham-2d ($p=0.04$) compared to controls; no other statistical differences were seen. However, there were trends for increased AT180-ir in the background of the left S2a and S2b ($p=0.06$) than the right homotypic regions. **Discussion**: Increased p-tau at Ser202/Thr205 (AT8-ir) expression is seen at the LFPI-2d group ipsilateral to the LFPI, but increase in Thr231 tau phosphorylation was limited to the left M1 region. Ongoing studies examine the p-tau expression at later timepoints. The data support the hypothesis that targeting p-tau might be a promising approach for the design of therapies with disease modifying and/or antiepileptogenic potential.

Disclosures: P.G. Saletti: None. **C.P. Lisgaras:** None. **W.B. Mowrey:** None. **Q. Li:** None. **W. Liu:** None. **P.M. Casillas-Espinosa:** None. **I. Ali:** None. **R.D. Brady:** None. **N. Jones:** None. **S.R. Shultz:** None. **T.J. O'Brien:** None. **S.L. Moshé:** None. **A.S. Galanopoulou:** None.

Poster

565. Brain Injury and Trauma: Brain: Histology and Cellular Markers of Brain Injury

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 565.10/N1

Topic: C.10. Brain Injury and Trauma

Support: NIH P30AG13846
NIH R01AG057902
National Center for PTSD

Title: CD8-expressing cell density is stage-specifically increased in chronic traumatic encephalopathy and comorbid Alzheimer's disease

Authors: *B. R. HUBER[1,2], I. MAHAR[2], D. KWASNIK[2], R. MATHIAS[1], V. ALVAREZ[1,2], C. JONATHAN[2], A. C. MCKEE[1,2]
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Abstract: BACKGROUND: Chronic traumatic encephalopathy (CTE) is associated with increased microglial activation that increases with disease stage. The presence of activated microglia suggests activation of the innate immune system, however little is known about activation of the adaptive immune system in CTE. Cytotoxic T-cells are part of the adaptive immune system and can target cells for apoptosis via the MHC1 antigen presentation system. Cytotoxic T-cells expressing CD8 are observed acutely after traumatic brain injury and in other neurodegenerative conditions such as Alzheimer's disease. Cytotoxic T-cell can induce apoptosis in neurons *in vivo*. However, the relationship between CD8-related neurotoxicity and the progression neurodegenerative disease is poorly understood, particularly for CTE. In the current study we quantify CD8 expressing cells in CTE and AD to determine if these neurodegenerations activate the adaptive immune system and are associated with disease stage in CTE. METHODS: Fixed frontal cortex samples were obtained from the VA-BU-CLF Brain Bank (N=183): controls, early (stage 1-2) CTE, late (3-4) CTE, and CTE with Alzheimer's disease (CTE-AD). Sections were stained and scanned, cortical subregions traced, and staining quantified using a Leica Aperio system. ELISA values were from a Neuroinflammation Panel (Meso Scale Diagnostics). Corpus callosum thickness was measured cross-sectionally. RESULTS: Late CTE and CTE-AD cases had greater sulcal CD8-expressing cell density. AT8 staining correlated with CD8 cell density, and increased between controls, early CTE, late CTE, and CTE-AD cases. Late CTE and CTE-AD cases had increased expression of ICAM1 and MDC, whereas early CTE cases showed increased IL-13. CD8-expressing cell density inversely correlated with and anterior corpus callosum thickness.CONCLUSIONS: CD8 cell density is not increased in early CTE cases (despite elevated pTau), but is in later CTE stages. This may be related to increases in inflammatory cytokines in late CTE and transient IL-13 expression in early CTE. CD8 cell density is associated with white matter loss, and may contribute to clinical symptoms.

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Poster

565. Brain Injury and Trauma: Brain: Histology and Cellular Markers of Brain Injury

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 565.11/N2

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant TL1TR001072
NIH Grant NS082432
Burroughs Wellcome Foundation (PUP program)
Dana Foundation

Title: White matter microstructural changes in the corpus callosum and external capsule following highly repetitive subconcussive impacts in the awake adolescent rat

Authors: *T. G. RUBIN[1], W. HOOGENBOOM[3], C. A. BRANCH[2], M. L. LIPTON[4]
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Abstract: In contact sports, such as a soccer and American football, players can accumulate thousands of subconcussive hits to the head over the course of a single season, and many more over a lifetime. Recent evidence has shown that these hits are associated with cognitive deficits and CNS symptoms independent of concussion, and may lead to long term behavioral and cognitive changes associated with chronic traumatic encephalopathy (CTE). While various animal models have been developed to reproduce the biomechanical, neurological, and pathological aspects observed in human concussive injury, subconcussive injury has been largely unexplored. Here we developed a new model of highly repetitive subconcussive injury to explore and characterize the changes in white matter and underlying mechanisms of injury. The protocol was approved by the IACUC at Albert Einstein College of Medicine. Young adult (~p35) male and female rats (n=6 experimental, n=6 sham) underwent subconcussive TBI induction without scalp incision or anesthesia using a Leica Impact One^{TM} Impactor, fit with a rubber impacting tip, 10 mm diameter impacting surface. Animals received 10 hits/day (1 minute apart), to the left parietal bone, midway between the ear and bregma, every day for 7 days, totaling 70 hits (impact velocity=2.5m/s, depth=5mm, dwell=100ms). Animals were mildly restrained in a cone-shaped plastic bag and placed in a foam cradle to ensure reproducible impact location while allowing the head to freely move following impact. Sham animals underwent the same procedures, but received no impacts. All animals underwent diffusion tensor imaging (DTI) prior to injury and 24 hours after the final injury. Animals were then sacrificed 24 hours after the final imaging or 3 months later for immunohistochemistry. All data were randomized and blinded before analysis. The corpus callosum, and bilateral external capsule were manually traced and quantified using MIPAV (v8.0.2). Animals showed no signs of gross pathology (e.g., skull fractures or hemorrhage) or overt behavioral abnormalities. In contrast to sham, impacted animals displayed numerically decreased mean FA (3-6%) for all regions of interest (ROIs) compared to preinjury levels. Also, axial and radial diffusivity (AD and RD) increased for all ROIs for both groups. Our data provide promising preliminary evidence that our model produces injury similar to that seen associated with human subconcussive injury and may be a viable tool for further exploring mechanisms of injury and recovery. Continuing studies will include characterization of behavioral changes as well as histopathology in the short- and long-term following subconcussive head impacts.

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Poster

565. Brain Injury and Trauma: Brain: Histology and Cellular Markers of Brain Injury

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 565.12/N3

Topic: C.10. Brain Injury and Trauma

Support: HIH Grant U54GM104942
NIH Grant P20GM109098
NIH Grant K01NS081014

Title: MitoNEET (CISD1) knockout mice have increased susceptibility to intracranial hemorrhage

Authors: *S. A. BENKOVIC, JR[1], C. M. BROWN[2], W. J. GELDENHUYS[3]
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Abstract: MitoNEET is an iron-containing protein localized to the outer mitochondrial membrane that regulates mitochondrial respiration, iron transport into the mitochondrion, and lipid and carbohydrate homeostasis. We previously observed that deletion of mitoNEET resulted in a loss of tyrosine hydroxylase immunoreactivity, dopamine depletion in striatum, increased ROS production, iron accumulation, increased inflammatory protein expression, and behavioral deficits that caused a phenotype similar to Parkinson's disease. Here, we evaluated the histopathological consequences of a heterozygous mitoNEET deletion in six-month old male mice and observed brain-wide microhemorrhages and subsequent reactive gliosis of both astrocytes and microglia in comparison to wild-type control mice. Immunohistochemical analysis of mitoNEET reactivity revealed a near complete absence throughout the brain of mutant mice. Perls' Prussian Blue (PPB) staining revealed hemosiderin of microhemorrhages in most brain regions including: hippocampus, thalamus, cortex, cerebellum, olfactory bulb, and brain stem; and the white matter tracts connecting these regions: corpus callosum, stria terminalis, and fimbria of the fornix. Large microhemorrhages were on the order of $2000\mu^2$. Chromagen-enhancement of the PPB stain revealed ferritin-bound iron which accumulated in the striatum. Distinct populations of blue and brown cells were observed in several brain regions. Microhemorrhages caused activation of both astrocytes and microglia with a different pathological profile. Astrocytic reactivity was greater in proximity to the vascular disruption while microglial reactivity appeared homogeneously throughout the parenchyma. Microglia were observed filled with phagocytic debris from older hemorrhages or in the process of ingesting debris from newer hemorrhages. These data suggest that loss of mitoNEET causes a pathological elevation of ferritin-bound iron, microhemorrhage, reactive gliosis and reduced integrity of the neurovasculature.

Disclosures: S.A. Benkovic: None. **C.M. Brown:** None. **W.J. Geldenhuys:** None.

Poster

565. Brain Injury and Trauma: Brain: Histology and Cellular Markers of Brain Injury

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 565.13/N4

Topic: C.10. Brain Injury and Trauma

Support: Effects of Neurotrauma Consortium Award No. W81XWH-13-2-0095

Title: Hippocampal and entorhinal cortex Alzheimer's disease-like pathology in human chronic traumatic encephalopathy: A chronic effects of neurotrauma consortium study

Authors: *C. M. KELLEY, M. NADEEM[1], F. C. CRAWFORD[2], A. C. MCKEE[3], S. E. PEREZ[1], E. J. MUFSON[1]
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Abstract: Chronic traumatic encephalopathy (CTE) is a progressive neurodegenerative condition resulting from repetitive mild trauma to the head, a circumstance prevalent in contact-sport athletes and military personnel. Although the regional spread of tau pathology in the CTE brain marks disease stage and severity (McKee et al., 2013), very little is known about the distribution and morphology of tau positive profiles within the hippocampal complex. Eighteen male Caucasian and African-American former professional contact-sport athletes from Stage II (n = 6, age at symptom onset 20-65 y; age at death 25-70 y), Stage III (n = 6, age at symptom onset 24-40 y; age at death 45-67 y), and Stage IV (n = 6, age at symptom onset 30-68 y; age at death 62-80 y) were obtained from Boston University School of Medicine. Paraffin blocks containing the hippocampus and entorhinal cortex (EC) were sectioned at 8 µm and mounted on slides, treated with citric acid, and immunolabeled with AT8 (an early pathological tau marker). In addition, amyloid pathology was evaluated with antibodies against the amyloid precursor protein and Aβ (6E10), Aβ1-40, and Aβ1-42. AT8-positive profile number and size were analyzed using a 60X oil-immersion lens controlled by a MicorBrightField software suite; presence of various Aβ species was examined with a Nikon Eclipse 80 microscope. Quantitative analysis revealed significantly more AT8-positive neurons in the CA1 and CA3 hippocampal subfields and the EC in Stage IV compared with Stage II (CA1, 12.6-fold; CA3, 11.5-fold; EC, 11.0-fold; Mann-Whitney U, $p < 0.01$). The EC and hippocampal subfields also displayed significantly smaller AT8-positive neuronal area in Stage IV compared to Stage II by an average 37.8 % (EC, 26.5 %; CA1, 35.1 %; CA3, 51.7 %; Mann-Whitney U, $p < 0.01$). Stage III displayed intermediate values for both AT8-positive neuron count and size, suggesting a transitional pathological stage. In contrast, minimal Aβ profiles were seen in the hippocampal-EC complex, primarily in Stage IV cases, suggesting that amyloid and an altered Aß species

profile are not a requisite co-condition of tau pathology in CTE. Data suggest that phosphorylated tau (AT8) protein levels may provide a biomarker to track and a drug target to slow the progression of CTE.

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Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.01/N5

Topic: C.10. Brain Injury and Trauma

Title: Putative dendritic correlates of repetitive traumatic brain injury: A quantitative Golgi study

Authors: A. WARLING[1], L. UCHIDA[1], V. NGUYEN[1], M. E. GARCIA[1], N. B. SHEA-SHUMSKY[1], S. SVIRSKY[2], T. D. STEIN[2], *B. G. JACOBS[1]
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Abstract: Repetitive traumatic brain injury (RTBI) may be a major risk factor for the neurodegeneration associated with chronic traumatic encephalopathy (CTE). Although TBI has been associated with acute dendritic and spine damage (Castejón et al., 2004), its potential enduring effects on cortical dendrites have not been investigated. Thus, the present study examined long-term changes in the dendritic systems of supragranular pyramidal neurons following RTBI, in cases with and without CTE diagnoses. Samples were obtained from the frontal and occipital poles of six males with a history of sports-related RTBI (M_{age} = 77 ± 13 years), five with CTE diagnoses and one without, and compared to tissue from 12 neurologically normal individuals (M_{age} = 72 ± 6 years; Jacobs et al., 1997). Tissue was prepared using a modified rapid Golgi technique, with 20 neurons sampled from each cortical region in post-RTBI tissue (n = 240), and 10 neurons sampled from each cortical region in control tissue (n = 240). Dendritic arbors were analyzed using computer-assisted morphometry. Compared to control tissue, quantitative dendritic and spine measures tended to be markedly decreased in all post-RTBI tissue regardless of CTE diagnosis. This decrease was observed in both cortical regions, with the prefrontal cortex being more severely affected than the visual cortex. Such dendritic declines following RTBI may have negative implications for cognitive functioning, with or without a specific neurodegenerative diagnosis.

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Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.02/N6

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 8UL1TR000055
USAMRMC Award W81XWH-12-1-0004
DOD Award W81XWH-14-1-0561

Title: Role of head impact exposure in concussion for college and high school football athletes

Authors: *B. D. STEMPER[1,2], A. SHAH[3], R. CHIARIELLO[4], A. WILD[4], M. MCCREA[4]
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Abstract: The biomechanical mechanism of concussion has long been understood to be head impact resulting in high rate head rotational accelerations. However, recent evidence identified a possible role of repetitive head impact exposure (HIE) in concussion onset in contact sport athletes and cognitive/imaging changes in non-concussed athletes. This study outlined the profile of HIE in high school and college football athletes, and identified differences in HIE between concussed and non-concussed athletes. The study protocol was IRB approved and informed consent was obtained from all athletes or their legal guardian. Athletes were recruited from 4 local high school and 4 NCAA Division III football teams. Head impact accelerations were monitored for all contact activities during the 2015-17 football seasons using the Head Impact Telemetry (HIT) System (Riddell SRS). Only impacts with peak resultant linear acceleration greater than 10 g were included for analysis. All concussions in enrolled athletes were identified and diagnosed by team medical staff according to a standardized protocol. Following injury notification, the study team used HIT System and video data, as well as the concussion report, to identify a single concussive head impact. HIE was quantified using a Cumulative Metric (CM) that was calculated by summing the concussive injury risk according to peak head accelerations for each head impact over the period of interest. As such, CM magnitude increased for a greater number of head impacts and higher severity head impacts. Average daily CM and CM on the injury date were calculated for concussed athletes up to and including the injury date, and for all non-concussed athletes on all days of participation in contact activities. A total of 149 high school and 405 college football athletes were enrolled. Eight high school athletes sustained concussion during the study (rate: 5.4%) and 35 college athletes sustained concussion (rate: 8.6%). Approximately 88% of concussed high school and college athletes had average daily CM that was greater than the 50th percentile CM for non-concussed athletes. However, the percentage

of high school athletes that exceeded the 50th percentile CM on the injury date and for the 5- and 10-day periods leading up to the injury date (~88%) was considerably greater than the percentages (83%, 77%, 67%, respectively) for college athletes. These findings highlighted a higher rate of concussion in college football athletes, that concussed football athletes at both levels sustained more severe HIE than non-concussed athletes, and that the difference in HIE between concussed and non-concussed athletes was greater at the high school level.

Disclosures: B.D. Stemper: A. Employment/Salary (full or part-time):; Medical College of Wisconsin, Zablocki VA Medical Center. **A. Shah:** None. **R. Chiariello:** None. **A. Wild:** None. **M. McCrea:** None.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.03/N7

Topic: C.10. Brain Injury and Trauma

Support: Liaison Committee between the Central Norway Regional Health Authority and the Norwegian University of Science and Technology
Norwegian National Advisory Unit for functional MRI

Title: Prospective study of blood biomarkers in mild traumatic brain injury patients

Authors: *A. K. HABERG[1], G. CLARKE[1], C. EINARSEN[1], T. FOLLESTAD[1], H. ZETTERBERG[3], K. BLENNOW[3], A. VIK[2], T. SKANDSEN, 7030[1]
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Abstract: The aims of the study were to assess the level of glial fibrillary acidic protein (GFAP) and Neurofilament Light-1 (NFL) at five time points after mild traumatic brain injury (MTBI), and examine the relationship between GFAP and NFL levels and acute injury severity and post-concussive symptoms at 2 weeks. 379 consecutive patients with MTBI aged 16-60, were prospectively recruited from the emergency departments at a level 1 trauma center and a municipal outpatient clinic. Blood samples were drawn acutely (71 patients, 73 controls), at 72 hours (134 patients, 6 controls), at 2 weeks (177 patients, 9 controls), 3 (170 patients, 100 controls) and 12 months (158 patients, 55 controls) after injury. Plasma analysis was performed using the Quanterix Neurology 4-plex panel. Acute CT and brain MRI at 3T within 72 h were acquired. Posttraumatic amnesia (PTA) was recorded as short (≤1 hour) or long (>1 hour). Post concussive symptoms assessed at 2 weeks with the Rivermead Post concussion symptom questionnaire, high symptom burden was ≥3 items rated >2. Separate mixed model analyses were performed with logarithmically transformed values of NFL and GFAP values over time in

MTBI patients versus controls. Mann-Whitney U-tests were used for comparing MTBI patients stratified based on clinical characteristics versus blood brain biomarkers levels. In MTBI, GFAP peaked in the acute phase, while NFL peaked at 2 weeks. There was a 95% difference in GFAP levels in the acute phase between MTBI and controls (p<.0001), and a 80% difference in NFL levels between MTBI and controls at 2 weeks (p<.0001). By 12 months, both NFL and GFAP levels had returned to control levels in MTBI. The interaction effect of group and time was statistically significant for both NFL (p<.0001) and GFAP (p<.0001). Patients with traumatic axonal injury had near significantly higher NFL than patients with other intracranial lesions (p=0.051). Median NFL values were 119.6 versus 22.5 pg/ml. Patients with long and short PTA had higher GFAP and NFL than controls (p=0.001). NFL was higher in patients with long PTA (p=0.038) while no significant difference was found in GFAP between PTA groups. Patients with high symptom burden at 2 weeks had lower GFAP and NFL than patients with low symptom burden (p=0.050 and 0.024). In this longitudinal study of MTBI, we detect GFAP and NFL levels in peripheral blood that were different from those of controls from the acute phase, returning to control levels at 12 months. GFAP and NFL appear to be useful as biomarkers of acute injury severity in MTBI. Surprisingly, we found a negative association between high symptom burden at 2 weeks and blood biomarkers.

Disclosures: A.K. Haberg: None. **G. Clarke:** None. **C. Einarsen:** None. **T. Follestad:** None. **H. Zetterberg:** None. **K. Blennow:** None. **A. Vik:** None. **T. Skandsen:** None.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.04/N8

Topic: C.10. Brain Injury and Trauma

Support: Howard Hughes Medical Institute

Title: Genetic basis of neurosurgically resected hemimegalencephaly and epilepsy

Authors: *C. GARCIA[1], H. MACHADO[1], W. JUNIOR[1], J. GLESSON[2]
[1]Univ. of São Paulo - USP, ribeirão preto, Brazil; [2]Howard Hughes Med. Inst., San Diego - CA, CA

Abstract: Hemimegalencephaly (HME) is a malformation of cortical development, characterized by enlargement of the convolutions and a cerebral hemisphere. It is considered the most common cause of refractory epilepsy in children. The clinical condition presents macrocephaly, delayed psychomotor development and hemiparesis. Studies demonstrated thatgenetic factors are involved in the HME. Essays on the molecular pathogenesis and cellular cortical malformations can reveal information about associated mechanisms and contribute to

new therapeutic approaches discovered for the treatment of symptoms. RCS patient, age 8, negative family history of genetic disorders, diagnosed with HME at two months of life, subjected to surgical treatment with a year of life. The microscopic exam show histological sections of the neocortex stained by H&E show áreas with Chaslin´s superficial cortical gliosis and extensive áreas of neuronal loss and neuronal bodies grouped, resulting in architectural distortion, mainly of the laminar distribution. Additionally, there are some rare foci of giant neurons, with large nuclei and wide cytoplasm. In some areas, the córtex presents reduction of the number of layers (four layers), with fusion of the gyri, some foci of rarefied neuropil in the córtex and White matter and some islets of cortical matter in the white matter. The patient was classified as dysplasia cortical focal Type IIA of the ILAE classification. He was taken to the medical genetics clinic for diagnostic investigation we performed the sequencing of the complete patient exoma in order to find mutations responsible for the clinical picture. Among the most relevant results, we found a mutation at position 1624 G>A in PI3KCA gene as a possible head of the HME and the results were valid for ddPCR and smMIPs. We conclude that patients with malformations of cortical development have genetic changes which may influence the individual's phenotype and this information can offer better pharmacological alternatives for treating these patients.

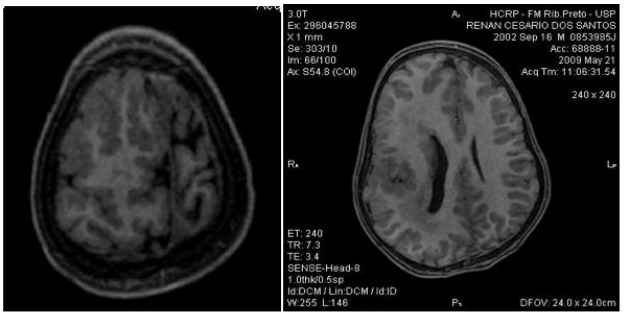


Figure 1: MRI scan show typical hemimegalencephaly peri-insular with polimicrogyria

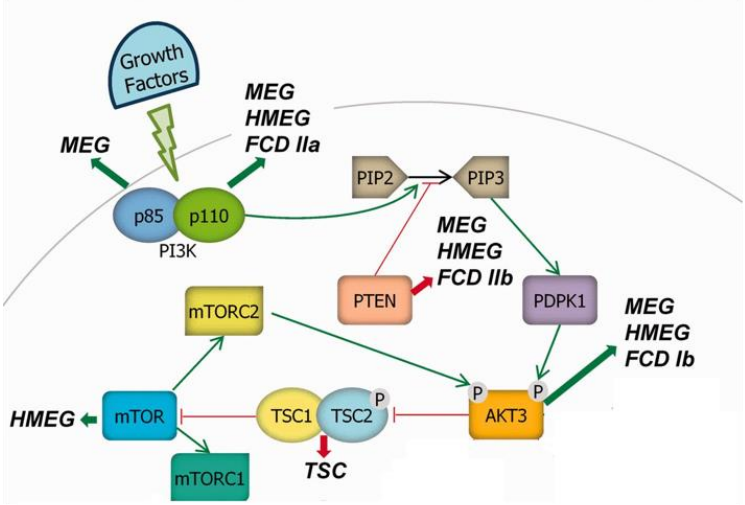


Figure 2: PI3K signaling pathway and its relevance in the cortical malformations.

Disclosures: C. Garcia: None. **H. Machado:** None. **W. Junior:** None. **J. Glesson:** None.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.05/N9

Topic: C.10. Brain Injury and Trauma

Title: Functional brain changes in patients with traumatic brain injury

Authors: *J. ASHLEY[1], N. G. HARRIS[3], M. J. ASHLEY[5], C. K. SINGH[5], M. ASHLEY[2,4], G. S. GRIESBACH[6]
[1]Res., [2]Ctr. for Neuro Skills, Bakersfield, CA; [3]Neurosurg., [4]Neurol., UCLA, Los Angeles, CA; [5]Ctr. For Neuro Skills, Bakersfield, CA; [6]Ctr. For Neuro Skills, Encino, CA

Abstract: Alterations in functional connectivity (fc) can be widespread in multiple brain networks following traumatic brain injury (TBI). We report data from a pilot study investigating network disruptions following TBI. T1 anatomical data and resting state functional magnetic resonance imaging were obtained from 10 TBI participants and 8 healthy controls (HC) using a 3T Siemens Prisma system. All subjects completed a battery of cognitive tests after each scan. Changes in fc and cognitive performance were evaluated at 2 different occasions. The second evaluation occurred an average of 60 days apart. Image data were pre-processed and warped to a template atlas in order to conduct pair-wise correlation analysis as an indicator of fc between regions within 35 sub networks and 170 specific brain regions of interest. The general linear model with correction (FDR $P<0.05$) and without ($P<0.005$) was used. Analysis revealed a significant effect of injury. Initial evaluation revealed that the TBI group had longer simple and complex reaction time and long delay recall deficits. The second evaluation indicated TBI associated deficits in reaction time were still present. We found group differences in fc at both time-points that were dominated by injury-associated hyperconnectivity (83% of significantly different connections $p<0.005$). These findings were observed in regions within the following networks: default mode, salience, dorsal attention, fronto-parietal and cerebellar ($P<0.005$). More robust findings that survived FDR correction were present within the dorsal-attention, language and front-parietal network. Moderate injury-related hypoconnectivity was also observed, and was confined mainly to circuits arising from seeds in sensory-motor, salience and default mode networks at both imaging sessions (4 of 24 connections and 5 of 30 connections at 1st and 2nd sessions, respectively). Overall changes across both imaging sessions showed greater changes in fc among the injured compared to the HC group ($P<0.005$). In particular, the TBI group showed temporal-associated decreases in fc in default mode and salience network and an increase in visual networks compared to HC ($P<0.05$ FDR). Deficits in reaction time were associated with

hyperconnectivity in the salience network and hypoconnectivity in the sensory-motor and default mode networks.

Disclosures: J. Ashley: A. Employment/Salary (full or part-time):; Centre for Neuro Skills. **N.G. Harris:** None. **M.J. Ashley:** A. Employment/Salary (full or part-time):; Centre for Neuro Skills. **C.K. Singh:** A. Employment/Salary (full or part-time):; Centre for Neuro Skills. **M. Ashley:** A. Employment/Salary (full or part-time):; Centre for Neuro Skills. **G.S. Griesbach:** A. Employment/Salary (full or part-time):; Centre for Neuro Skills.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.06/N10

Topic: C.10. Brain Injury and Trauma

Support: Norris Foundation

Title: In search of biomarkers for late recovery of patients with traumatic brain injury: A multimodal approach

Authors: *E. ROSARIO[1], J. DIVINE[2], M. JOHNSON[3], C. SCHNAKERS[4]
[1]Res. Inst., Casa Colina Hosp. and Centers For Healthcare, Pomona, CA; [2]Casa Colina Hosp. and Centers for Healthcare, Pomona, CA; [3]UCLA, Los Angeles, CA; [4]Casa Colina and Centers for Healthcare, Pomona, CA

Abstract: Research Objective: To provide a preliminary database of neural profiles that can be related to cognitive and functional outcome measures in chronic patients with moderate to severe traumatic brain injury (TBI). **Design:** Prospective longitudinal study. **Setting:** Casa Colina Hospital and Centers for Healthcare Research Institute (Pomona, CA). **Participants:** Six TBI patients (5 males; age range: 21-44 years old; 12-21months post-injury) were included in this study. **Main Outcome Measures.** The following data were collected at monthly intervals over 6 months: ***a) Blood sample*** to detect changes in molecular blood-based biomarkers such as neuron-specific enolase (NSE), glial fibrillary acid protein (GFAP), S-100β protein, myelin basic protein (MBP), cleaved tau protein (C-tau), spectrin breakdown products (SBDPs), ubiquitin C-terminal hydrolase-L1 (UCH-L1). Blood-based biomarkers are determined using ELISA. ***b) Magnetic Resonance Imaging (MRI)*** recordings were performed on a Siemens Magnetom Verio 3T to assess structural changes. MRI data are analyzed in collaboration with the UCLA Department of Psychology, using FMRIB Software Library (FSL). ***c) Electrophysiological recordings*** of 2 types were performed, using a B-Alert wireless system, to assess changes in electrical activity and processing: 5 minutes resting state and P300 auditory oddball (listening versus counting). EEG/ERPs analyses are performed using EEGLAB. ***D) Neuropsychological assessments*** were

performed by trained clinicians to assess cognition and functional recovery. **Results.** Significant correlations between functional abilities, neuroimaging and blood-based biomarkers were observed. Results also suggest both transient and enduring changes in neural activity over the 6-month study period of recovery. **Conclusion.** Our preliminary findings will help characterize the diverse range of brain activity patterns that occur as a result of chronic TBI and potentially lead to the development of adapted and tailored treatment plans for these patients.

Disclosures: J. Divine: None. **M. Johnson:** None. **C. Schnakers:** None.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.07/N11

Topic: C.10. Brain Injury and Trauma

Title: A distinct population of cholinergic neurons in the human parabrachial nucleus

Authors: *S. DE LACALLE
Biomed. Sci., Heritage Col. of Osteo. Med., Athens, OH

Abstract: Over the last 40 years, neurons of the Parabrachial Nucleus (PBN) were shown to play an important role in the processing and relaying of somato- and viscero-sensory information. A recent overview of clinical correlations (Benarroch, 2016) has presented additional evidence for the involvement of the human PBN in multiple modulatory functions. More recently, the question has been raised as to whether parts of the PBN belong to the ascending reticular arousal system. We investigated the distribution of cholinergic perikarya and putative cholinergic terminal fields in the human PBN, to provide a neurochemical background against which more accurately interpret the results obtained from human imaging and interventional studies of clinical relevance, such as the fMRI study on gustatory pathways, on brainstem respiratory control, on temperature, and on pain. Human brain tissue obtained at routine autopsy from neurologically intact individuals was sectioned on the coronal plane, and examined using light microscopy, following standard immunocytochemical techniques with polyclonal antibodies against Choline Acetyl Transferase (ChAT). Sketch overlay drawings were created to aid in visualizing immunoreactive elements and anatomical features of the region. Photographs were compared with current atlases of the human brainstem to define anatomical landmarks. ChAT-ir cells were medium-sized, oblong or triangular in shape, with 2-3 prominent dendrites. In double-stained sections they were found overlapping (but clearly distinct from) previously described CGRP-containing regions of the PBN. Most were scattered within the medial and lateral PBN, with two prominent clusters: one, at the level of the rostral tip of the superior cerebellar peduncle in the dorsolateral parabrachial area, and a second ventrocaudal cluster, in the external lateral nucleus of the PBN. Many, but not all, also contained CGRP-ir. These findings suggest a small,

widespread and distinct population of cholinergic neurons within the human PBN, lending morphological support to the possible involvement of this region in coma and sleep apnea, the pathophysiology of chronic pain, and other central autonomic regulation processes already described in other mammals. Additional refinements in neuroimaging and neuropathological analysis will help establish specific relationships in support of the clinical relevance of the PBN in neurologic disease.

Disclosures: S. de Lacalle: None.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.08/N12

Topic: C.10. Brain Injury and Trauma

Support: University research funding of the German Sports University, Cologne

Title: Cumulative effects of sport related concussions on functional brain oxygenation

Authors: *I. HELMICH, J. COENEN, S. SCHUPP, C. WAGNER, M. WERNKE, J. RUEHLING, S. EICH, E. PARDALIS, S. HENCKERT, H. LAUSBERG
German Sports Univ., Cologne, Germany

Abstract: Objectives: Sport related concussions (SRC) are a risk factor for cognitive impairment and its underlying brain functions. Previous studies reported hypometabolism in frontal brain regions during working memory tasks in athletes suffering from postconcussive symptoms. In addition, hypermetabolism in response to moderate working memory processing loads has also been described. We therefore investigated functional brain oxygenation during difficult and easy working memory tasks in athletes with the history of a SRC differentiating between high and low symptomatic individuals. Methods: 91 athletes with a SRC were investigated regarding their brain oxygenation in frontal cortices using functional NearInfraRed Spectroscopy (fNIRS) during a working memory (wm) task with a difficult and an easy task condition. The amount of concussions was included as a covariate in the analysis. Results: Individuals with a post-concussion symptom (PCS) score > 10 showed less correct answers and slower response times in the wm tasks and specifically, in the difficult wm task decreased brain oxygenation in frontal cortices. However, the same individuals showed increased brain oxygenation patterns during easy wm tasks. Furthermore, the amount of experienced concussions correlated negatively with the degree of brain oxygenation during wm tasks. Conclusion: Athletes with a history of concussion showed brain oxygenation patterns that were altered in relation to the present symptomatology. Whereas high wm processing loads lead to decreased brain oxygenation in symptomatic individuals, low processing loads lead to increased functional brain oxygenation.

Furthermore, the more concussions an athlete experienced, the lower the degree of functional brain oxygenation was observed. Thus, SRC present cumulative effects on functional brain oxygenation.

Disclosures: I. Helmich: None. **J. Coenen:** None. **S. Schupp:** None. **C. Wagner:** None. **M. Wernke:** None. **J. Ruehling:** None. **S. Eich:** None. **E. Pardalis:** None. **S. Henckert:** None. **H. Lausberg:** None.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.09/O1

Topic: C.10. Brain Injury and Trauma

Support: Fund Scientific Research Flanders - Grant G087213N
Special Research Fund, KU Leuven - OT/14/127 3M140230

Title: Functional connectivity of the sensorimotor network is influenced by the corticospinal tract wiring pattern in unilateral cerebral palsy

Authors: *C. SIMON-MARTINEZ[1], E. JASPERS[2,1], K. ALAERTS[1], E. ORTIBUS[1], K. KLINGELS[1,3], N. WENDEROTH[2], H. FEYS[1]
[1]KU Leuven, Leuven, Belgium; [2]ETH Zurich, Zurich, Switzerland; [3]Hasselt Univ., Hasselt, Belgium

Abstract: Introduction. In unilateral cerebral palsy (uCP), the development of the corticospinal tract (CST) can be affected, resulting in different wiring patterns (contralateral, ipsilateral or bilateral). The CST wiring has been put forward as an important factor determining sensorimotor function and treatment response. Here, we explored differences in functional connectivity (FC) of the sensorimotor network between different CST wiring patterns, using resting state functional MRI (rs-fMRI). **Patients and methods.** Individuals with uCP due to periventricular white matter lesions underwent a rs-fMRI scan and a single-pulse Transcranial Magnetic Stimulation session (n=24, mean age (SD): 13y3m (±4y6m), 15 females; CST wiring: 9 contralateral, 8 ipsilateral, 7 bilateral). FC was determined for 8 bilateral sensorimotor regions of interest (ROIs) (primary motor (M1) and sensory (S1) cortex, secondary sensory cortex (S2), dorsal and ventral premotor cortex (dPMC, vPMC), supplementary motor area (SMA), thalamus, and putamen) and correlated to all other voxels in the brain (seed-based analysis). SPM one-way ANOVA models were used to contrast differences in interhemispheric and intrahemispheric FC between the CST wiring groups (height and cluster threshold p-uncorrected<0.01). **Results.** Within the non-lesioned hemisphere, FC between cortical seeds (M1, S2, dPMC, SMA) and primary sensorimotor and association sensory areas was increased in the bilateral CST group compared to

the other two groups. Similarly, within the lesioned hemisphere, those in the bilateral CST group showed increased FC between cortical seeds (M1, S2) and the primary and association sensory areas compared to the other groups. Interhemispheric FC between dPMC and the primary sensorimotor area was highest in the bilateral CST group, followed by the ipsilateral group, and lowest in those with contralateral CST wiring. Lastly, seeds placed in the thalamus and putamen resulted in higher FC with the cortical and subcortical structures within the lesioned hemisphere in the contralateral CST group, compared to the other two groups. **Discussion and conclusion.** Our results indicate that the CST wiring affects the long-range FC pattern of individuals with uCP, whereby bilaterally wired individuals have higher FC between cortical structures, whilst the contralaterally wired individuals have higher FC with subcortical structures. These results are a first step toward a better understanding of the underlying pathophysiology of white matter lesions by combining FC measures and CST wiring pattern in individuals with uCP.

Disclosures: C. Simon-Martinez: None. **E. Jaspers:** None. **K. Alaerts:** None. **E. Ortibus:** None. **K. Klingels:** None. **N. Wenderoth:** None. **H. Feys:** None.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.10/O2

Topic: C.10. Brain Injury and Trauma

Support: Henry Jackson Foundation

Title: Delayed frontal responses discriminate malingered individuals from patients with brain injury

Authors: *S. STROTHKAMP[1,2], J. NEAL[2], E. BEDINGAR[2], B. WAGNER[2], V. VAGNINI[2], Y. JIANG[3]
[1]Lexington, KY; [2]Dept. of Behavioral Sci., Univ. of Kentucky Col. of Med., Lexington, KY; [3]Dept. of Behavioral Sci., Univ. of Kentucky Chandler Med. Ctr., Lexington, KY

Abstract: Traumatic brain injury (TBI) is a major public health concern in the United States. Neuropsychologists report that up to 40% of individuals undergoing evaluations for TBI may be malingering neurocognitive deficits. The current malingering tests can be manipulated via coaching. Thus, a malingering test involving measures of brain activity is needed for validating TBI while identifying malingerers. We hypothesize that, due to active mental manipulation, healthy malingers' frontal brain responses are delayed compared to those who have brain injury. The memory-related brain potentials were compared among three groups: individuals with moderate or severe TBI (n=14), individuals who are healthy but malingering neurocognitive deficit (age-matched, n=15) and individuals who are healthy and honest (age-matched, n=13).

The scalp electrophysiological signals and memory performance were recorded during an Old-New memory recognition task. The EEG signals were recorded with a 32-channel scalp EEG cap. The latency of frontal event related potentials indicative of cognitive processing, known as P3, were analyzed using EEGLAB by calculating fractional latencies of bilateral of frontal sites. Results show a significant delay in P3 fractional latencies in recognizing studied items (Old) in malingerers when compared to brain injured subjects in central (Fz) and left frontal electrodes (FP1, F3). A significant delay was also shown during old tasks in malingerers when compared to honest subjects in bilateral frontal electrodes F3 and F4. There were no significant differences in posterior sites. These findings, matching our previously reported reaction time performances, indicate the presence of additional processing time and effort in the brain activity of malingering individuals when compared to healthy honest and brain injured individuals.

Disclosures: S. Strothkamp: None. **J. Neal:** None. **E. Bedingar:** None. **B. Wagner:** None. **V. Vagnini:** None. **Y. Jiang:** None.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.11/O3

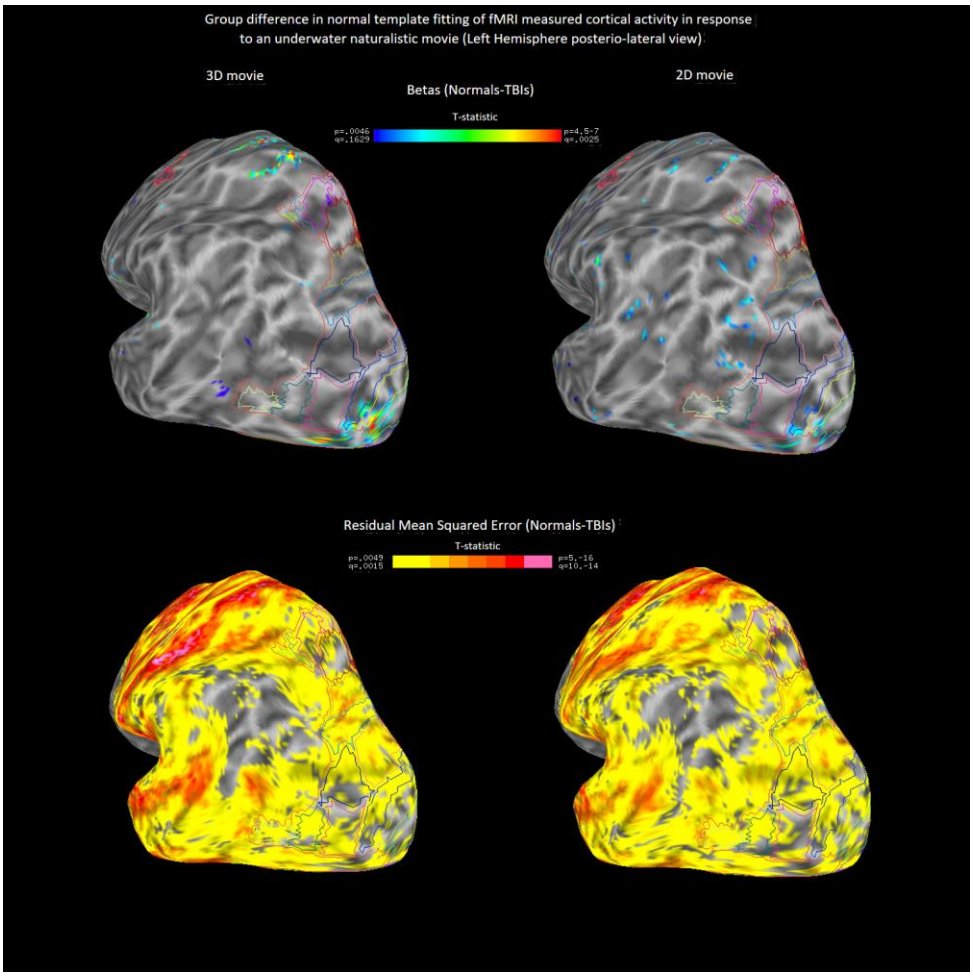
Topic: C.10. Brain Injury and Trauma

Title: Cortical responses to 3D natural stimuli distinguishes between concussed and normal brains

Authors: *T. RUIZ[1], R. FARIVAR-MOHSENI[2]
[1]McGill MUHC, Montreal, QC, Canada; [2]Ophthalmology, McGill Univ., Montreal, QC, Canada

Abstract: Traumatic brain Injury (TBI) affects more than 2,000,000 people in North America yearly, and patients report visual complaints that can last for months. We have shown that visual performance is altered by TBI, as patients have higher visual noise, and an abnormal contrast sensitivity profile. Higher level visual dysfunctions that could explain TBI patients symptoms have yet to be discovered, although resting-state studies have hinted at a potential cortical synchronicity problem. Here, we explore the consequences of mTBI on cortical activity while patients watch a naturalistic movie, to investigate functional visual disturbances during scene and object representation. Seventeen mTBI patients were shown two 5 minutes clips of an underwater movie in a 3T Siemens MRI (TR=2000ms, Resolution=3mm3), once in 3D and once in 2D. Each control and mTBI cortical activity was fitted to an assigned normal template (obtained by averaging the time-series of the controls in a leave-one out procedure) and the betas and residual error distributions were compared across groups (t-test) to assess scaling differences as well as the variance explained by the template model. We found that mTBIs showed significantly lower betas than controls in the foveal area of the early visual cortex, and in the

sensory-motor cortices, especially for the 3D movie, which could suggest that mTBIs need less transformation to fit the normal template than the normal themselves. However, combined with the residual error differences, our results show that the traumatically injured brain does not fit the normal template adequately, except for the early visual areas. Our result suggest that tightly stimulus-driven cortical activity of the injured brain is constrained to a close-to-normal synchronicity in these regions, a synchronicity that does not propagate to the rest of the brain as it does in the control group. Interestingly, the low amount of scaling necessary to fit the model in the sensory-motor areas is accounted for by particularly high residual error, suggesting that the model simply does not explain these voxels' activity.



Disclosures: T. Ruiz: None. **R. Farivar-Mohseni:** None.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.13/O5

Topic: C.10. Brain Injury and Trauma

Title: Heart rate variability during exercise is a biomarker distinguishing between subjects with post-concussive syndrome following mild traumatic brain injury and healthy volunteers

Authors: *R. C. DUGGAN[1], E. DHAMALA[2], B. E. KOSOFSKY[2]
[1]Weill Cornell Med. Col., New York, NY; [2]Joan and Sanford I Weill Med. Col. of Cornell Univ., New York, NY

Abstract: The autonomic nervous system (ANS) plays a crucial role in maintaining homeostasis throughout the body. In a circulatory process called cerebral autoregulation, the ANS regulates the cardiovascular system and modulates cerebral blood flow in response to changes in metabolic demand within the central nervous system. Research has shown that cerebral autoregulation can be noninvasively assessed by measuring heart rate variability (HRV), the variation in time between heartbeats. In studies comparing athletes to sedentary people of comparable age and weight, athletes were found to have higher HRV, indicating a link between physical health and HRV. Similarly, decreased HRV has been associated with cardiac dysfunction, sepsis, and increased mortality.

Cerebral autoregulation has been shown to be particularly vulnerable to impairment by traumatic brain injury (TBI). This is due to an uncoupling of cerebral blood flow (CBF) with task-induced brain activity following TBI resulting in inducible headaches. This has been proposed as one of the mechanisms underlying some of the persistent symptoms of post-concussive syndrome (PCS). Physical exertion increases demand for CBF, though as a result of impaired autoregulation, the ANS may be unable to couple such demand with appropriate brain perfusion, leading to headaches and other symptoms of PCS. However, aerobic exercise below the threshold for PCS symptom onset has been shown to increase CBF and improve autoregulation, as measured by increased HRV (Clausen, M., *et al.*, 2015). Thus aerobic exercise shows therapeutic promise as a method to reduce PCS symptom severity, for which HRV may serve as a biomarker.

In this study, we enrolled 20 subjects > 14 years of age with mTBI and 20 healthy volunteers, all of whom underwent a graded-exercise test on a recumbent stationary bicycle, using a variation of the Buffalo Concussion Treadmill Test (Leddy, J.J., 2013). We collected measurements of heart rate and HRV with simultaneous ECG recordings and wrist-based beat-to-beat HR monitoring. Correlation analysis and power spectrum analysis of the HRV data found that participants with mTBI who became symptomatic during exercise had significantly reduced HRV compared to healthy control participants, consistent with prior research (Leddy, J. *et al.*, 2017).

These findings have informed our development of an ongoing longitudinal study investigating whether an at-home, remotely-monitored graded exercise regimen can reduce symptom severity in mTBI patients with ongoing persistent PCS symptoms.

Disclosures: R.C. Duggan: None. **E. Dhamala:** None. **B.E. Kosofsky:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); b2d2.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.14/O6

Topic: C.10. Brain Injury and Trauma

Support: N/A

Title: Longitudinal exRNA profiles in patients with aSAH

Authors: *A. COURTRIGHT[1], I. MALENICA[1], A. YERI[1], E. HUTCHINS[1], E. ALSOP[1], B. MEECHOOVET[1], T. BEECROFT[1], E. CARLSON[1], P. NAKAJI[2], M. S. KALANI[3], K. R. VAN KEUREN-JENSEN[1]
[1]Translational Genomics Res. Inst., Phoenix, AZ; [2]Barrows Neurolog. Inst., Phoenix, AZ; [3]Univ. of Virginia, Charlottesville, VA

Abstract: Subarachnoid haemorrhage (aSAH) is life threatening injury that carries significant societal and financial burdens. One of the leading causes of an SAH is an aneurysm, in which a weakness in the wall of a blood vessel ruptures. aSAH patients that survive are at risk of having delayed cerebral ischemia (DCI), vasospasm. This secondary injury results in restricted blood flow that can lead to significant neurological impact or mortality. Predicting this secondary injury cascade could help doctors treat and mitigate the damage. Despite extensive research and efforts to improve the outcomes of patients with SAH, there has been little change in patient outcomes and rates of morbidity or mortality.
A major obstacle to improving the care of this patient population has been the inability to sample affected tissues in patients, and the lack of validated animal models able to reproduce human conditions. Extracellular RNAs (exRNAs), contained within vesicles and RNA-binding proteins, are released from all tissues in the body, including the brain. ExRNAs are released into biofluids, such as cerebrospinal fluid and blood, and provide us with the opportunity to sample brain-related information from patient biofluid samples. They offer a promising means of identification of RNA elements that could predict outcomes and/or explain unclear pathological processes. The recognition of predictors of specific outcomes and early indicators of pathologic processes in patients with brain insults could lead to earlier and better treatments while improving the overall

understanding of the disease biology. Samples were collected from each patient over a ten day period, staring from day 1. CSF was collected from the ventriculostomy that patients have to relieve the pressure on the brain and blood was also collected and spun down to separate out the plasma. Both of these biofluids where sequenced for both microRNA and mRNA to evaluate the the exRNA. We have promising data that exRNAs can be used to stratify the risks for complications in patients with hemorrhagic events of the brain and that these candidate exRNAs can lend new insight into the disease process for the development of novel therapeutic targets.

Disclosures: A. Courtright: None. **I. Malenica:** None. **A. Yeri:** None. **E. Hutchins:** None. **E. Alsop:** None. **B. Meechoovet:** None. **T. Beecroft:** None. **E. Carlson:** None. **P. Nakaji:** None. **M.S. Kalani:** None. **K.R. Van Keuren-Jensen:** None.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.15/O7

Topic: C.10. Brain Injury and Trauma

Support: VA Research Service

Title: Prevalence of homelessness in veterans of operation enduring freedom (OEF) and operation Iraqi freedom (OIF) with traumatic brain injury from blast versus non-blast exposure

Authors: *K. L. PANIZZON[1], A. HARTOONIAN[2], N. CHOOTHAKAN[2], J. RAVAYI[2], B. ARYANFAR[2], R. A. WALLIS[2,3]
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Abstract: OBJECTIVE: To evaluate the prevalence of homelessness in veterans of Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) who have had blast versus non-blast traumatic brain injury (TBI). BACKGROUND: Homelessness has been defined as not having a "fixed, regular or adequate night-time residence", which includes moving frequently between different types of accommodations and staying in homeless shelters and places not meant for human habitation such as vehicles or abandoned buildings. Among the general population, homelessness has been a social, economic, and public health issue in the United States for decades. Homelessness is also a concern because it is associated with a wide range of serious medical problems, such as mental health and substance abuse problems, premature mortality, and frequent hospitalizations. According to the Los Angeles Homeless Services Authority, in Los Angeles County there were a total of 57,794 homeless people, with 8% (4,828) of that being veterans. Within that group, there were 2,102 (4%) veterans that were chronically homeless. One of the signature injuries for veterans deployed in the OEF and OIF conflicts is

that of TBI, particularly blast TBI. In addition, homelessness is a relatively frequent issue particularly for patients with disabilities, such as TBI. We were interested in determining the prevalence of homelessness in veterans with TBI from blast versus non-blast exposure. METHODS: We conducted a pilot, retrospective chart review study of patients with TBI seen at the Poly-Trauma Clinic of the VA Greater Los Angeles Healthcare System. We collected data regarding blast versus non-blast TBI in OEF/OIF veterans. In addition, we collected data regarding homelessness in this patient population. RESULTS: A total of 737 charts were reviewed. Of these, 300 were identified as OEF/OIF subjects with a confirmed diagnosis of TBI. The racial/ethnic background of these subjects was 42.0 % Caucasian, 12.7% African-American, 27.0% Hispanic-American, 10.0% Asian-American, and 8.3% Other. We found that a mean 59.3% ± 4.3 (n = 178) of subjects had suffered blast-TBI and 40.7% ± 3.9 (n = 122) had non-blast TBI. The mean age of subjects with blast TBI was 24.5 ± 1.3 years, while those with non-blast TBI was 25.5 ± 1.4 years. The prevalence of homelessness following TBI in this patient group was 21.3% ± 3.4 (n=64). Homelessness was found in a mean of 19.7% ± 3.2 (n = 35) subjects with blast TBI, and 23.8% ± 3.7 (n = 29) in those with non-blast TBI. CONCLUSION: In this population of OEF/OIF veterans with TBI, homelessness was reported at a relatively high frequency following both blast and non-blast TBI.

Disclosures: A. Hartoonian: None. **N. Choothakan:** None. **J. Ravayi:** None. **B. Aryanfar:** None. **R.A. Wallis:** None.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.16/O8

Topic: C.10. Brain Injury and Trauma

Title: Altered brain functional connectivity after chemotherapy in men with gastric cancer

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Abstract: Objective: In cancer patients, chemotherapy is essential for increasing survival rate of cancer. However, cognitive impairments such as executive functions, verbal memory, and motor function have been reported after chemotherapy. We investigated the effect of chemotherapy on gray matter and neural network features of gastric cancer patients.
Methods: 19 gastric cancer patients with adjuvant chemotherapy(C+), 14 gastric patients without adjuvant chemotherapy(C-), and 11 healthy controls(HC) were evaluated. We performed neuropsychological studies, voxel-based morphometric analysis, and resting-state functional

magnetic resonance imaging analysis twice at pre- and post-chemotherapy. Intrinsic resting state networks were examined with seed-based analysis method and correlation between brain connectivity patterns and results of neuropsychological studies were analyzed.
Results: Results of neuropsychological studies showed decrease in executive function after adjuvant chemotherapy in C+ group. The results of the analysis indicate decrease in functional connectivity in the default mode network at 3 months after chemotherapy. However, chemotherapy did not cause structural change.
Conclusion: Adjuvant chemotherapy did not cause structural changes in the brain, but the results showed decrease in default mode connectivity, which is associated with decrease in the executive function. Our results suggest that chemotherapy for gastric cancer patients has altered neural networks for executive control.

Disclosures: J. Ahn: None. **Y. Jung:** None.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.17/O9

Topic: C.10. Brain Injury and Trauma

Support: Contract to BrainScope Company, Inc. from US Army Medical Research and Material Command, #W911QY-14-C- 0098

Title: Classifying concussion in university athletes using diffusion tensor imaging

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Abstract: Sports-related concussions, or mild traumatic brain injuries (mTBI), are extremely common and a major concern for athletes, athletic trainers, and coaches. Though quick concussion assessments can be conducted on the field to determine ability to return to play, there is a lack of consensus on the diagnostic criteria for mTBI. Due to its heterogeneous course, there is a need for objective diagnostic measures of concussion that are predictive of outcome so that individuals can receive appropriate care and treatment. While mTBI results in diffuse axonal injury, this is difficult to detect using conventional structural magnetic resonance imaging (MRI). However, the results may be able to be detected using diffusion tensor imaging (DTI), a measure of structural connectivity. The aim of this study is to generate a classifier using MRI and DTI to identify concussion. Thirty-four university athletes (15 diagnosed with concussion, 19 controls)

received structural MRI and DTI, the concussed athletes within 72 hours of injury. Diffusion weighted images were motion, eddy, and distortion corrected using TORTOISE. Anatomical images were reconstructed using Freesurfer. Eighteen major white-matter pathways were automatically reconstructed using global probabilistic tractography constrained by anatomical priors (TRACULA). Logistic regression identified nine contributive tracts for classifying concussion using fractional anisotropy (bilateral anterior thalamic radiations, bilateral cingulum - angular bundles, left cingulate gyrus, left corticospinal tract, right inferior longitudinal fasciculus, and bilateral superior longitudinal fasciculi) and eight contributive tracts using mean diffusivity (bilateral anterior thalamic radiations, bilateral cingulum - angular bundles, right cingulate gyrus, left corticospinal tract, right inferior longitudinal fasciculus, and left superior longitudinal fasciculus). Two types of classifiers (one linear, one nonlinear) were trained using 32 randomly sampled athletes and tested with the remaining 2, with 1000 iterations each. Logistic regression (22 degrees of freedom) achieved an overall accuracy of 72% for MD and 71% for FA. A nonlinear support vector machine classifier achieved an overall accuracy of 75% for MD and 74% for FA. These results indicate that DTI measures may be useful for identifying mTBI, though they may be most reliable in conjunction with other behavioral and MRI measures of injury. As concussion is a complex injury, clinicians will increasingly benefit from integrating biological, electrophysiological, and behavioral measures of injury in diagnosis.

Disclosures: M. Ly: None. **S. Scarneo:** 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.; Brainscope Company, Inc. contracted from US Army Medical and Material Command. **A. Lepley:** None. **K. Coleman:** 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.; Brainscope Company, Inc. contracted from US Army Medical and Material Command. **C. Chen:** None. **D.J. Casa:** 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.; Brainscope Company, Inc. contracted from US Army Medical and Material Command.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.18/O10

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R21DC009900

Title: Abnormal cerebral hemodynamic responses to postural change during acute concussion

Authors: *J. LIU[1], M. FAVRE[1,2], A. KNOX[1,2], K. BREWER[1,2], M. FALVO[1,2], J. M. SERRADOR[1,2]
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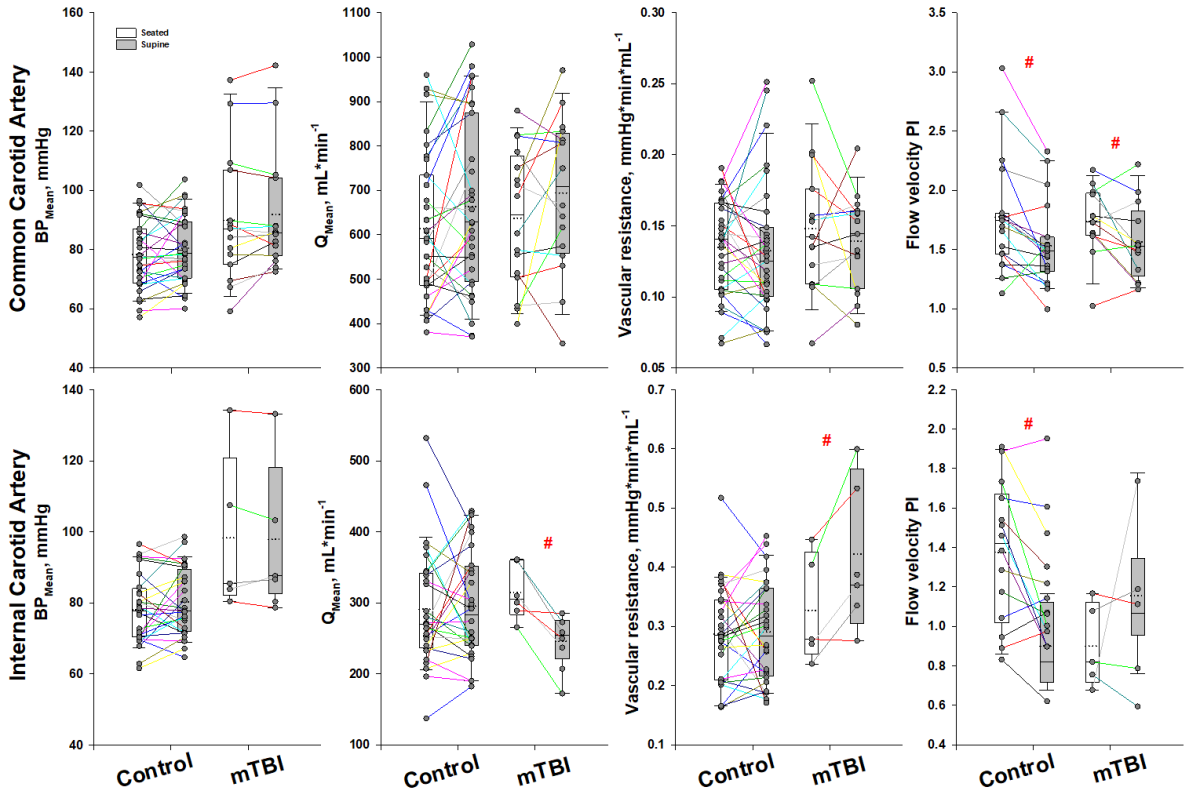
Abstract: Objectives: To determine the impact of concussion (i.e. mild traumatic brain injury, mTBI) on cerebral hemodynamic responses to postural change in its acute phase (<25 hrs). **Methods:** Sixteen rugby players (8 females) with acute (10~1482 min, median = 52 min) concussion and 37 non-concussed teammates (10 females) (as controls) participated in the study. The diameter and blood flow velocity were continuously recorded for 1 min at common (CCA) and/or internal carotid arteries (ICA) using duplex ultrasonography, along with a beat-by-beat blood pressure (BP) recording. The mean volumetric flow (Q_{Mean}) was calculated offline. Vascular resistance (VR) was derived as BP_{Mean}/Q_{Mean}; and the pulsatility of BP and flow velocity was assessed by the pulsatility index [PI = (Systolic - Diastolic)/Mean]. All data were collected on the field in both seated and supine positions. **Results:** The BP_{Mean} in concussed players was about 13 mmHg (6~20 mmHg for 95%CI) higher than controls, but no posture-related changes were observed in BP_{Mean} and BP pulsatility. At CCA, no significant change in VR was found from seated to supine postures, but a slightly increased Q_{Mean} and a decreased PI were observed in the absence of between-group differences regarding these responses. However at ICA, only controls showed responses similar to CCA; While in in concussed players, the Q_{Mean} was decreased (314±39 to 246±40 $mL \cdot min^{-1}$, P=0.006) and VR was increased (0.327±0.092 to 0.423±0.137 $mmHg \cdot min \cdot mL^{-1}$, P=0.038) with no significant change in PI from seated to supine postures. The repeated-measures ANOVA confirmed significant Position×Group interactions in these ICA hemodynamic responses. **Conclusions:** Following acute concussion, abnormal hemodynamic (Q_{Mean}, VR and PI) responses to postural change are likely found at the cerebral vessel (i.e. ICA), but not the peripheral vessel (considering the $Q_{ECA} \approx Q_{CCA} - Q_{ICA}$; ECA = external carotid artery), indicating an impaired postural CBF regulation. These metrics are related to the CBF regulation, and may provide a point-of-care assessment of important clinical relevance.



Disclosures: J. Liu: None. **M. Favre:** None. **A. Knox:** None. **K. Brewer:** None. **M. Falvo:** None. **J.M. Serrador:** None.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.19/O11

Topic: C.10. Brain Injury and Trauma

Support: MOST104-2923-B-038-001-MY3

Title: Post-traumatic epilepsy in childhood increases the risk to develop attention deficit hyperactivity disorder: A 9-year follow-up study in Taiwan

Authors: *J. WANG[1], L.-Y. YANG[2], W.-C. LO[3], C.-C. HUANG[4]
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Abstract: Traumatic brain injury (TBI) in children (≤12 yr) may cause neurobehavioral developmental disorder. Our previous study has demonstrated that childhood TBI is associated with a greater likelihood of developing attention-deficit/hyperactivity disorder (ADHD). ADHD is also commonly observed in children with epilepsy. The aim of this study was to determine whether post-traumatic epilepsy (PTE) is an independent risk factor for the increased risk of ADHD during 9-year follow-up period. Using the National Health Insurance Research Database of Taiwan population, we included 488 newly diagnosed children with PTE (aged ≤12 y) from 2001 to 2002 . We randomly selected the TBI patients matched with sex, age, and registry year for each PTE patient (n=4,880). Cox proportional hazard regressions were performed to analyze the 9-year ADHD-free survival rate between these two cohorts. A total of 19 PTE patients (4.45%) developed ADHD during the 9-year follow-up period and 39 patients (1.83%) from the control cohort. TBI patients were found to be 2.03-fold ($p<0.001$) more likely to develop ADHD during the follow-up period than the control cohort. In conclusion, our data indicate an increased risk of ADHD in childhood PTE patients. This finding suggests that children who had suffered PTE were more susceptible to delayed ADHD. Clinicians and family members should be alert to ADHD in children with PTE history. Intensive monitoring or early treatment for delayed ADHD would be needed for TBI children with PTE.

Disclosures: J. Wang: None. **L. Yang:** None. **W. Lo:** None. **C. Huang:** None.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.21/O13

Topic: C.10. Brain Injury and Trauma

Support: National Institute on Alcohol Abuse and Alcoholism R01 AA-016780
South African National Research Foundation
Lycaki/Young Fund, State of Michigan
NIAAA Collaborative Initiative on FASD U01 AA014790

Title: The morphology of the intraparietal sulcus of children prenatally exposed to alcohol and its role on number processing

Authors: *M. GREEFF[1,2], E. M. MEINTJES[2], S. W. JACOBSON[4,2,3], C. D. MOLTENO[3], J. L. JACOBSON[4,2,3], F. L. WARTON[2], C. M. R. WARTON[2]
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Abstract: Introduction
The intraparietal sulcus (IPS) plays a critical role in number processing.(Dehaene *et al.*, 2003;

Ashkenazi *et al.*, 2008) a domain particularly sensitive to prenatal alcohol exposure (PAE).(Meintjes *et al.*, 2010; J. L. Jacobson *et al.*, 2011; Woods *et al.*, 2015) Although smaller parietal lobes have been reported in PAE(Archibald *et al.*, 2001) , the lateral and medial walls of the IPS have not been studied separately. Using "gold standard" manual tracing, we investigated whether PAE is related to morphological changes in IPS and whether these changes are related to number processing. This study was conducted in Cape Town, South Africa, where heavy prenatal alcohol use and fetal alcohol spectrum disorders (FASD)are prevalent in the Cape Coloured community.

Methods

52 right-handed children recruited from our cross-sectional (*n*=32)(Jacobson *et al.*, 2008; Meintjes *et al.*, 2010) and longitudinal (*n*=20)(S. W. Jacobson *et al.*, 2011) cohorts were scanned at 9-14 yr with a 3T Siemens Allegra MRI. The children were diagnosed by expert FASD dysmorphologists as fetal alcohol syndrome (FAS)/partial FAS (FAS/PFAS;*n*=15), non-syndromal heavily exposed (HE;*n*=13), or controls (*n*=24).

Manual tracing (coronal sections) involved tracing (1) all medial walls of the IPS (MIPS) and (2) the entire sulcus—medial and lateral (LIPS) walls. LIPS volume was calculated by subtracting MIPS from total sulcal volume. The occipital portion was excluded.

Results

Left LIPS volumes were smaller in children with FAS/PFAS compared to controls (F=4.854, p=0.012), effects that remained a trend after adjustment for TIV, sequence and sex (F=2.645, p=0.082). Larger left and right MIPS were related to better WISC Arithmetic Scaled scores (r=0.346, p=0.012; r=0.304, p=0.029, respectively), and larger left LIPS to poorer proximity judgment (PJ; r=-0.250; p=0.098). Relations to MIPS volumes survived adjustment for alcohol exposure, sex and age, while the association of LIPS with PJ became stronger (ß=-0.337, p=0.053).

Discussion

LIPS volume is smaller in FAS/PFAS than controls. While larger MIPS was associated with better WISC Arithmetic, smaller LIPS was associated with better PJ, which is surprising since PAE is related to lower levels of activation in regions of the IPS involved in number processing.(Woods *et al.*, 2015) Additional research is needed to clarify why a larger volume of the LIPS might be related to poorer number processing performance.

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Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.22/O14

Topic: C.10. Brain Injury and Trauma

Title: Changes in functional connectivity are associated with one season of head-to-ball exposure in male collegiate soccer athletes

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Abstract: Objectives: Recovery following mild traumatic brain injury (mTBI) depends on characteristics of both the injury and the injured, and the relationships between these characteristics are not well understood. Not all head impacts are immediately symptomatic or clinically recognized as mTBI. Studying the effects of these impacts may be informative. Autonomic dysregulation is thought to underlie many of the multi-dimensional symptoms following mTBI and may derive from altered connectivity in the brain central autonomic network (CAN). We sought to establish a relationship between non-symptomatic head-to-ball impacts (HBIs) and CAN connectivity in Male NCAA Division I soccer athletes.
Methods: 21 male NCAA Division I athletes (age: 20.2±1.5 years) served as participants.11 soccer athletes were monitored by athletic training staff throughout one season for HBIs. 3 cross-country athletes and 7 golfers served as controls. All participants underwent a resting-state fMRI pre- and post-season. 20 ROIs were selected based on regions previously implicated in control and modulation of basal autonomic function. Graph theoretical analysis was used to probe changes in network architecture among nodes (regions) with edge weights above threshold (|cost| >.3). Connectivity maps thresholded by seed and connection ($p < .05$ uncorrected) were examined to clarify changes in network connectivity. Contrasts were performed to test for changes across the season that were explained by individual differences in HBI exposure ($p < .05$ uncorrected).
Results: Within the 20 node network, HBIs accounted for reduced degree centrality of the left and right insular cortex and right putamen [$t(18)>2.36$, $p<.02$], increased degree and betweeness centrality in the left anterior and right posterior parahippocampal gyri [$t(18)<-2.22$, $p<.03$], and increased betweeness centrality in the anterior cingulate cortex [$t(18)=-3.11$, $p=.006$]. HBIs were associated with reduced functional connectivity among the anterior cingulate cortex [$F(5,14)=5.08$, $p=.0073$)], right hippocampus, left putamen, and left insular cortex [$t(18>2.23$, $p<.04$].
Conclusion: A contemporary hypothesis is that chronic exposure to repeated non-symptomatic head impacts has neurological effects. We demonstrate that soccer athletes sustaining the greatest number of HBIs also experienced the greatest altered connectivity among regions associated with autonomic function. Future work should consider the importance of impact magnitude and correlate network changes with peripheral measures of autonomic function at rest and in response to standardized stressors.

Disclosures: D.C. Monroe: None. **D.B. Keator:** None. **R.S. Blumenfeld:** None. **J.W. Hicks:** None. **S.L. Small:** None.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.23/O15

Topic: C.10. Brain Injury and Trauma

Support: Canada Excellence Research Chair: 215063

Title: Identifying electrophysiological components of covert awareness in patients with disorders of consciousness

Authors: *G. LAFORGE, A. M. OWEN, B. STOJANOSKI
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Abstract: A small but significant number of those who survive acute brain injury will transition into a state of unconscious wakefulness known as the vegetative state (VS). Patients in a VS remain behaviourally non-responsive during clinical examination and, despite exhibiting clear evidence of wakefulness, do not appear to possess conscious awareness. However, recent neuroimaging research has identified a subpopulation of these patients who reliably produce neural markers of "covert" awareness. Indeed, imagined motor-imagery paradigms have identified covert, rather than behavioural command-following ability in nearly 20% of patients tested and naturalistic movie-watching tasks in fMRI have been used to index higher-order "executive" processing, a proxy of awareness, in patients who appear to be entirely unconscious. Here, we demonstrate the utility of bedside EEG recorded during a naturalistic audio paradigm to detect higher-order stimulus processing in VS patients and others diagnosed with so-called disorders of consciousness (DoC). We used a correlated components analysis to calculate global and time-resolved inter-subject neural synchronization, an established measure of stimulus engagement, in healthy volunteers and patients with DoC while they listened to a suspenseful auditory narrative. At the group level, no significant differences were observed in global inter-subject correlations (ISC) between the intact narrative and a scrambled narrative condition in healthy controls, $t(14) = 1.22$, $p > .05$. However, we did find significantly more time points in the audio with significant ISC for the intact audio condition than the scrambled condition, $\chi^2(1, N = 152) = 4.52$, $p < .05$, that corresponded to suspenseful moments in the clip. As a group, patients with DoC had fewer time windows with significant ISC during the intact audio condition than either the intact, $\chi^2(1, N = 152) = 21.35$, $p < .0001$, or scrambled audio conditions $\chi^2(1, N = 152) = 7.35$, $p < .01$, in healthy controls. We then compared the neural response to the clip from individual DoC patients to healthy controls to quantify the similarity of their executive processing. We found that the correlated component topography and the degree of patient-to-group ISC could differentiate a demonstrably aware locked-in patient from those with DoC. Crucially, we found evidence of preserved narrative processing in one VS patient who has

previously demonstrated some residual awareness in fMRI. Together, these results suggest that EEG recorded during naturalistic auditory stimulation may provide a sensitive, low-cost, and portable means to assess the neural correlates of covert awareness in patients with DoC.

Disclosures: G. Laforge: None. **A.M. Owen:** None. **B. Stojanoski:** None.

Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

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Program #/Poster #: 566.24/O16

Topic: C.10. Brain Injury and Trauma

Support: VA Merit Grant I01-CX000499
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Naval Medical Logistics Grant N62645-11-C-4037

Title: Functional deficits in combat-related mild traumatic brain injury revealed by MEG working memory N-back task

Authors: *M. HUANG[1], S. L. NICHOLS[3], A. ROBB-SWAN[1], A. ANGELES-QUINTO[1], D. L. HARRINGTON[1], A. DRAKE[8], C. W. HUANG[4], T. SONG[5], M. DIWAKAR[9], V. B. RISBROUGH[6], S. MATTHEWS[10], R. CLIFFORD[2], C.-K. CHENG[7], J. W. HUANG[11], K. A. YURGIL[12], Z. JI[1], I. R. LERMAN[13], R. R. LEE[1], D. G. BAKER[2]
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Abstract: Introduction: Combat-related mild traumatic brain injury (mTBI) is a leading cause of sustained cognitive impairment in military service members and Veterans. However, the mechanism of persistent cognitive deficits including working memory (WM) dysfunction is not fully understood in mTBI. Few studies of WM deficits in mTBI have taken advantage of the temporal and frequency resolution afforded by electromagnetic measurements. Using magnetoencephalography (MEG) and an N-back WM task, we investigated functional

abnormalities in combat-related mTBI. Method: Study participants included 25 symptomatic active-duty service members or Veterans with combat-related mTBI and 20 healthy controls with similar combat experiences. MEG source-magnitude images were obtained for alpha (8-12 Hz), beta (15-30 Hz), gamma (30-90 Hz), and low-frequency (1-7 Hz) bands. High resolution MEG source imaging technique, Fast-VESTAL, was used in creating MEG source magnitude images for the responses of N-back task. For each frequency band, a voxel-wise repeated measure ANOVA was performed to create F-value maps for examining the group differences (i.e., mTBI versus control groups), with 1-, 2-, and 3-back conditions treated as repeated measures. Family-wise error across voxels was corrected using cluster analysis for the F-value maps at a corrected $p<0.01$ level. Result: Compared with healthy combat controls, mTBI participants showed increased MEG signals (i.e., hyper-activations) across frequency bands in rostral prefrontal cortex (rPFC) including frontal pole (FP), ventromedial prefrontal cortex, orbitofrontal cortex (OFC), and anterior dorsolateral prefrontal cortex (dlPFC), but decreased MEG signals (i.e., hypo-activations) in posterior dlPFC and anterior cingulate cortex. Hyper-activations in FP, OFC, and anterior dlPFC were associated with slower reaction times. MEG hyper-activations from the lateral FP area were also associated with worse performance on neuropsychological tests that measure processing speed and executive functions (i.e., letter sequencing, verbal fluency, and digit symbol coding). These findings suggested that aberrant neuronal activity in combat-related mTBI, especially in PFC, was functionally significant, relating to individual differences in cognitive proficiency. Conclusion: Aberrant activations during WM were revealed for the first time in combat-related mTBI using MEG source magnitude imaging. Among all the abnormalities in the PFC, the profound hyper-activations in rPFC (mainly the FP, but also vmPFC, and OFC) revealed by the present study suggests that the aberrant rPFC is an important feature in combat-related mTBI.

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Poster

566. Brain Injury and Trauma: Human Studies II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 566.25/P1

Topic: C.10. Brain Injury and Trauma

Support: USAMRMC grant W81XWH-13-2-0095
VA RR&D grant I01RX002172-01
Mid-Atlantic Mental Illness Research, Education and Clinical Center

Salisbury VA Health Care System

Title: Influence of primary blast exposure on development of PTSD following deployment

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Abstract: Service members are frequently exposed to blasts or explosions during deployment. These events may or may not be accompanied by acute symptoms of mild traumatic brain injury (mTBI). The long-term effects of primary blast exposure on veterans of the wars in Iraq and Afghanistan are currently unknown. As part of this study, we developed a structured interview that evaluates lifetime blast exposures and connects blast events to mTBI events. Posttraumatic stress disorder (PTSD) diagnosis was determined using the Clinician Administered PTSD Scale – 5 (CAPS-5). The Salisbury VAHCS IRB approved this study to ensure that the privacy of research subjects was maintained and their welfare protected. Participants included 165 combat-exposed post-deployment veterans who passed performance and symptom validity measures. Chi-Square analyses were conducted to analyze differences in categorical variables. ANOVA were used to analyze differences in continuous variables. Logistic regression was used to evaluate the contributions of variables to PTSD diagnosis or recovery. Most blast exposure events (71%) occurred during combat and relatively few (19%) were associated with acute symptoms indicative of mTBI. Primary blast exposure was associated with higher rates of both current ($p < .026$, Cramer's V=0.173) and lifetime ($p < .001$, Cramer's V=.296) PTSD. Deployment mTBI was associated with higher rates of lifetime PTSD ($p < .001$, Cramer's V=.276). When participants with deployment mTBI were removed from the analysis, blast exposure remained associated with increased rates of lifetime PTSD ($p < .001$, Cramer's V=0.378). In addition, higher severity of blast exposure remained associated with higher rates of both lifetime ($p < .032$, Cramer's V=.227) and current ($p < .017$, Cramer's V=.252) PTSD. Logistic regression was used to predict lifetime and current PTSD diagnosis from deployment mTBI and blast exposure. The model did not significantly predict current PTSD diagnosis, but significantly predicted lifetime PTSD diagnosis. An interaction was observed between blast exposure and deployment TBI ($p < .053$) such that experience of either or both increased the likelihood of a lifetime PTSD diagnosis. For the model including higher severity blast exposure, only blast exposure significantly predicted either current or lifetime PTSD. These results indicate that primary blast exposure increases risk for developing PTSD even when the blast exposure was not associated with acute TBI symptoms.

Disclosures: K.H. Taber: None. **J.A. Rowland:** None. **E. Epstein:** None. **S.L. Martindale:** None. **H.M. Miskey:** None. **R.D. Shura:** None.

Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.01/P2

Topic: C.10. Brain Injury and Trauma

Support: NRF Grant NRF-2017M3C7A1028945

Title: Upregulation of lysosome by EGF-triggered endocytosis or trehalose attenuated zinc neurotoxicity via the increase of buffering capacity for intracellular free zinc

Authors: *J.-W. EOM, Y.-H. KIM
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Abstract: Zinc is an essential metal ion for almost all of the organisms, and maintaining intracellular free zinc homeostasis is vitally required. To keep a balance of cytoplasmic free zinc, excess zinc is sequestrated into intracellular organelles, such as lysosome and mitochondria, as well as binds to metal-binding proteins, metallothionein. However, too much increase of intracellular free zinc provoked lysosome membrane permeabilization (LMP) and then cathepsin-mediated neuronal death.
Therefore, we tried to upregulate the number of lysosomes to reduce zinc neurotoxicity by augmentation the buffering capacity for intracellular free zinc through epidermal growth factor (EGF)-triggered endocytosis or pre-treatment of trehalose, known as a disaccharide, which increases lysosome biogenesis via mTOR-independent manner.
First, we examined whether EGF-induced endocytosis elevated the number of lysosome in mouse cortical cultures. Within 30 min after EGF treatment, LAMP-1/LAMP-2, as well as a mature form of cathepsin D, were increased in Western blot. Secondly, we observed that pre-exposure to EGF significantly attenuated zinc neurotoxicity, suggesting the possibility that increase of lysosomes by EGF-triggered endocytosis expand the handling capacity for intracellular free zinc. So, we next tried to see that chemical inhibitions of EGF-induced endocytosis and endosomal trafficking pathway affect zinc-induced cell death. Supporting the critical action of endocytosis and endosomal trafficking pathway in attenuation of zinc neurotoxicity by EGF, chlorpromazine, the chemical clathrin-dependent endocytosis blocker, or methyl-β-cyclodextrin, the chemical caveolin-dependent endocytosis blocker, almost completely reversed EGF-mediated neuroprotection against zinc toxicity. We also observed that the specific tyrosine kinase inhibitor of EGF, compound 56 or AG1478, blocked EGF-mediated neuroprotection against zinc toxicity as well as EGFR endocytosis after EGF treatment.
As another strategy for upregulation of lysosome, we treated Trehalose. As expected, Trehalose significantly reduced zinc neurotoxicity and noticeably increased LAMP-1 and TFEB in Western blot. Taken together, quantitative regulations of lysosome through EGFR endocytosis or

exposure of trehalose play a critical role in zinc homeostasis, and it ultimately could be a therapeutic target for acute brain injury.

Disclosures: J. Eom: None. **Y. Kim:** None.

Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.02/P3

Topic: C.10. Brain Injury and Trauma

Support: NINDS 5R01 NS083405
NINDS 5R01 NS084857
NINDS F30 NS096876

Title: Neuroprotective strategies following experimental traumatic brain injury: Inhibition of mitochondrial permeability transition, lipid peroxidation-derived neurotoxic aldehyde scavenging and monoamine oxidase inhibition

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Abstract: In the US, there are over 5 million people suffering from a traumatic brain injury (TBI)-related disability. Unfortunately, due to the complex pathophysiology that occurs following injury, there are no neuroprotective FDA-approved pharmacotherapies for TBI. Mitochondrial dysfunction and lipid peroxidation, including generation of lipid peroxidation-derived neurotoxic aldehydes, are central to the TBI secondary injury, and therefore, make promising therapeutic targets for prevention of neuronal death and dysfunction following TBI. The purpose of these studies was threefold. 1) Evaluate the neuroprotective effect of cyclosporine A (CsA), on synaptic and non-synaptic mitochondria. Mitochondria are heterogeneous, consisting of synaptic and non-synaptic mitochondria, which have distinct properties. Our results indicate that compared to non-synaptic mitochondria, synaptic mitochondria sustain greater damage 24h following severe controlled cortical impact injury in young male rats, and are protected to a greater degree by CsA, an FDA-approved immunosuppressant, capable of inhibiting mitochondrial permeability transition. 2) Evaluate the neuroprotective effects of a 72h subcutaneous continuous infusion of CsA combined with phenelzine (PZ), an FDA-approved monoamine oxidase inhibitor (MAOI) class anti-depressant capable of scavenging neurotoxic aldehydes compared to monotherapy. Our results indicate that 72h post-TBI (the peak of mitochondrial dysfunction and lipid peroxidation) that individually

CsA or PZ attenuate lipid peroxidation-derived neurotoxic aldehyde formation, PZ maintains mitochondrial respiratory control ratio and cytoskeletal integrity, but together, PZ + CsA, do not maintain neuroprotective effects. 3) Although neurotoxic aldehyde scavenging, a PZ mechanism of action, has proven neuroprotective properties, the effect MAO inhibition has on pathology following TBI is unknown. Therefore, the ability of PZ (aldehyde scavenger, MAOI), hydralazine (HZ, aldehyde scavenger, non-MAOI) and pargyline (PG, non-aldehyde scavenger, MAOI) to improve learning and memory 3 – 7 days post-injury was evaluated using Morris water maze (MWM). Although neither PZ, HZ, nor PG were able to improve retention memory, PZ animals did not learn during the acquisition phase and lost more weight compared to other groups, potentially due to high levels of norepinephrine or serotonin. In fact, when HPLC was utilized to evaluate monoamine and metabolite levels of our PZ, HZ, PG dosing paradigm in naïve rats, PZ showed a significant increase in norepinephrine and serotonin compared to other groups.

Disclosures: I.N. Singh: None. **J.A. Dunkerson:** None. **J.A. Wang:** None. **P.F. Huettl:** None. **R.L. Hill:** None. **R. Smith:** None. **E.D. Hall:** None.

Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.03/P4

Topic: C.10. Brain Injury and Trauma

Support: Massey TBI Grand Challenge Award
American Epilepsy Society Junior Investigator Award
NIH BRAIN Initiative Grant R03MH111316

Title: Biophysical modeling reveals efficacious drug combinations for improved neuroprotection immediately after traumatic brain injury

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Abstract: Traumatic brain injury (TBI) is a long-standing public health concern in the United States with an estimated 5.3 million people currently living with a TBI-related injury. Depending on the severity of the injury, TBI can lead to a wide range of functional deficits. The altered ionic milieu, synaptic changes, and network dynamics following TBI create excitotoxicity resulting in cell death and changes in brain volume. Currently, there are no pharmacological drugs that offer complete neuroprotection immediately after TBI, and numerous clinical trials have failed to find a consistently efficacious drug. Antiepileptic drugs ($GABA_A$ agonists) that are often used to treat epilepsy are not effective in reversing the excitotoxicity following TBI. Using computational

modeling of a neocortical regular spiking neuron, we hypothesize that this could be due to the reversal of the chloride gradient caused by the upregulation of Na-K-Cl transporter (NKCC1) immediately following the injury. Our modeling results suggest that restoring the altered chloride gradient by blocking the NKCC1 transporter would release the neuron from depolarization block and therefore facilitate the neuroprotection provided by $GABA_A$ agonists. We subsequently tested the neuroprotective efficacy of a $GABA_A$ agonist in combination with a NKCC1 transporter blocker, using a controlled cortical impact (CCI) model of TBI in rats. This combination of drugs led to significantly reduced cortical damage compared to controls. Thus, biophysically-principled drug design might offer a rational way to identify ideal drug combinations for the treatment of TBI.

Disclosures: S. Sudhakar: None. **T. Choi:** None. **V. Hetrick:** None. **O. Ahmed:** None.

Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.04/P5

Topic: C.10. Brain Injury and Trauma

Support: NIH F31 NS101741
NIH R21 NS098009
DOD ERP W81XWH-17-1-0531

Title: Glycolytic inhibition with 2-deoxyglucose preserves inhibitory cortical network function following traumatic brain injury

Authors: *J. B. KOENIG[1], D. CANTU[1], C. S. LOW[2], D. KONG[1], C. G. DULLA[1]
[1]Neurosci., Tufts Univ. Sch. of Med., Boston, MA; [2]Tufts Univ., Boston, MA

Abstract: Following a traumatic brain injury (TBI), post-traumatic epilepsy (PTE) can occur. Chronic seizures can be a significant cause of disability for TBI patients, especially when the seizures are refractory to currently available anticonvulsant therapies. The latent period between a TBI and the onset of PTE provides a therapeutic opportunity to prevent the pathophysiological changes that result in a seizure-prone network. Post-traumatic epileptogenesis may be explained by a loss of network inhibition resulting in an excitatory/inhibitory imbalance of the network. By targeting the metabolic changes following TBI, namely acute increases in glycolysis, we may be able to prevent the downstream loss of inhibitory interneurons and resulting hyperexcitability. We hypothesize that 2-deoxyglucose (2DG), a glucose analog that competitively inhibits glycolysis, preserves cortical network function following TBI.
To study TBI, we have used a model known as controlled cortical impact (CCI) in mice. Using this approach we found cortical network hyperexcitability, increased glutamatergic signaling,

and a loss of parvalbumin and somatostatin inhibitory interneurons following injury. To examine the effects of glycolytic inhibition on post-TBI pathophysiology, we treated animals with vehicle or 2DG through systemic intraperitoneal injection daily for 7 days after CCI. We show that *in vivo* 2DG treatment after CCI prevented the development of epileptiform activity following injury and preserved the input-output relationship observed in healthy cortex. We also show that 2DG attenuated losses of parvalbumin expression in cortical inhibitory interneurons adjacent to the lesion site and rescued changes in excitatory and inhibitory postsynaptic currents. The protective effects of *in vivo* 2DG are independent of changes in CCI lesion volume or systemic metabolic effects (such as change in body temperature). Finally, we propose that glycolytic inhibition with 2DG may act through differential effects on the excitability of different neuronal subtypes, and are currently exploring potential metabolic differences between excitatory and inhibitory neurons.
Our research supports a role for glycolytic inhibition in the preservation of inhibitory network function and the prevention of epileptogenesis following TBI. Thus, 2DG has significant potential as a translational intervention for patients after TBI. Finally, our work reveals a possible mechanism of action for 2DG -- a novel cell type-specific coupling of metabolism to neuronal excitability.

Disclosures: J.B. Koenig: None. **D. Cantu:** None. **C.S. Low:** None. **D. Kong:** None. **C.G. Dulla:** None.

Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.05/P6

Topic: C.10. Brain Injury and Trauma

Support: NIH grant MH063663
NIH grant MH087001
Azevan Pharmaceuticals

Title: Effects of an orally active, highly selective, arginine vasopressin V1a receptor antagonist on cerebral edema after moderate traumatic brain injury

Authors: *T. R. MORRISON[1], N. G. SIMON[2,3], S.-F. LU[2], Z. CHENG[2], C. F. FERRIS[1,4], P. P. KULKARNI[1,4]
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Abstract: In the US, TBI is a contributing factor to a third of injury-related deaths, one of the leading causes of death and disability in persons under 35 and over 65, and carries an annual economic burden of ~$86 billion. Arginine vasopressin (AVP) is a chemical signal that influences brain water permeability and a major driver of the pathophysiology of brain edema after TBI. These cerebrovascular effects are mediated by interactions between AVP receptor signaling and aquaporin-4 channels. Previously, we showed that treatment with AVN576, a highly selective and orally active arginine AVP V1a receptor antagonist, for 5 days beginning 24 hrs after moderate head injury significantly reduced edematous enlargement of the lateral ventricles, and eliminated cognitive deficits following moderate TBI in rats.
In this experiment, we tested how shortening the time interval between injury and treatment altered the efficacy of AVN576 after a moderate TBI. We also examined whether AVN576 treatment affected biomarkers of injury severity, and if this change correlated with ventricular volume and/or hippocampal functional connectivity. A moderate TBI was produced using the momentum exchange model in male SD rats. Experimental animals were treated 6 hrs later with AVN576 and thereafter twice daily for 5 days. SHAM and concussed+vehicle treated rats served as controls. Prior to TBI, animals were pre-scanned for baseline functional connectivity and ventricular volume (T2 relaxivity), and then scanned again 5 hrs, 24 hrs, and 7 days after injury. Blood samples were taken in parallel with scans and evaluated for injury severity markers. All images were registered to a 3D MRI rat atlas to assess differences between groups across 171 brain areas. Scans showed that edema persisted 7 days after injury in untreated animals, and that AVN576 significantly reduced edema. Moreover, we also report changes in connectivity, and alterations in the relationship between biomarkers of injury severity and scanning parameters. These results, in accord with our previous findings, suggest that V1a antagonism may represent a novel approach for reducing or eliminating cerebral edema that significantly affects mortality and morbidity following moderate/severe TBI.

Disclosures: N.G. Simon: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Simon holds equity in Azevan Pharmaceuticals, Inc. F. Consulting Fees (e.g., advisory boards); Paid consultant to Azevan Pharmaceuticals, Inc. **S. Lu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Lu holds equity in Azevan Pharmaceuticals, Inc.. **Z. Cheng:** None. **C.F. Ferris:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ferris holds equity in Azevan Pharmaceuticals, Inc. **P.P. Kulkarni:** None.

Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.06/P7

Topic: C.10. Brain Injury and Trauma

Title: Fibroblast growth factor 21 enhances the therapeutic potential of mesenchymal stem cells in a mice model of traumatic brain injury

Authors: *R. A. SHAHROR[1], Y. WANG[3], G. R. LINARES[4], Y.-H. CHIANG[1], D.-M. CHUANG[5], K.-Y. CHEN[2]
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Abstract: Abstract
Traumatic Brain Injury (TBI) is a progressive and complex brain injury that results in many adverse and long-term neurological consequences. TBI triggers a cascade of molecular and cellular changes that can lead to development of post-traumatic co-morbidies, such as epilepsy and Alzheimer's disease. Mesenchymal stem cell (MSC)-based therapies have recently emerged as a promising, reliable and safe cell therapy approach for managing and treatment and of many neurodegenerative diseases, and may be effective in targeting the pathophysiology of TBI. MSCs have therapeutic effects possibly by their secretome at the site of brain injury results in regulate the damage and promoting the repair processes. The focus of this project is to investigate whether MSCs modified to secrete fibroblast growth factor 21 (FGF-21), a novel metabolic regulator that has emerged as potent neuroprotective agent, can further enhances the recovery in controlled cortical impact (CCI) TBI mice model.. Our preliminary results have shown MSCs-secreting FGF-21 enhance their migration ability using an in vitro assay. Moreover, mice that received an Intracerebroventricular (ICV) injection of MSC-FGF21 contralateral to the injury side after 24 hours of the CCI-TBI injury, showed significantly improved functional outcomes compared with mice treated with empty MSCs- Empty Vector (EV) or vehicle (PBS). The functional outcomes have been demonstrated improvements in cognitive functions (using Morris Water Maze test (MWM), and Novel Object Recognition test (NOR)), motor functions (Using Beam Walking test), emotional and behaviour functions (using Open Field test (OFT) and Forced Swim Test (FST)) in MSC-FGF21 treated mice compared with MSCs- Empty Vector (EV) or vehicle (PBS) treatment. The improved functional outcome might be explained by the enhanced neurogenesis and axonal remodelling following MSC-FGF21 transplantation. These data suggest that fgf-21 can enhances the potential of MSCs based therapy might be a useful approach to consider for treatment of TBI.

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Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.07/P8

Topic: C.10. Brain Injury and Trauma

Support: MOTIE, Korea Grant 10067378

Title: Combined effects of suicidal gene-expressing mesenchymal stem cells and chemotherapy in experimental glioblastoma model

Authors: *J. HAN, D. JANG, H. SUH-KIM, S. KIM
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Abstract: The intrinsic heterogeneous and infiltrative nature of glioblastoma cells to the adjacent normal brain parenchyma, as well as their resistance to most chemotherapeutic agents available currently, are the main obstacles to the treatment of GB. Human mesenchymal stem cells (MSCs) have emerged as attractive cellular vehicles to deliver therapeutic genes for ex-vivo therapy of diverse diseases; this is in part because they have the capability to migrate into tumors. Previously, we showed that MSC can deliver a bacterial suicide gene, cytosine deaminase (CD), and remove the glioblastoma multiform (GBM) via bystander effects. Here, we report that a potential application of MSC/CD in combination with temozolomide (TMZ), which is an oral alkylating agent used widely in the clinical treatment of high-grade gliomas. Glioblastoma LN229 cells were stably transduced to express a green fluorescence protein utilized for in vivo and in vitro experiments. Treatment with MSC/CD with 5-FC effectively suppressed the GBM and such effects were higher in the presence of TMZ. Therefore, we propose that combined treatment of MSC/CD with chemotherapy can be used to treat patients with GBM during the immediate postoperative period by sensitizing the GBM.

Disclosures: J. Han: None. **D. Jang:** None. **H. Suh-Kim:** None. **S. Kim:** None.

Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.08/P9

Topic: C.10. Brain Injury and Trauma

Support: Mallinckrodt Pharmaceuticals

Title: Inhaled nitric oxide protects cerebral autoregulation through prevention of impairment of ATP and calcium sensitive K channel mediated cerebrovasodilation after traumatic brain injury

Authors: *W. M. ARMSTEAD[1], P. PASTOR[2], V. CURVELLO[2], H. HEKIERSKI[2]
[1]Univ. of PA, Philadelphia, PA; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Introduction: Hypotension and low cerebral perfusion pressure are associated with low cerebral blood flow, cerebral ischemia, and poor outcomes after traumatic brain injury (TBI). Cerebral autoregulation is impaired after TBI, contributing to poor outcome. Since ethical considerations constrain mechanistic studies in humans, we use an established porcine model of fluid percussion brain injury (FPI) to understand this pathology. In prior studies, ERK mitogen activated protein kinase (MAPK) and ET-1 had been observed to be upregulated and contribute to impairment of cerebral autoregulation and histopathology after FPI in the pig. Activation of ATP and Calcium sensitive (Katp and Kca) channels produce cerebrovasodilation and contribute to autoregulation, both impaired after TBI, contributory to poor outcome. Upregulation of ERK MAPK and ET-1 produces K channel function impairment after CNS injury. Inhaled nitric oxide (iNO) has recently been observed to prevent impairment of cerebral autoregulation and hippocampal CA1 and CA3 neuronal cell necrosis after FPI in pigs via block of upregulation of ERK MAPK and ET-1. We presently investigated whether iNO prevented impairment of Katp and Kca-mediated cerebrovasodilation after FPI. **Methods:** Lateral FPI was produced in anesthetized pigs. Pial artery reactivity was measured via a closed cranial window. Data (N=5) were analyzed by repeated measures ANOVA, with significance determined at $P < 0.05$. **Results:** Results show that pial artery dilation in response to the Katp agonist cromakalim, the Kca agonist NS1619, PGE2 and the NO releaser sodium nitroprusside (SNP) were blocked by FPI, but such impairment was prevented by iNO administered at 30 min or 2h post-injury. Protection lasted for at least 1h after iNO administration was stopped, indicating that protection was durable. **Discussion:** Using vasodilation as an index of function, these data indicate that iNO prevents impairment of cerebral autoregulation and limits histopathology after TBI through protection of K channel function via blockade of ERK MAPK and ET-1.

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Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.09/P10

Topic: C.10. Brain Injury and Trauma

Support: DICBR
HJF

Title: Aging in mice with repeated concussive injuries: Implications of n-3 polyunsaturated fatty acid deficiency

Authors: *A. DESAI[1], H. CHEN[1], K. KEVALA[2], H.-Y. KIM[1]
[1]Natl. Inst. On Alcohol Abuse and Alcoholism, Bethesda, MD; [2]Natl. Inst. on Alcohol Abuse and Alcoholism, Bethesda, MD

Abstract: Repeated head injuries can have protracted effects on neuropathology and behavior. Chronic traumatic encephalopathy has been observed in post-mortem brains of humans who had sustained multiple head injuries. The concentration of n-3 polyunsaturated fatty acids (n-3 PUFA) in diet and of docosahexaenoic acid (DHA) in the brain also impact the recovery in many models of acute brain injury. The present study was designed to explore if the effect of repeated head injuries during adulthood (4-5 months age) is sustained in aged mice (17-18 months age) and to investigate whether brain DHA concentration is relevant in this paradigm. Pregnant C57Bl/6N mice (E14) were fed with n-3 PUFA deficient diet. At weaning, the pups were either continued on the same diet (deficient group) or given n-3 PUFA adequate diet (adequate group). At 4-5 months age, the mice were given one head injury daily for three days using the closed head impact model of engineered rotational acceleration (CHIMERA). Behavioral studies including tests for anxiety- and depression-like behavior and learning/memory were performed after 2 months of injury (adult mice) or 17-18 months of age (aged mice). The mice were euthanized and their brains were collected for histology and biochemical studies. Mice fed with deficient diet had lower brain DHA concentration than the adequate group. The aged mice with repeated head injuries had a clear trend for increased activity compared to the sham. The deficient diet group of mice appeared to show anxiety-like behavior in the elevated plus maze test. Increased gliosis was also observed after injury, which was exacerbated by DHA deficiency. The results indicate that mice can show differences in behavior and brain pathology even after 13 months of having repeated head injuries and that the brain DHA status affects injury-related brain pathology.

Disclosures: A. Desai: None. **H. Chen:** None. **K. Kevala:** None. **H. Kim:** None.

Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

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Program #/Poster #: 567.10/P11

Topic: C.10. Brain Injury and Trauma

Support: NIH-NS40125

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VAI01RX001127
1-F32NS090748
THE PITTSBURGH FOUNDATION

Title: Lithium improves striatal dopamine neurotransmission and synaptic dopaminergic protein abundance following traumatic brain injury

Authors: *S. W. CARLSON, C. DIXON
Neurosurg., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Experimental models of traumatic brain injury (TBI) recapitulate neurobehavioral impairments and the development of secondary injury sequela observed in TBI patients. Previous work from our lab shows that TBI reduces formation of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, protein machinery important for vesicular fusion, contributing to impaired neurotransmission in the weeks post-injury. In the hippocampus, lithium treatment increases SNARE monomeric protein abundance and SNARE complex formation, and promotes the recovery of cognitive function after controlled cortical impact (CCI). However, the effects of TBI on the SNARE complex formation have not been studied in the striatum, a region exhibiting deficits in evoked dopamine neurotransmission. The objective of this study was to evaluate the effect of lithium treatment on SNARE complex formation and dopamine neurotransmission in the striatum. To test this, anesthetized male Sprague-Dawley rats received CCI (2.7mm) or sham injury, and injected daily (i.p.) with vehicle or 1.0mmol/kg/ml lithium chloride for 7d, beginning 5 minutes post-injury. Daily treatment with lithium significantly improved high-potassium evoked striatal dopamine release at 7d post-injury (n=6-7/group). In a separate cohort, animals received CCI or sham surgery as described and the brains were dissected at 7d post-injury and processed to produce synaptosomal lysates for immunoblotting (n=6/group). CCI significantly reduced cysteine string protein alpha, VAMP2 and SNARE complex formation in striatal synapses. Treatment with lithium did not increase SNARE protein abundance or SNARE complex formation. However, lithium increased the abundance of alpha synuclein, D2 receptor and phosphorylation of tyrosine hydroxylase. These findings demonstrate treatment with lithium improves striatal neurotransmission, and suggests that lithium may increase the abundance of multiple dopaminergic proteins after TBI.

Disclosures: S.W. Carlson: None. **C. Dixon:** None.

Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

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Program #/Poster #: 567.11/P12

Topic: C.10. Brain Injury and Trauma

Support: DOD Grant W81XWH-15-1-0283 (JDL)
DOD Grant W81XWH-15-1-0284 (MS)

Title: Purinergic agonists reduce cerebral damage in a preclinical mouse model of Blast-induced traumatic brain injury

Authors: *E. BOZDEMIR KURBANOV[1], F. A. VIGIL[1], V. BUGAY[1], S. H. CHUN[1], S. KHOURY[1], L. ESPINOZA[1], D. M. HOLSTEIN[1], H. AKAL[2], I. SANCHEZ[1], M. HOBBS[1], R. ELLIOT[3], C. SPRAGUE[3], G. RULE[3], J. CAVAZOS[1], B. LUND[3], M. SHAPIRO[1], R. BRENNER[1], J. D. LECHLEITER[1]
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Abstract: Rationale- Blast concussions are a too common occurrence for many soldiers. Although blast trauma is correlated with epilepsy development, it is unknown if blast injury causes early seizures that contribute to epilepsy. As a first step towards treatment, we developed a mouse model of post blast seizures and investigated purinergic receptor agonists as potential therapeutics.
Method- We used the shock tube apparatus located at the Sensory Trauma Division, US Army Institute for Surgical Research (USAISR) to produce pressure waves having the characteristic "Friedlander" waveform of a free-field blast wave. 10-week-old C57BL/6J mice were subjected to repeated blasts (3X in 3 days, 14 kPa) directed head-on. 30 minutes following trauma, mice were treated with either vehicle or purinergic agonist (AST-004 0.2 mg/kg or MRS2365 0.85 mg/kg). The day after the last blast exposure, one group of mice was implanted with screw-type EEG electrodes and allowed 24 hours to recover. They were then recorded continuously over the following 48 hours. The rest of the mice were not implanted with EEG electrodes. All mice were sacrificed 7 days after the initial blast exposure and brains harvested for brain slice electrophysiology, biochemistry or histology. GFAP and Iba1, established markers for astrocytes and microglia, were used as biomarkers for injury. We also used Sholl Analysis to investigated astrocyte morphology.
Results- Blast injured mice demonstrated a significant increase in GFAP and Iba1 levels as well as morphology changes indicating astrogliosis. Purinergic treatments significantly reduced all injury markers, primarily in the hippocampal region. EEG recordings showed that 5/10 mice exhibited generalized seizures ranging in frequency from 1/day to 9/day, with durations of 5-36 sec per seizure (average 22 sec). Seizures correlated with behavioral arrest in some, but not all animals. Patch clamp recording from hippocampus dentate gyrus granule neurons indicated blast injury increased intrinsic excitability. Biochemical studies indicated a significant increase in phospho- tau (S202), with no change in total Tau, which was reduced by purinergic treatment.
Conclusions- Repetitive moderate blast injuries cause early seizures and neuronal hyper-excitability. Reactive astrogliosis, microglial activation and phospho-tau are elevated after blast injury. Pharmacological activation of purinergic receptors substantially protected against blast injury as indicated by reversal of GFAP and phospho-tau levels.

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Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.12/P13

Topic: C.10. Brain Injury and Trauma

Title: A novel self-guided rehabilitation task activates limbic memory circuitry and preserves cognitive performance after diffuse traumatic brain injury in the rat

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Abstract: Traumatic brain injury (TBI) is not a transient event from which all people recover; the resulting damage can evolve into neurological disease. As with patients, experimental TBI disrupts rodent memory circuits, evident as impaired cognitive performance. Experimental rehabilitation strategies, such as enriched environment and exercise, have partial success in alleviating symptoms. New rehabilitation strategies are necessary to demonstrate therapeutic efficacy and explore cellular mechanisms that promote recovery. Diffuse brain injury by midline fluid percussion leads to cognitive impairments by 1 month post-injury, permitting a timeframe to implement and investigate delayed interventions. We hypothesize that rehabilitation targeting the spatial and contextual memory circuit will prevent the onset of injury-induced memory impairments by specifically activating rodent limbic circuitry. Rehabilitation occurs in a box with a peg-board floor that allows for 10cm plastic pegs to be inserted at 2.5cm intervals in designated layouts; termed Peg Forest Rehabilitation (PFR). Uninjured and brain-injured rats were exposed to PFR (15 min/day), allowing free navigation through random layouts of the peg-filled arena for 10 days over 2 weeks or for 5 days starting one or two weeks post-injury. Controls were exposed to an open-field arena (15 min/day) or served as caged-controls. One-month post-injury, cognitive performance was tested for short-term, long-term, and working memory. Brain-injured animals exposed to PFR performed no different than uninjured rats on all three cognitive assessments. Brain-injured control rats performed significantly worse than shams on all three cognitive assessments, demonstrating injury-induced cognitive impairment in the absence of rehabilitation. In naïve rats exposed to PFR, neurons in the limbic memory circuit exhibited increased cFos expression compared to caged controls, confirming specific circuit activation. Results following a single week of rehabilitation preliminarily indicate that 2 weeks of PFR are necessary for spared cognitive performance. Thus, passive, intermittent rehabilitation targeting specific circuitry can prevent cognitive symptomatology. The Peg Forest is a viable

rehabilitation strategy to explore cellular and molecular mechanisms to preserve neurological function.

Disclosures: L. Law: None. **D.R. Griffiths:** None. **J. Lifshitz:** None.

Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.13/P14

Topic: C.10. Brain Injury and Trauma

Title: Minocycline plus N-acetylcysteine improves structure and function of distal brain regions even when dosed days after closed head injury

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Abstract: Patients with moderate to severe traumatic brain injury (TBI) receive treatment within hours. Patients with mild TBI are more likely to delay seeking treatment and therefore will need drugs with a longer therapeutic time window. Drugs to treat experimental TBI lose potency as the therapeutic time window increases. Previous studies have shown that the drug combination of minocycline (MINO) plus N-acetylcysteine (NAC) loses little potency when first dosed at 1 or 12 hours. The therapeutic time window in mice was further examined after a first dose of MINO plus NAC at 12 hours (MN12) or 72 hours (MN72) after closed head injury (CHI). Behavioral recovery was assessed with an active place avoidance task, which requires two functioning hippocampi and the connections between them; and Barnes maze which only requires one functioning hippocampus. Both gray and white matter injury was assessed histologically. MN12 improved both Barnes maze and active place avoidance as well as preventing white and gray matter injury in the hippocampi ipsilateral to the impact site. MN72-treated mice acquired and retained Barnes maze, despite histological damage to ipsilateral brain regions. The ability to acquire Barnes maze suggests that MN72 restored synaptic plasticity in the contralateral hippocampus. This hypothesis was tested by examining hippocampal synaptic plasticity in MN72-treated mice. The atypical protein kinase C, PKMζ, is needed for long-term potentiation (LTP) of Schaffer collateral-CA1 synapses. CHI damaged cellular ultrastructure, impaired LTP and decreased PKMζ levels in both hippocampi; MN72 treatment restored LTP and PKMζ levels only in the contralateral hippocampus. MN72 treatment also maintained neuronal structure and synaptic density in the contralateral, but not the ipsilateral hippocampus. These data show that MN12 treatment prevents both white and grey matter damage proximal to the injury. There is a

loss of potency when MINO plus NAC is dosed at 72 hours, yet it still prevents gray matter damage and restores synaptic function distal to the impact site. When dosed soon after injury, MINO plus NAC may have sufficient potency to treat moderate and severe TBI. The long therapeutic time window of MINO plus NAC suggests it may be effective to treat mild TBI.

Disclosures: K. Whitney: None. **M.A. Sangobowale:** None. **A. Alexis:** None. **E. Nikulina:** None. **D.L. Dickstein:** None. **T.C. Sacktor:** None. **P.J. Bergold:** None.

Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.14/P15

Topic: C.10. Brain Injury and Trauma

Support: Chuck Noll Grant

Title: Towards developing methods for non-invasive and online suppression of cortical spreading depolarization simulated on a mesoscale model

Authors: *A. CHAMANZAR, S. GEORGE, A. MENON, S. KELLY, M. CHAMANZAR, P. GROVER
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Abstract: Rationale: Cortical spreading depolarizations (CSDs) are waves of silencing of normal brain activity which propagate across the cortical surface [Zandt, et al., 2015]. Evidence shows that a near-complete neuronal energy depletion during CSD propagation can cause secondary brain damages after a wide range of neurological diseases, e.g. TBI, stroke, and hemorrhages [Dreier, et al., 2018][Lauritzen, et al., 2011]. This motivates us to examine possible solutions to stop this devastating wave.Commonly used techniques for CSD suppression involve invasive and/or pharmacological methods. These include (i) lesion extraction or decompression after TBI and hemorrhages to remove clotted blood and stop CSDs [Hartings, et al., 2014], and (ii) Injecting vasodilating drugs such as L-arginine to maintain enough oxygen and energy supply to the neurons [Scheckenbach et al, 2006].
Due to the side effects of surgery and chemical injections in brain, and the time it takes to stop CSDs using these methods, we are motivated to explore physiological mechanisms of CSD propagation and suppression, which is the first step towards a broader goal of designing non-invasive techniques, e.g. through transcranial current stimulation, to suppress CSDs.
Methods: We use a CSD model [Tuckwell, 2008] that is based on reaction and diffusion of components that are involved in CSD propagation, i.e. ions (K+, Ca++, Na+, Cl-) and neurotransmitters (GABA and glutamate) in intra and extracellular space. Using this model and inspired by a recent theoretical work on seizure suppression [Zhang, et al., 2017], we vary

parameters of neurons, e.g. calcium conductance, glutamate pump strength, and membrane cut-off potential, across space and time, to examine their effect individually on CSD propagation.Results: Our simulation results suggest that changing these neural parameters can indeed suppress CSD waves completely and in real time. We attempt three different types of changes in the region which contains the entire CSD wave at the initial stage of propagation: (i) constant change (increase or decrease) in the parameter values, (ii) spatially randomized Gaussian parameters, and (iii) 2D sinusoidal perturbations. We observe that changing glutamate pump strength using method (i) and (ii), calcium conductance using all three methods, and membrane cut-off potential using only method (i), can suppress CSDs.Conclusions: Our simulation results suggest that perturbing neural parameters can be used to suppress CSD waves. It remains to be fully understood if these spatial patterns of perturbations can be implemented and used for CSD suppression using available non-invasive stimulation techniques.

Disclosures: A. Chamanzar: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent holder: Carnegie Mellon University. **S. George:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent holder: Carnegie Mellon University. **A. Menon:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent holder: Carnegie Mellon University. **S. Kelly:** None. **M. Chamanzar:** None. **P. Grover:** 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.; Chuck Noll grant.

Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.15/Q1

Topic: C.10. Brain Injury and Trauma

Support: Charles H. Skip Smith Endowment Fund to Gong Chen

Title: Brain repair after traumatic injury through NeuroD1-mediated astrocyte-to-neuron conversion

Authors: *Z. LEI, F. ZHANG, G. CHEN
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Abstract: Background
Traumatic brain injury (TBI) is one of the leading causes of death in the US, especially among young people. So far there is no effective treatment to repair the damaged brain after TBI.

Our recent studies on in vivo reprogramming have shown that internal glial cells can be directly converted into functional neurons in the central nerves system in situ, which may shed lights on potential treatment for TBI.
Method
A closed head injury (CHI) model is set up to mimic brain concussion injury such as that suffered by football players. The impact can be controlled precisely to cause a focal blunt injury over the intact skull of a mouse, which will trigger further damages to its brain tissue and neural network. Then NeuroD1 or control viruses will be applied through intracranial injection into the injury site to investigate the effect of in situ astrocyte-to-neuron conversion.
Results
There is no evident internal neurogenesis in the control adult mouse cortex after TBI. However, NeuroD1 treatment can regenerate a large number of newborn neurons from astrocytes throughout the injured mouse cortex. These newborn neurons showed a transitional stage in-between an astrocyte and a neuron in early time points, and later became fully functional mature neurons with integration into the internal neural networks. Furthermore, with the regeneration of new neurons and reconstruction of damaged neural networks, the astrocyte-to-neuron (AtN) conversion induced by NeuroD1 also has substantial beneficial effects in ameliorating the local environment.
Conclusion
NeuroD1-mediated neuroregeneration and neuroprotection can be a novel therapeutic treatment for TBI.
(This work is supported by Charles H. Skip Smith Endowment Fund to Gong Chen.)

Disclosures: Z. Lei: None. **F. Zhang:** None. **G. Chen:** Other; Gong Chen is a co-founder of NeuExcell Therapeutics inc.

Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.16/Q2

Topic: C.10. Brain Injury and Trauma

Support: NIH/NINDS Grant U54 NS100064

Title: The use of pharmacokinetic and pharmacodynamic modeling and simulation to facilitate the screening and early-stage development of new therapies for post-traumatic epilepsy

Authors: *L. COLES[1], C. K. LISGARAS[2], W. LIU[2], P. G. SALETTI[3], P. CASILLAS-ESPINOSA[4,5], S. SHULTZ[4,5], N. JONES[4,5], I. ALI[4,5], R. BRADY[4,5], J. CLOYD[1], T. O'BRIEN[4,5], S. L. MOSHE[6], A. S. GALANOPOULOU[7]
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Abstract: Background: EpiBioS4Rx aims to identify new therapies to prevent post-traumatic epilepsy (PTE) following traumatic brain injury (TBI). The EpiBioS4Rx project 2 has created a multicenter preclinical therapy screening platform to enhance reproducibility and implemented pharmacokinetic (PK) and pharmacodynamic (PD) studies early in the preclinical screening process to optimize treatment delivery. PK and PD modeling can accelerate drug discovery and development by helping to identify lead compounds, optimizing drug delivery, scaling doses across species, and guiding the design of human studies. In EpiBioS4Rx a multi-disciplinary team is screening compounds for antiepileptogenic effects using the lateral fluid percussion injury (LFPI) rat model of TBI in which PTE is well documented. **Objective**: To utilize PK and ultimately PK-PD modeling and simulation to select the best treatment protocols in the rat LFPI model. Here we tested levetiracetam and sodium selenate. **Methods:** Male 11-week old Sprague Dawley rats were used as either naïve controls or following LFPI. induction at the left parietal region, using a 5mm craniotomy and injury parameters optimized to induce moderate/severe TBI with a mortality of ~30%. Rats received either a bolus injection (intraperitoneal or subcutaneous (SC)) given in controls or immediately after LFPI or a bolus followed by SC minipump placement (ALZET 2ML1) 1hr later. The minipump was removed after 7 days. Blood was collected from the lateral tail vein at specified timepoints bracketing 0 and 24 hours after the bolus, or between minipump placement and 2hr after pump removal to study drug exposure and washout. Parietal cortical samples were collected at similar timepoints. Levetiracetam concentrations in plasma and brain were measured using validated HPLC-MS/MS methods and sodium selenate and selenium were measured using ICP-MS systems. A population-based PK and PD modeling approach was utilized. **Results:** Brain-to-plasma ratios ranged from 0.8-1 for levetiracetam, ~1 for sodium selenate and <0.1 for selenium. Levetiracetam and sodium selenate concentrations were well fit by one-compartment, first-order absorption PK models with and apparent clearances of ~130 and 16.5mL/hr/kg and volumes of distribution of ~400 and 10 mL/kg, respectively. **Conclusions:** Obtaining PK information early in the screening allows us to develop PK/PD models and simulate the effect of drugs on efficacy and safety measurements, guiding optimal treatment protocols in EpiBioS4Rx. These models will be validated in future antiepileptogenic studies and ultimately used to inform design of human clinical studies.

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Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.17/Q3

Topic: C.10. Brain Injury and Trauma

Support: The Moody Project for Translational Traumatic Brain Injury Research
CONACYT-COPOCYT
ConTex
Fundación Marrón Cajiga
The Coalition for Brain Injury Research
The John S. Dunn Foundation

Title: From cancer to neurotrauma, potential for therapeutic repurposing of a clinically approved PARP1 inhibitor

Authors: *J. ALLENDE LABASTIDA[1], J. GAO[2], T. J. DUNN[2], H. ZHANG[4], P. R. KLEIN[5], A. AHMAD[3], J. GUPTARAK[3], M.-A. MICCI[6], D. S. PROUGH[8], C. SZABO[3], P. WU[7]
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Abstract: Poly (ADP-polymerase) 1 (PARP1) is activated in response to DNA damage induced by reactive oxygen species and excitatory amino acids, all of which are important effectors in the pathophysiology of TBI, and has been shown to occur in the pericontusional neurons after brain trauma. Activation of PARP1 promotes the depletion of NAD and ATP, inducing mitochondrial dysfunction and cell death; and is implicated in pro-inflammatory signaling. On the other hand, PARP1 deficient mice exhibit a neuroprotective phenotype, and PARP1 polymorphisms in humans correlate with differential neurological outcomes after TBI. Recently, two potent PARP1 inhibitors have been approved as chemotherapeutics in ovarian cancer, allowing the possibility of repurposing for non-oncological diseases. Here, we examined the effect of olaparib (Lynparza), a clinically approved PARP inhibitor, in both in vitro and in vivo models of TBI. Our *in vitro* studies showed that olaparib effectively reverses oxidant-induced cell death in a differentiated B35 neuroblastoma cell line, and significantly reduces apoptosis of neurons and astrocytes that were derived from human fetal brain neural stem cells in a stretch injury model. In an *in vivo* study using a closed-skull weight drop TBI model, treatment with olaparib reduced TBI-induced hippocampal reactive astrogliosis and improved cognitive function in mice (as assessed by novel object recognition test). In conclusion, PARP may be a potential target for intervention to protect against neuronal injury in the acute phase of TBI. Further studies are needed to determine whether olaparib is also effective to prevent the later-stage neurological deterioration secondary to acute or repetitive TBI.

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Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.18/Q4

Topic: C.10. Brain Injury and Trauma

Support: CCF RPC 196, 2017

Title: Lateral cerebellar nucleus stimulation promotes motor recovery and suppresses neuroinflammation in a fluid percussion injury rodent model

Authors: *H. H. CHAN[1,2], C. A. WATHEN[3], N. D. MATHEWS[2], O. HOGUE[4], J. P. MODIC[2], R. KUNDALIA[2], C. WYANT[2], H.-J. PARK[3], I. M. NAJM[5], B. D. TRAPP[2], A. G. MACHADO[3], K. B. BAKER[6]
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Abstract: Many traumatic brain injury (TBI) survivors live with persistent disability from chronic motor deficits despite contemporary rehabilitation services, underscoring the need for novel treatment. We have previously shown that deep brain stimulation (DBS) of the lateral cerebellar nucleus (LCN) can enhance post-stroke motor recovery and increase the expression of markers of long-term potentiation in perilesional cerebral cortex. We hypothesize that a similar beneficial effect will be for motor deficits induced by unilateral fluid percussion injury (FPI) in rodents through long-term potentiation- and anti-inflammatory based mechanisms. Male Long Evans rats with a DBS macroelectrode in the LCN underwent FPI over contralateral primary motor cortex. After 4 weeks of spontaneous recovery, DBS treatment was applied for 4 weeks, with the pasta matrix, cylinder, and horizontal ladder tests used to evaluate motor performance. All animals were euthanized and tissue harvested for further analysis by histology, immunohistochemistry, RNA microarray assay and Western Blot. LCN DBS-treated animals experienced a significantly greater rate of motor recovery than untreated surgical controls, with treated animals showing enhanced expression of RNA and protein for excitability related genes, suppressed expression of pro-inflammatory genes, suppressed microglial and astrocytic activation, but proliferation of c-fos positive cells. Finally, our data suggest a possible role for anti-apoptotic effects with LCN DBS. LCN DBS enhanced the motor recovery following TBI, possibly by elevating the neuronal excitability at the perilesional area and mediating anti-apoptotic and anti-inflammatory effects.

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Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.19/Q5

Topic: C.10. Brain Injury and Trauma

Support: (KSCHIRT) 14-12A
NIH R01 NS072302-02S1
R01 NS072302
T32 NS077889
P30 NS051220

Title: Insulin-like growth factor-1 overexpression promotes survival of adult-born neurons and improved cognition following traumatic brain injury

Authors: *E. LITTLEJOHN[1], D. SCOTT[1], K. E. SAATMAN[2]
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Abstract: The pathology associated with traumatic brain injury (TBI) manifests in motor and cognitive dysfunction following injury. Immature neurons residing in the neurogenic niche of the dentate gyrus (DG) in the hippocampus, a brain structure required for learning and memory, are particularly vulnerable to TBI. The inability to restore this population of hippocampal immature neurons following TBI has been causally linked to cognitive impairment. Insulin-like growth factor-1 (IGF-1) is a potent neurotrophic factor capable of mediating neuroprotective and neuroreparative processes. We have shown that elevating brain levels of IGF1 stimulates hippocampal neurogenesis, enhancing the recovery of immature neuron numbers after severe TBI in mice. However, little is known about the effectiveness of IGF1 to promote long-term survival of neurons born after injury. To this end, astrocyte-specific IGF1 conditionally overexpressing mice (IGF1-TG) and wild-type (WT) mice received controlled cortical impact (n=9/genotype) or sham (n= 2/genotype) injury and 50 mg/kg BrdU (i.p.) twice daily for 7 days following TBI. At six weeks following injury, total numbers of proliferated cells ($BrdU^+$) and the subset expressing a mature neuronal marker ($NeuN^+/BrdU^+$) were counted at the injury epicenter (3 sections/animal). IGF1 significantly increased $NeuN^+/BrdU^+$ cell density at 6 weeks post-injury ($p<0.05$, compared to WT injured mice. IGF1 overexpressing mice had improved cognitive flexibility during radial arm water maze reversal learning. These data suggest that IGF1 stimulates end-stage survival of posttrauma-born neurons and improves long-term cognition.

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Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.20/Q6

Topic: C.10. Brain Injury and Trauma

Support: Merit Review Award # B78071, B1005-R & RCSA B7345S, from the United States (U.S.) Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D)

Title: Therapeutic tms reduces tbi-induced cognitive, anxiety, spasticity, and balance disabilities

Authors: *F. J. THOMPSON[1,2,3], J. HOU[1,2], R. NELSON[1], S. TSUDA[1,2], G. MUSTAFA[1,2], J. WATTS[1,2], N. MOHAMMAD[1], A. LERNER[1], J. PEDRAZA[1], P. BOSE[1,2,4]
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Abstract: TBI can initiate enduring cognitive, anxiety, and sensory/motor disabilities that significantly impact the quality of life. Therapies are urgently needed that can provide safe and effective reduction in these long-term disabilities without negatively impacting cognitive recovery. During the course of CNS function evaluations in rats following TBI, we observed potentially significant therapeutic benefits induced by a recruitment ladder evaluation protocol using TMS. The objective of the current studies was to systematically evaluate potential therapeutic benefits induced by this TMS protocol on measures of cognitive performance, anxiety, spasticity, and balance functions following TBI in adult rats. Closed head (impact acceleration) TBIs were produced by a modification of the Marmarou procedure (450g x 1.25m drop height). The TMS treatments consisted of single pulse cranial surface TMS (Mgstim, 25mm figure 8 coil). An intensity ladder from 30% through 70% maximal intensity was delivered three times/week for one month. At the completion of treatment, cognitive performance was assessed using a serial learning paradigm in a Morris water maze (MWM). Spasticity was quantitated using velocity dependent ankle torque and triceps surae EMGs. Anxiety was assessed using time spent and entry patterns in open and closed areas of an elevated plus maze (EPM). Balance was tested using a rotorod balance beam protocol. Compared with intact animals, the TBI animals revealed significant increases in: a) escape latency during serial learning in a MWM, b) time spent in closed portions of the EPM, c) velocity dependent ankle torque and stretch evoked triceps surae EMGs, and d) significant decreases in time spent on the rotating beam of the rotorod. Compared with the untreated TBI animals, the TMS treated TBI animals revealed significant reduction in MWM escape latency, time spent in the closed portions of the EPM, spasticity, and balance deficits. Immunohistochemistry of neural tissues associated with these functions (hippocampus, amygdala, motor cortex, lateral vestibular nucleus, showed significant

increases in trophic and neuromodulatory factors (BDNF, DβH, $GABA_b$), in treated, compared with untreated TBI animals. The locus coeruleus (which plays a vital role in regulation of excitability, immune responses, blood brain barrier integrity) showed significant inflammation (NFkB, MMP-9, GFAP, OX-42), and cell loss in TBI animals. Inflammatory marker expressions were significantly less in the TBI-TMS treated animals. These preliminary studies indicate a significant therapeutic reduction in long-term TBI disability measures.

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Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

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Program #/Poster #: 567.21/Q7

Topic: C.10. Brain Injury and Trauma

Support: Merit Review Award # B78071, and B1005-R from the United States (U.S.) Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D)

Title: Effects of the iron chelator on the blood-brain barrier (BBB) disruption and inflammatory responses following traumatic brain injury (TBI) in rats

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Abstract: Each year, over 10 million people in the world suffer mortality or hospitalization due to traumatic brain injuries (TBIs). The majority of these injuries are closed-head TBIs (cTBIs) of mild/moderate severity. There is a growing concern that in addition to specific TBI-disabilities, even mild TBI may significantly elevate risk factors for long-term chronic inflammation-induced progressive diseases. Acceleration/deceleration TBIs induce damage of micro-vessels which results in endothelial shear injury, BBB dysfunction, and micro-bleeding. Microbleeds-derived iron can provide an enduring promotion of inflammation, further breakdown of the BBB tight junctions, and cell death through multiple inflammatory pathways. Thus, removal of toxic iron is potentially an important therapeutic design for TBI treatment and rehabilitation. The current studies were initiated to examine the BBB disruption, neuronal viability, and inflammatory

responses as well as test the safety and efficacy of a novel iron chelator (Hexadentate monosodium salt, NaHBED) on these pathological complications following a moderate cTBI (450g/1.25m) in rats. BBB disruption following cTBI was detected using Evans blue dye (1 ml; 4%) injection into the left ventricle. In a separate cohort of animals, immunofluorescence studies were performed on coronal brain sections of intact and cTBI animals that were labelled with antibodies against dopamine beta-hydroxylase, neuronal nuclei, inflammatory markers (e.g. IL5, TNFα) , and key molecules involved in the BBB disruption (NF-kB, MMP-9 etc.). To detect the neuronal cell deaths, the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays were also performed. Our data to date, indicate that following TBI, the number of viable neurons in certain brain regions (e.g., locus coeruleus, mesencephalic nucleus of trigeminal nerve, etc.) was significantly reduced. In addition, the contents of the Evans blue dye, the expressions of molecules involved in the BBB disruption (NF-kB, MMP-9, etc.), and inflammatory responses (e.g. IL5, TNFα) were significantly elevated following cTBI. These detrimental pathological complications were significantly attenuated by iron chelator (NaHBED) treatment. These results suggest that: 1) iron deposit via cTBI-induced BBB disruption accelerates neuroinflammation and inflammation-mediated neuronal cell death (i.e., pyroptosis), and 2) these can be attenuated by an iron chelator treatment. The present study provides new information regarding the understanding of cTBI-induced neuropathology that can contribute to the development of a novel therapy.

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Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.22/Q8

Topic: C.10. Brain Injury and Trauma

Support: R21-NS096515
Arizona Alzheimer’s Consortium

Title: Remote ischemic conditioning attenuates the peripheral component of neuroinflammation and improves chronic behavioral outcomes in diffuse brain injured female mice

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Abstract: Introduction: Remote ischemic conditioning (RIC) is intermittent restriction of blood flow to a limb or non-vital organ. This therapeutic strategy protects major organs from ischemia-reperfusion injury, reduces cognitive impairments in vascular dementia models, and halts the increase of acute biomarkers after severe traumatic brain injury (TBI). Though the mechanism of RIC is unknown, RIC may modulate the inflammatory response, which shows sex differences after experimental TBI. We hypothesize that post-injury RIC reduces the population of peripheral macrophages in the diffuse-injured brain acutely, with sustained therapeutic effect on cognitive performance and anxiety, and protects against secondary inflammatory challenge more effectively in males than females.
Methods: Diffuse brain injury by midline fluid percussion or sham injury was performed on adult mixed sex C57BL/6 mice. After 1-hour, mice received 4x5 minute sessions of RIC (tourniquet on thigh) with 5-minute reperfusion between each session or anesthesia control. Blood, spleen, and brain were collected at 3 and 7 days post-injury (DPI), and processed for flow cytometry to quantify inflammatory monocytes in the spleen and blood ($Ly6c^{high}Cd115+$) and peripheral macrophages in the brain ($Cd11b+CD45^{high}$). Ongoing studies will complete the analysis of therapeutic efficacy on cognitive performance and anxiety over 90DPI. Protection against secondary inflammatory challenge (10 mg/kg LPS, i.p.) at 100DPI and will be measured using open-field and neuroinflammation using immunohistochemistry.
Results: After TBI, female mice had significant decreases in peripheral monocyte populations in the blood and spleen after RIC treatment compared to non-RIC treated TBI controls at 3DPI. These findings extended to the brain; RIC reduced peripheral macrophage populations in RIC-treated mice compared to non-RIC treated controls ($F(1,11) = 8.046$, $p<0.05$). Data from male mice showed a similar trend without significant differences in the peripheral macrophage response after TBI or RIC treatment. These population changes resolved by 7DPI for both sexes.
Conclusions: RIC modulated the peripheral macrophage and monocyte response to TBI in a sex-dependent, time-dependent manner. Behavioral outcomes and secondary immune challenge will determine whether therapeutic efficacy extends to recovery of neurological deficits. RIC remains a practical, personalized therapy for TBI, in part by reducing neuroinflammation.
Funding: R21-NS096515 and Arizona Alzheimer's Consortium

Disclosures: M. Saber: None. **Y. Hur:** None. **K.R. Giordano:** None. **I. Christie:** None. **R.K. Rowe:** None. **J. Lifshitz:** None.

Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

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Program #/Poster #: 567.23/Q9

Topic: C.10. Brain Injury and Trauma

Support: NIH NIMH R01, MH083804

NIH NIMH R01, MH070596
Hirschl/Weill-Caulier Research Award

Title: RNA aptamers for FGFR3 to modulate glia function after brain injury

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Abstract: Glial cells include astrocytes and oligodendrocytes, both of which are critical for normal brain function and in response to brain injury. Recently, our lab has implicated FGF signaling in astrogliosis and oligodendrogenesis, both in the injured and uninjured cortex. Specifically, for astrocytes, we have shown that activation of FGF signaling keeps astrocytes quiescent both in the normal and injured cortex. For oligodendrocytes, we observed an increase in oligodendrogenesis after activation of FGF signaling in the subventricular zone and in the cortex after a demyelination injury. Both these processes were observed with a constitutively active form of FGFR3. To confirm these genetic findings and in order to modulate these processes pharmacologically, we sought to develop novel ligands specific for FGFR3 since there are no specific agonists or antagonists for the FGFRs. Because aptamers are molecules that tend to bind targets that have heparin binding domains, we systematically evolved nuclease stabilized RNA aptamers that specifically bind FGFR3. In our functional screen of these aptamers, we found that one aptamer specifically, NK01, demonstrates high affinity for FGFR3 and inhibits FGF2 from binding FGFR3. Interestingly, upon dimerizing NK01, the aptamer reverses its role and behaves as an agonist, mimicking FGF2 in its function. I have further characterized these molecules in a primary culture model to 1) test for specificity and 2) test how these ligands effect downstream signaling factors of FGFR3, particularly phospho-ERK. I have locally delivered the drugs to the injured cortex to modulate both astrocyte activation and oligodendrogenesis. My preliminary results suggest that we see an increase in oligodendrocyte precursor cells at the site of injury. I am currently working on analyzing the astrocytic response upon delivery of the drug and after injury.

Disclosures: N. Kamatkar: None. **M. Levy:** None. **J. Hébert:** None.

Poster

567. Brain Injury and Trauma: Pre-Clinical Therapeutic Strategies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 567.24/Q10

Topic: C.10. Brain Injury and Trauma

Support: UW Department of Neurosurgery

Title: Prolonged dexamethasone release from gelatin/PLGA hydrogel for suppression of neuroinflammation after traumatic brain injury

Authors: T. ZHAO[1], N. GONZALEZ[2], J. JOHNSON[1], *R. SAIGAL[3]
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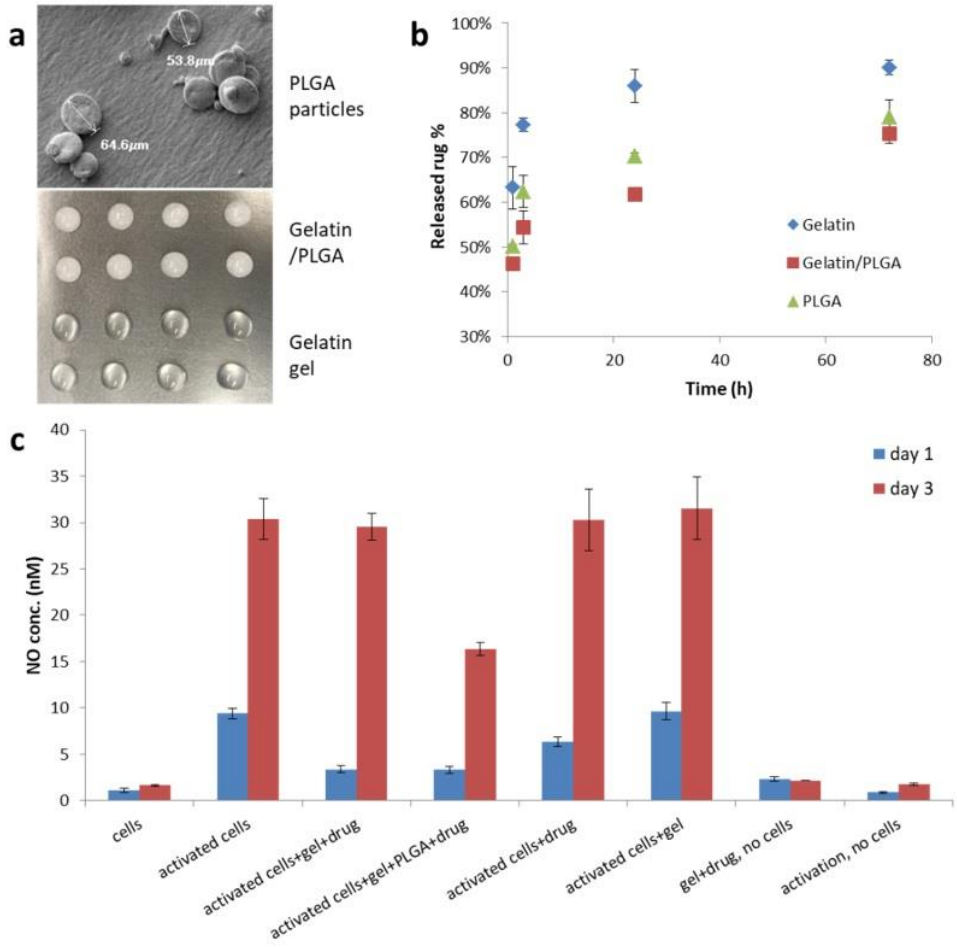
Abstract: Traumatic brain injury (TBI) is a significant public health concern that affects individuals in all demographics. Neuroinflammation can cause secondary injury after TBI. Dexamethasone, an FDA-approved corticosteroid, has been shown to produce neuroprotective effects by inhibiting inflammation. However, delivery of large systemic doses of steroids is limited by side effects, such as sepsis and pneumonia. Therefore, a localized delivery system may be favored to allow for a therapeutic dose at the injury site. Gelatin-based films without added drug delivery are already in use as an absorbable implant after decompressive craniectomy to avoid tissue adhesion. Our work focuses on developing a gelatin-based hydrogel system that can be implanted for both tissue isolation and a prolonged local drug release of dexamethasone. Zero length cross-linker was used to fabricate a fully absorbable gelatin gel. Cross-linker concentration was carefully optimized to form a stable low-swelling/shrinking hydrogel. For prolonged drug release, PLGA, a biodegradable polymer was first blended with dexamethasone to form microparticles, which were then encapsulated in the gelatin gel (Figure 1a). The gelatin/PLGA hydrogel system provides a sustained release profile for over 72 hour and showed slower, controlled release compared to the gelatin alone (Figure 1b). After incubating the gelatin/PLGA hydrogel with activated BV2 microglial cells in vitro, nitric oxide (NO), an inflammatory cytokine up-regulated at the injury site, was measured using the Griess assay. Significant reduction of NO production was observed from the gelatin/PLGA group, especially after 3 day's incubation compared to the no treatment control and the other faster releasing systems (Figure 1c). This indicates the gelatin/PLGA system provides a prolonged anti-inflammation function. CyQUANT cell proliferation assay showed no toxic effects on the microglia. This gelatin/PLGA system thus shows great promise as a local, sustained drug release system for treatment of neuroinflammation after TBI.



Disclosures: T. Zhao: None. **N. Gonzalez:** None. **J. Johnson:** None. **R. Saigal:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

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Program #/Poster #: 568.01/Q11

Topic: C.11. Spinal Cord Injury and Plasticity

Support: KSCHIRT #14-5
DOD #W81XWH-15-1-0656

Title: Telemetric monitoring of penile pressure during mating in rats with different spinal cord contusion injury severities

Authors: *C. J. STEADMAN, C. H. HUBSCHER
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Abstract: Sexual dysfunction is rated a top priority quality of life issue amongst the spinal cord injury (SCI) population. In SCI males, erectile function, ejaculation, and fertility are severely impaired. Currently, limited research is exploring the mechanisms underlying sexual dysfunction after SCI. The present study utilized a telemetric pressure transducer implanted into the corpus cavernosum of the penis to examine the differences in erectile function and pre-determined mating parameters during awake mating behavior for various injury severities using a rat contusion model. After pre-injury mating experience, animals received either a sham laminectomy or a mild (150 kD) or moderate (175 kD) or moderate-severe (210 kD) contusion injury. Animal groups were given two weeks of recovery post contusion, then underwent a weekly mating behavior paradigm for six weeks. The set mating behavior paradigm examined the counts, average pressure, and average duration of mating parameters including mounts, intromissions, ejaculations, partial erectile events (30 mmHg < baseline pressure < 130 mmHg), and full erectile events (>130 mmHg above baseline pressure). Animals in the mild and moderate contusion groups showed partial deficits in the mating parameters examined compared to intact animals, but less deficits than moderate-severely injured animals. Such deficits in examined mating behaviors and erectile function is likely due to disruption of the descending bilateral reticulospinal projections within the spinal cord. Pressure deficits of injured animals as compared to intact animals suggests alterations to the sensory circuitry at the level of the erectile center at the L6-S1 spinal cord. Differences in the duration of the mating parameters suggests a disruption of the descending and/or local input to the penile musculature, including the bulbospongiosus and the ischiocavernosus muscles that are responsible for maintenance of erection. Utilizing telemetric pressure transducers to record erectile event in an awake, behaving animal model may further elucidate the mechanism by which sexual function deficits occur after SCI.

Disclosures: C.J. Steadman: None. **C.H. Hubscher:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.02/Q12

Topic: C.11. Spinal Cord Injury and Plasticity

Support: DOD W81XWH-15-1-0614
NY SCIRB DOH01-ISSCI6-2016-00018
NY State ECRIP Fellowship

Title: Longitudinal profiling of peripheral myeloid cells in a person with traumatic spinal cord injury

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Abstract: Life expectancy for persons with traumatic spinal cord injury (SCI) has not improved in decades and is lower than for able-bodied persons. Infections are the leading cause of death after SCI. Inflammation is also common in persons with SCI, where it may promote common medical consequences of SCI. Infections and inflammation may oppose neurological recovery, particularly in the first year after SCI. The cellular mechanisms contributing to infection susceptibility and inflammation after SCI remain unknown. Dendritic cells (DCs) are the most potent antigen presenting cells, linking innate to adaptive immune responses. Here, we profiled functional recovery and circulating DCs from a person at day 4 and then at 3, 6 and 12 months after SCI. The standard of care physical exam, the International Standards for Neurological Classification of SCI, classifies motor and sensory function throughout the body. The SCI was classified as American Spinal Injury Association Impairment Scale (AIS) grade D, indicating a neurologically incomplete injury. Due to changes in sensory scores, the neurological level of injury changed over time and was C8, C1, C7 and T5 at day 4 and at 3, 6 and 12 months after SCI. The Neuromuscular Recovery Scale measured the person's ability to perform tasks related to mobility, standing and walking. The person's phase improved over time and was 3B, 3C and 4A, indicating good recovery of function, at 3, 6 and 12 months after SCI. The Spinal Cord Independence Measure (SCIM) evaluates activities of daily living on a 100-point scale. The SCIM scores were 87, 89, and 89 at 3, 6 and 12 months. By flow cytometry, the percentage of activated CD11c+HLADR++ myeloid DCs increased over time and was 1.7, 6.7, 5.5, and 8.5% of CD11c+ CD16- CD3-CD56- cells at day 4 and at 3, 6 and 12 months after SCI. The percentage of CD123+ HLADR+ plasmacytoid DCs increased over time and was 0.25, 0.74, 0.65, and 0.69% of CD56-CD3- cells at day 4 and at 3, 6 and 12 months after SCI. In the future, we will profile gene expression of peripheral blood leukocytes in this and other individuals to determine if markers of immunity and inflammation are changed over time.

Disclosures: O. Bloom: None. **M.A. Bank:** None. **M.D. Gallo:** None. **D. Griffin:** None. **A.B. Stein:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

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Program #/Poster #: 568.03/Q13

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NYS SPINAL CORD INJURY RESEARCH BOARD

DEPARTMENT OF VETERANS AFFAIRS MERIT AWARD
CRAIG H. NEILSEN FOUNDATION

Title: Spinal electromagnetic stimulation induces modulation of M-wave and H-reflex responses and recovery of frequency-dependent depression of H-reflex in chronic spinal cord injured rats

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Abstract: The main reason for functional loss after SCI is the lack of propagation of signals through not only damaged/cut axons, but through survived fibers as well. Using animal models and intracellular recordings from individual motoneurons, we have previously demonstrated that administration of several treatments that have been designed to improve transmission in damaged spinal cord usually associated with recovery of function after SCI. In clinics, however, evaluation of neurophysiological parameters is limited to measuring EMG, H-reflex and M-wave responses evoked by peripheral electric stimulation. Our recent results of animal studies demonstrate that spinal electromagnetic stimulation (SEMS) is capable of enhancing synaptic transmission in damaged spinal cord by increasing the function of NMDA receptors at the neuronal networks. Additionally, our recent human studies revealed that administration of repetitive SEMS induced long-lasting modulation of M-wave and H-reflex responses in SCI participants. However, the mechanisms underlying these effects of SEMS in humans remain understudied and require investigation using animal models. In this study, we have examined effects of SEMS on M-wave and H-reflex responses using an animal model. Non-injured adult rats and rats that received chronic mid-thoracic contusion injury were used to investigate effects of SEMS. Effects of SEMS on M-wave and H-reflex responses and on frequency-dependent depression of H-reflex (FDD), as well as possible mechanisms underlying these effects have been examined. Our results demonstrate that SEMS induces significant changes in excitability at spino-muscular circuitry in both non-injured and SCI rats. A single train of SEMS (25 minutes, 0.2Hz, total 400 pulses) induces long lasting facilitation of both of M-wave and H-reflex responses and leftward shift of threshold intensities, i.e. lower threshold intensities required to evoke these responses. These changes are long-lasting and are sustained for approximately 3 hours post SEMS administration. Consistent with literature, the FDD rate was decreased (i.e. it was less depression) in SCI animals. Importantly, our results revealed that SEMS was able to recover frequency-dependent depression (FDD) of H-reflex in chronic SCI rats. Using intraspinal injections of the NMDA receptor blocker MK-801, we have identified NMDA receptors as an important contributor in the induction of SEMS induced changes in the properties of H-reflex. These results identify SEMS as a novel non-invasive tool for long-lasting modulation of neuro-muscular circuits, and importantly, modulation of spinal networks after chronic spinal cord injuries

Disclosures: H.A. Petrosyan: None. **L. Liang:** None. **A. Tesfa:** None. **C. Zou:** None. **S. Sisto:** None. **V.L. Arvanian:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

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Program #/Poster #: 568.04/Q14

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Langford Trust PhD Studentship Grant
Kennel Club Charitable Trust Grant

Title: 'Implant-host tissue matching' using ultrasound elastography for olfactory ensheathing cell transplantation in spinal cord injury: Measuring the stiffness of injured spinal cord using intraoperative ultrasound elastography in a natural canine model provides a target to create matched stiffness collagen hydrogels encapsulating olfactory ensheathing cells, which can increase cell survival after transplantation into sites of chronic spinal cord injury

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Abstract: Spinal cord injury (SCI) can cause irreversible paralysis and incontinence. Intraspinal transplantation of olfactory ensheathing cells (OECs) improves walking in dogs and is promising in humans, but inconsistent return of function implies that refinements are necessary. Experimentally, increased cell transplant number is associated with improved functional outcomes and hydrogel biomaterials improve cell survival. However, to be safely delivered in patients, hydrogel stiffness must be compatible with host tissue to avoid iatrogenic damage or increased inflammatory responses. This is of particular concern in the central nervous system given its soft structure and intricate cellular architecture that is easily disrupted by mechanical stress. However, there is no data on *in vivo* stiffness after SCI.
We aimed to determine spinal cord stiffness in a spontaneous canine model of SCI and create a cell-hydrogel construct of the same stiffness. Further, we tested if a hydrogel of matched SCI stiffness could improve cell survival.
We recruited 15 dogs with SCI undergoing decompressive surgery after acute intervertebral disc herniation at the University of Bristol Veterinary School (ethical approval VIN/15/036). We measured spinal cord stiffness at the lesion epicentre and lesion periphery non-invasively using ultrasound elastography. We determined a target stiffness for injured cord of 18.3kPa (IQR 11.6-31.1kPa) and found surrounding spinal cord to be significantly stiffer (median 47.9kPa, IQR 32.6-81.7kPa).
We measured the stiffness of varying concentrations of collagen hydrogels (1.5-8.5mg/mL). Encapsulating OECs consistently increased hydrogel stiffness by 80±1%; for example, the

stiffness of 7.5mg/mL hydrogels increased from 10.2±1.4kPa to 19.2±1.0kPa (n=4), a stiffness that is comparable to injured canine spinal cord.
As a proof of principle, we injected OECs expressing green fluorescent protein (GFP) into chronic dorsal column crush lesions in rats within either matched stiffness collagen hydrogel (n=8) or media (n=6). The percentage of surviving GFP-positive OECs at 2 weeks after transplantation was significantly higher in hydrogel transplanted animals (4.3±2.6%) compared to media controls (0.72±0.35%).
We therefore provide evidence that: (i) spinal cord stiffness can be non-invasively determined intraoperatively; (ii) hydrogels encapsulating OECs can match this stiffness; (iii) matched stiffness hydrogel constructs increase OEC survival. This could address a safety concern of hydrogel implant and suggests that OEC transplantation in hydrogel may improve neurological outcome after SCI by increasing cell survival.

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Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

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Program #/Poster #: 568.05/R1

Topic: C.11. Spinal Cord Injury and Plasticity

Support: KSCIRC #14-5
DoD #W81XWH-15-1-0656

Title: Activity-based training effects on upper urinary tract function following spinal cord injury

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Abstract: Common urinary dysfunction deficits that arise after spinal cord injury (SCI) include polyuria, urinary tract/bladder infections, detrusor-sphincter dyssynergia, incontinence, and urinary retention. For this reason, bladder dysfunction is ranked as a top priority. Using a contusion male rat model for SCI, we have previously demonstrated that activity-based training (ABT) can lead to improvements in both upper and lower urinary tract function, although the mechanisms are currently unknown. In the current study, using one of the kidneys from each rat for Western blot, we demonstrate that vasopressin (AVP) V2 receptor density significantly decreases following chronic SCI while natriuretic peptide receptor density (NPR-A) significantly increases. Furthermore, these levels are normalized (relative to sham surgical controls) in groups of rats receiving either of two different forms of ABT, fore-limb only stepping or quadrupedal

stepping on a treadmill for 1 hour daily. Using immunohistochemical analyses of the other kidney from each rat, support is obtained for elevated atrial natriuretic peptide levels. Further, a significant amount of glomerular loss is observed in the kidneys of non-trained animals, suggesting an exercise-induced effect that maintains glomerular integrity. Thus changes in the upper urinary tract following activity based training offer a potential mechanism through which exercise can have a positive effect on urinary complications experienced following SCI.

Disclosures: J. Gumbel: None. **L.R. Montgomery:** None. **C.H. Hubscher:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

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Program #/Poster #: 568.06/DP06/R2

Topic: C.11. Spinal Cord Injury and Plasticity

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Wings for Life Foundation (ARF)

Title: Open data commons for spinal cord injury (ODC-SCI_{beta}): Community-driven datasharing infrastructure for research

Authors: *C. A. ALMEIDA[1], M. S. BEATTIE[1], J. L. BIXBY[2], J. C. BRESNAHAN[1], A. CALLAHAN[3], J. S. GRETHE[4], J. HAEFELI[1], J. HUIE[1], V. LEMMON[2], M. E. MARTONE[4], D. S. MAGNUSON[5], D. M. MCTIGUE[6], J. L. NIELSON[7], P. G. POPOVICH[6], J. SCHWAB[6], W. TETZLAFF[8], A. TORRES ESPÍN[9], K. FOUAD[9], A. R. FERGUSON[1,10]
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Abstract: Spinal cord injury (SCI) involves changes to the cellular, molecular, and tissue integrity of the spinal cord. SCI results in deficits in of motor control and mobility, and sensory and autonomic dysfunction. The complexity of SCI limits reproducibility of findings across laboratories and translation of new treatments from bench-to-bedside. The SCI research field has a ‘big-data’ problem; there are too many variables, metrics, and symptoms associated with SCI

to identify a single mechanistic target that generalizes across the full heterogeneity of the SCI syndrome. Data analytics, machine learning, and contemporary data science tools have the potential to enable researchers to query and extract patterns from complex data to form hypotheses and make new discoveries. To use these tools large volumes of data are needed, requiring sample sizes exceeding those typically collected within a single laboratory. Cultural skepticism to data sharing has provided a major barrier to pooling data, limiting the potential of data science. Yet in the past 8 years the SCI research community has demonstrated a growing willingness to share data and work collaboratively, pooling data across 13 laboratories to form the VISION-SCI repository, now housing subject-level data from over 3000 rodents with SCI. A collective of SCI researchers are now focused on building a scalable, structured data sharing platform that enables users to upload, query, and download data: the Open Data Commons for Spinal Cord Injury (http://ODC-SCI.org), a partnership with the Neuroscience Information Framework (part of the NIH Neuroscience Blueprint Initiative). The ODC-SCI platform is compatible with NIH-endorsed data stewardship principles that biomedical data be made FAIR (Findable, Accessible, Interoperable, and Reusable) and the NINDS Common Data Elements (CDE) project for neurotrauma. The ODC-SCI (beta) accommodates raw data underlying published figures as well as unpublished data and metadata. The portal helps to harmonize and democratize data, and grant users access to large volumes of data that are otherwise inaccessible. ODC-SCI has the potential to improve reproducibility across laboratories, and hasten new discoveries within SCI research with a data-driven approach.

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Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.07/R3

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Department of Neurological Surgery
RRF at UW
Craig H. Neilsen Foundation
DoD CDMROP

Title: Loss of perfusion measured by ultrafast contrast enhanced ultrasound (CEUS) predicts injury severity following acute spinal cord injury

Authors: *Z. Z. KHAING[1], L. CATES[1], J. HYDE[1], R. HAMMOND[2], M. F. BRUCE[2], C. P. HOFSTETTER[3]
[1]Neurolog. Surgery, [2]Univ. of Washington, Seattle, WA; [3]Dept of Neurolog. Surgery, Univ. of Washington, Seattle, WA

Abstract: Traumatic spinal cord injury (tSCI) often leads to debilitating loss of sensory and motor function at and below the site of injury. Acutely, tSCI causes an almost complete loss of blood flow at the site of injury (primary injury), followed by significant ischemia in the penumbra of the injury, which may contribute to progressive cell death over time (secondary injury). Neuroprotective treatment strategies seek to limit secondary injury. However, techniques to simultaneously monitor temporal and spatial patterns of blood flow in the contused spinal cord are lacking. Here, we utilized a pre-clinical tool enabling visualization of local perfusion changes in real time in a rat tSCI model. Contrast-enhanced ultrasound imaging (CEUS) using Definity® microbubbles provides high resolution and real-time information of local blood perfusion changes in and around regions of tSCI. Using a research ultrasound device (Verasonics Vantage, USA) combined with a 15MHz linear array transducer (Vermon, France), plane-wave nonlinear Doppler acquisitions enabled the visualization of blood flow in the rat spinal cord using 10KHz pulse repetition frequencies. Serial US imaging of the spinal cords was performed at pre-injury, ~15 minutes post, and 8 weeks post contusion injury (Infinite Horizon at T7/T8; 150 or 200 kDyne). Images were acquired over 300 milliseconds at an effective 400 frames per second. Acutely, moderate contusion resulted in a significantly smaller area of perfusion deficit (1.79 ± 0.14 mm^2) compared to animals that sustained a severe contusion injury (2.88 ± 0.38 mm^2)($p<0.03$). In addition, ultrafast CEUS imaging of bolus kinetics found a delay (~ 1 second) in arrival time of microbubbles to tissue adjacent to the hypoperfused area wihtin the injury site, in both moderate and severe injuries. Correlation analysis between area of hypoperfusion and locomotor behavioral scores (BBB test) at 5 days post injury revealed a significant correlation between the extent of perfusion deficit and functional recovery ($r^2 = -0.82$; $p<0.005$). At 8 weeks post injury, there was still a difference in areas of hypoperfusion between mild (1.04 ± 0.42 mm^2) and moderate (1.77 ± 0.27 mm^2), although the difference was no longer significant ($p = 0.18$). Interestingly, there was a significant correlation between area of acute perfusion deficit and chronic (8 week post injury) BBB scores ($r2 = -0.14$; $p<0.001$). These data suggest that local blood perfusion data obtained using ultrafast CEUS imaging can be used to predict lesion severity and functional deficits. Development of intra-operative ultrafast CEUS imaging may be useful in the clinic to determine injury extent and severity in patients.

Disclosures: Z.Z. Khaing: None. **L. Cates:** None. **J. Hyde:** None. **R. Hammond:** None. **M.F. Bruce:** None. **C.P. Hofstetter:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.08/R4

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Gillson Logenbaugh Foundation
Mission Connect

Title: Relationship of gender and inflammation to depression in a rodent model of spinal cord injury

Authors: *K. BRAKEL, M. TERMINEL, S. KAPLER, K. NOVAK, M. HOOK
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Abstract: Major depressive disorder (MDD) is a significant, but understudied, consequence of spinal cord injury (SCI). Approximately 11-24% of SCI patients experience MDD, compared to 8% in the general population. However, in the general population, females are twice as likely to develop depression as males. While this trend would be expected to persist in the SCI population, epidemiological data has so far proved inconclusive. As the importance of gender specific treatments becomes more apparent throughout medicine, it also becomes critical to consider gender differences in research. Previously, we have shown that approximately one-third of male spinally injured rats exhibit behavioral, physiological, and immunological correlates of depression. This incidence is commensurate with the clinical population. Here, we compare depression in age-matched male and female Sprague Dawley rats. An array of depression-like behaviors (social activity, sucrose preference, forced swim, open field activity, burrowing) were examined prior to injury and for twenty-four days post-injury. Females had higher sucrose preference at the end of the study, indicating lower depression, but males and females also exhibited behavioral differences before injury. Males had higher center time activity in an open field, while females displayed higher social activity. Additionally, females expressed lower levels of serum pro-inflammatory cytokines TNFα, IL-17, and IL-18 than did the males before injury, and 10 days post-injury, female cytokine levels increased to levels commensurate with the males. However, by 24 days post-injury, anti-inflammatory cytokines IL-2 and IL-4 had also increased to levels higher than those of the males. These results indicate that there is a gender-specific response on a molecular level after SCI. They also reiterate the importance of considering gender-specific treatments after SCI, as pre-existing differences in biology and behavior may exist and persist throughout recovery.

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Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.09/R5

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NHMRC Grant RM09884

Title: Effects of a transection to the dorsolateral funiculus on ulnar nerve excitability in the rat

Authors: *B. WILD, R. ARNOLD, R. MORRIS
Sch. of Med. Sci., Univ. of New South Wales (UNSW Sydney), Sydney, Australia

Abstract: Assessment of nerve excitability with the use of electrophysiological techniques is an essential tool for investigating the function of peripheral nerves following spinal cord injury (SCI). One significant outcome of a SCI is the abolishment of the supraspinal input to the motor neurons below the level of the transection, therefore, resulting in paralysis. Clinical studies investigating the underlying mechanisms of these deleterious effects have been impeded by the heterogeneity of patients and limitations of conventional electrophysiological procedures. Nerve excitability testing (NET) is a sophisticated electrophysiological method that enables the indirect assessment of peripheral nerve function. More specifically, NET provides information regarding pathophysiological and biophysical changes that precede irreversible degenerative events after SCI. This study aimed to examine whether transections to the dorsolateral funiculus (DLF) induce abnormalities in ulnar nerve function that could be detectable by NET. NET was performed on 13 adult Long Evans rats one week prior and after DLF transections. Comparing the nerve excitability results between pre- and post- SCI demonstrated significant differences in multiple parameters. Post-SCI axons required significantly greater stimulus intensity (mV) to elicit a 50% compound muscle action potential (CMAP) response ($p<0.05$*).Post-SCI axons also showed a significantly greater threshold change to long hyperpolarising currents (hyperpolarising threshold electrotonus (-119.17 ± 7.41) when compared with the control values (-161.62 ± 4.86, $p<0.01$**). The resting current-threshold parameter was significantly reduced on the post-SCI recording compared to the baseline ($p<0.05$**). Both the resting current-threshold and threshold electrotonus parameters are sensitive measures of membrane potential. Taken together, these results suggest increased threshold for activation and with evidence of hyperpolarisation following a transection to the DLF. In conclusion, this study demonstrates that a DLF transection has a significant effect on ulnar nerve excitability properties, specifically nerve hyperpolarisation, following the loss of input to the ulnar nerve resulting from the DLF transection at the cervical level.

Disclosures: B. Wild: None. **R. Arnold:** None. **R. Morris:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.10/R6

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H Neilsen Foundation 457328

Title: A translationally relevant model of inducible pneumonia after spinal cord injury

Authors: *A. R. FILOUS[1], B. BROMMER[2], J. M. SCHWAB[1]
[1]The Ohio State Univ., Columbus, OH; [2]F.M.Kirby Neurobio. Ctr., Childrens Hosp. Boston, Boston, MA

Abstract: After spinal cord injury (SCI), patients become immunocompromised, making them highly susceptible to infections. Not only is pneumonia the leading cause of death after SCI, but even patients that survive the infection have a reduced potential to regain function, compared to SCI patients that have not suffered an infection. The mechanism is unclear as to how infections during an early stage after injury can lead to chronic changes in recovery potential. We have developed a mouse model of inducing pneumonia in a controlled fashion after SCI, as well as a scale to assess and track their sickness severity after infection. This model is translationally relevant, as it mimics many aspects of human SCI, and it can be used to explore the underlying mechanisms of this impaired recovery after infection.

Disclosures: A.R. Filous: None. **B. Brommer:** None. **J.M. Schwab:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.11/R7

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NJDOH Grant CSCR15FEL002

Title: Resting state functional connectivity of the thalamus in complete spinal cord injury

Authors: *K. KARUNAKARAN[1], R. YUAN[2], J. HE[3], J. ZHAO[4], J.-L. CUI[3], Y.-F. ZANG[5], Z. ZHANG[3], B. B. BISWAL[1]

[1]Dept. of Biomed. Engin., New Jersey Inst. of Technol., Newark, NJ; [2]Dept. of Psychiatry and Behavioral Sci., Stanford Sch. of Med., Stanford, CA; [3]Hebei Med. Univ. Third Affiliated Hosp., Shijazhuang, China; [4]Dept. of Radiology, Armed Police Force Hosp. of Sichuan, Leshan, China; [5]Ctr. for Cognition and Brain disorders, Hangzhou Normal Univ. Affiliated Hosp., Hangzhou, China

Abstract: Spinal Cord Injury (SCI) is often a result of compression to the spinal cord leading to neurological changes in both anterograde and retrograde direction from site of injury. Neuroimaging studies in SCI have mostly examined the functional organization of the cortex with limited attention to the sub-cortical substrates of the injury. Further, recovery rate of SCI population is relatively poor, often with secondary complications such as chronic pain, phantom limb and spasticity etc. Prevailing theories of chronic pain in the central nervous system indicates a dysfunction in the spinothalamo-cortical pathway. Besides, the modern view of the thalamus as both a driver and modulator demands the investigation of individual thalamic sub-nuclei to gain insight into thalamic neuroplasticity following deafferentation. To accomplish this, we used resting-state functional magnetic resonance imaging to perform both data driven and model driven connectivity analysis of the different thalamic sub-nuclei. A non-parametric two-sample t-test with permutations was performed for each of thalamic nuclei to compute functionality connectivity (FC) differences between 19 healthy controls (10 females) and 17 complete SCI (3 females) subjects with paraplegia. Results using data driven technique showed thalamic nulcei corresponding to anterior default mode network to exhibit decreased network strength with prefrontal cortex in SCI group. Results using model driven technique showed bilateral mediodorsal nucleus in SCI group to exhibit reduced functional connectivity with right middle temporal gyrus, dorsal anterior cingulate cortex, and insula. Additionally, left pulvinar nucleus of SCI group demonstrated a significant increase in connectivity with left inferior frontal gyrus and at a lower threshold with regions of the frontoparietal network. This is the first study to explore thalamic functional connectivity following SCI in humans. Our study establishes the use of resting-state fMRI to examine the functional alterations of the different thalamic sub-nuclei following SCI.

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Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.12/R8

Topic: C.11. Spinal Cord Injury and Plasticity

Support: French National Research Agency (ANR-Carnot Institute)

Fondation Motrice
Fondation de l'Avenir
Fondation Philanthropique Edmond J. Safra

Title: Preliminary results of long term stability epidural ECoG recordings in Human with two wireless WIMAGINE implants

Authors: *T. COSTECALDE[1], S. COKGUNGOR[2], T. AKSENOVA[2], A. YELISYEYEV[2], F. SAUTER-STARACE[2], G. CHARVET[2], A.-L. BENABID[2]
[1]CEA, Grenoble, France; [2]Univ. Grenoble Alpes, CEA, LETI, CLINATEC, MINATEC Campus, Grenoble, France

Abstract: Since several years, several Brain Computer Interface (BCI) approaches were used from EEG to single unit recordings, or ECoG (ElectroCorticoGram) recordings [1]. The WIMAGINE® implant was developed to record ECoG signals for long term clinical applications. This implantable medical device [2] is composed of an array of 64 electrodes, on hermetic titanium housing including the electronic boards, and an antennae for wireless transmission of data and remote power supply.
Bilateral implants were inserted epidurally in a patient using two 50mm craniotomies (https://clinicaltrials.gov/show/NCT02550522). Control ECoG recordings throughout the surgery were obtained. ECoG recordings were also performed up to three times a week during several months.
Results: WIMAGINE® allowed us to perform chronic ECoG recordings for a period of several months after implantation. No change in signal quality has been observed, analysis of evolution in time of ECoG showed a stable signal in frequency and amplitude along time, which is an important criterion for signal processing and treatment with sophisticated algorithms like those developed at Clinatec [3].
Conclusion: Long term signal recordings were obtained for the first time using two novel wireless WIMAGINE® implants, providing further support for BCI trials.
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Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.13/R9

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Paralyzed and non-paralyzed muscle: A comparative analysis in an acute rat spinal cord injury model

Authors: *M. E. HARRIGAN, A. R. FILOUS, T. WARNER, J. M. SCHWAB
Neurol., The Ohio State Univ., Columbus, OH

Abstract: Spinal cord injury (SCI) is a devastating condition that engenders severe disability and affects 250,000-500,000 people annually worldwide. Recent findings suggest that maintenance of muscle mass, the 'substrate' for proprioceptive input back into the spinal cord, plays a substantial role in functional recovery (1) and avoidance of secondary SCI consequences (2,3). To date, explanations for muscle wasting post-SCI have been confined to direct consequences of chronic paralysis, primarily disuse, which offer nominal therapeutic potential. Utilizing thoracic (T3) transection (Txn), to ensure complete paralysis of the lower extremity, in adult male Sprague Dawley rats we systematically investigated for: 1) acute muscle wasting - an optimal time point for intervention, and 2) wasting in non-paralyzed muscle, suggesting targetable systemic mechanisms of atrophy. Harvested Triceps brachii (upper extremity, non-paralyzed) and Gastrocnemius (lower extremity, paralyzed) wet muscle weights established significant early systemic muscle wasting (sarcopenia) also affecting non-paralyzed muscles. We hypothesize that atrophy of paralyzed muscle is likely driven by a combination of upper motor neuron (UMN) injury and systemic consequences of SCI. In contrast, wasting of non-paralyzed muscle, which retains UMN innervation, provides an avenue to pinpoint uniquely systemic consequences. Here we provide a characterization between paralyzed and non-paralyzed muscle, which will inform future mechanistic studies of this early and systemic phenomenon. Differential analysis includes Hematoxylin & Eosin (H&E) and Succinic Dehydrogenase (SDH) enzymatic histology to investigate morphological changes and metabolic fiber type shifting/rearrangement. Motor unit number estimation (MUNE) electromyography was used to determine whether UMN injury and/or systemic SCI effects affect function of lower motor neuron units. Lastly, we measured *in vivo* and *in situ* muscle contractility to quantify the functional impact of atrophy on muscle function.

Ref:
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recovery and circuit reorganization after spinal cord injury. Cell. 2014 Dec 18;159(7):1626-39.
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3. Sezer N, Akkuş S, Uğurlu FG. Chronic complications of spinal cord injury. World J Orthop. 2015 Jan 18;(1):24-33.

Disclosures: M.E. Harrigan: None. **A.R. Filous:** None. **T. Warner:** None. **J.M. Schwab:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.14/R10

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation

Title: Spinal cord injury differentially modifies peripheral and central BDNF and TrkB expression

Authors: *S. PARVIN[1], S. M. GARRAWAY[2]
[1]Physiol., Emory Univ., Atlanta, GA; [2]Physiol., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Although chronic neuropathic pain is a clinically challenging outcome of spinal cord injury (SCI), the underlying neurobiological mechanisms are not fully elucidated. The neurotrophin BDNF and its receptor TrkB have been implicated in central sensitization and nociceptive plasticity, particularly associated with inflammatory pain. However, whether BDNF or TrkB plays a role in neuropathic pain after SCI is less understood. In prior studies, we showed noxious stimulation that increased the expression of mechanical allodynia after SCI reduced spinal expressions of BDNF and TrkB, suggesting that pain after SCI may even be independent of central BDNF-TrkB signaling [Garraway et al. *Neuroscience 199: 86-102; 2011* and reviewed by Garraway & Huie JR; *Neural Plast. 2016*]. It was recently shown that low threshold mechanoreceptors (LTMRs) include a population known as Aδ-LTMRs. Aδ-LTMRs innervate hairy skin and signal directional touch. They express TrkB and require BDNF-TrkB signaling for normal function. The genetic identification of Aδ-LTMRs provides a prospective mechanism by which BDNF-TrkB signaling can contribute to pain after SCI, one involving Aδ-LTMR dysfunction. In this study, we assess central and peripheral changes in BDNF and TrkB expression after SCI in adult $TrkB^{CreER}$ mice, which allow for selective targeting of Aδ-LTMRs. The mice received a contusion SCI at T10 or a sham surgery. At 21 days after surgery, they were assessed for mechanical sensitivity with von Frey hairs, following which cellular assays were undertaken to measure BDNF and TrkB expression in the injured spinal cord and adjacent trunk

skin. SCI mice showed significant hind-paw sensitivity compared to pre-surgery baselines ($p<.0001$; *paired t test*) and sham controls ($p<.001$; *t test*). qRT-PCR showed that TrkB and BDNF mRNA were significantly reduced in the lesioned spinal cord, but significantly increased in most trunk skin sites examined ($p<.05$; *t test*), Similarly, western blot analyses showed that compared to sham subjects, TrkB protein was increased in the skin ($p<.05$; *t test*), although BDNF protein expression was unchanged. Preliminary histological studies in TrkB::tdtomato mice also showed a redistribution of TrkB expression after SCI, with a trend towards an increase in expression in the trunk skin. Current studies are assessing changes in BDNF and TrkB expression at an earlier time-point after SCI. Overall, these data suggest that peripheral BDNF-TrkB mechanisms which may involve Aδ-LTMRs' dysregulation, is likely contribute to pain hypersensitivity after SCI.

Disclosures: S. Parvin: None. **S.M. Garraway:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.15/R11

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Department of Defense (DoD) Spinal Cord Injury Research Program (SCIRP) Grant

Title: Behavioral conditioning approaches to investigate and reverse effects of peripheral afferent stimulation in a mouse model of neuropathic pain after spinal cord injury

Authors: *D. J. NOBLE, R. DONGMO, S. M. GARRAWAY
Physiol., Emory Univ., Atlanta, GA

Abstract: Recently, our lab has been investigating cutaneous afferents known as C-LTMRs that innervate hairy skin and normally encode for pleasant, affiliative touch. These afferents may be converted to transduce mechanical allodynia following spinal cord injury (SCI). We recently found that mechanical stimulation delivered at the level of injury and tuned to selectively recruit C-LTMRs evoked acute increases in respiratory rate (RR) in adult mice 1 week after SCI ($p < .05$). We have now shown that mice with a contusion SCI also avoid a context associated with this stimulation in a conditioned place aversion (CPA) setup. This increase in preference for the light "escape" chamber progressively developed over the course of 5 weeks, reaching significance at 21, 28, and 35 days post injury ($p < .05$ in each case). Given the different timelines of RR and behavioral changes, early RR increases could predict the emergence of affective pain. Here, we performed a series of pilot studies to assess the efficacy of a novel feedback-based strategy to reverse RR increases after SCI. Adult C57BL/6 or Th::ChR transgenic (for optogenetic targeting of C-LTMRs) mice received a T10 contusion SCI (70

kdynes, IH impactor) or sham surgery and were assessed starting 1 week after surgery. At weekly time points, repeated truncal stimulation (once/min for 10 mins) was administered in a modified CPA paradigm, either with a small brush or blue laser to mechanically or optically activate C-LTMRs. We then tested the feasibility of slow respiratory rate (SRR) training using a paradigm developed in uninjured rats to lower RRs over time and potentially reduce reactivity to stressful and nociceptive stimuli. Mice underwent 10-15 daily 2-hour SRR training sessions, during which RR was continuously recording via remote electric field sensors. Recorded data was processed by a customized interface in LabVIEW to monitor breathing and provide real-time LED feedback (aversive strobe light) that turned off whenever RR $\leq$ 240 breaths/min. SCI mice significantly decreased their RR from baseline by the second SRR training session ($p < .05$) and spent ~80% of each session below the target RR. Control animals trained using reversal conditioning procedures (rewarded for RR $\geq$ 220 breaths/min) did not experience a similar decrease. Furthermore, post-training RRs in SCI mice were statistically indistinguishable from resting RRs in a cohort of age-matched, experimentally naïve mice. These results demonstrate adaptability of SRR conditioning procedures to mice for studies into neuropathic pain following SCI. Ongoing studies are examining the impact of SRR training on C-LTMR-mediated pain aversion and stress-associated behaviors.

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Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.16/R12

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Helmsley Center for Restorative Medicine Grant G2746

Title: Assessment of bowel function after human spinal cord injury

Authors: *A. N. HERRITY[1,2], K. JOHNSON[1,2], T. ABELL[3], S. J. HARKEMA[1,2], C. HUBSCHER[1,4]
[1]Kentucky Spinal Cord Injury Res. Ctr., [2]Neurolog. Surgery, [3]Gastroenterology, [4]Anatom. Sci. and Neurobio., Univ. of Louisville, Louisville, KY

Abstract: Spinal cord injury (SCI) results in profound changes to sensorimotor function and autonomic systems, including bowel dysfunction, which is ranked as a top problematic issue affecting quality of life. The prevalence of bowel dysfunction includes dysmotility, chronic constipation and difficulty with evacuation, unexpected episodes of fecal incontinence, as well as autonomic dysreflexia associated with a distended rectum. Many individuals living with SCI are also dependent on a caregiver to assist with toileting procedures and previous studies have

indicated that time dedicated to one's bowel program may take up to 2-3 hours in SCI populations. Based off questionnaires with participants using the International Spinal Cord Injury Data Set for bowel function, we previously reported a significant improvement in participants' time required to complete their bowel program following activity-based, task-specific locomotor training. To begin understanding mechanisms underlying this and other subjective improvements reported in questionnaires, physiological measurements of bowel function were obtained in this feasibility study via anorectal manometry testing. Individuals (n=15) having either complete or incomplete (AIS A and B) SCI participated. Measurements of internal and external anal sphincter pressure, squeeze increase pressure, presence of the recto-anal inhibitory reflex, anorectal sensations, and balloon expulsion time were obtained. Mean squeeze pressure and squeeze increase pressure values were found to be below recommended guidelines in both AIS A and B participants. Sensations of rectal fullness and urge were noted to occur at lower thresholds in AIS B participants as compared to AIS A participants. These data provide quantifiable evidence for the occurrence of anorectal dysfunction following chronic SCI, including a deficit in the ability to generate sufficient squeeze pressure necessary for preventing fecal incontinence as well as for expulsion of contents, necessitating the use suppositories and/or digital evacuation. Delays in sensation underscores the importance of maintaining regularly scheduled bowel programs to prevent constipation.

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Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.17/R13

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Helmsley Restorative Medicine Pilot Grant

Title: Development of a comprehensive protocol for detecting bowel dysfunction after spinal cord contusion in wistar rats

Authors: *R. F. HOEY[1], C. HUBSCHER[2]
[1]Anatom. Sci. and Neurobio., Univ. of Louisville Sch. of Med., Louisville, KY; [2]Dept Anatom. Sci. & Neuro, Univ. Louisville Sch. Med., Louisville, KY

Abstract: Bowel dysfunction is highly prevalent after spinal cord injury (SCI) with 95% of patients requiring some method to induce defecation. Symptoms of bowel disruption are typically: reduced motility, constipation, fecal impaction, anal sphincter dysfunction, incontinence, and autonomic dysreflexia. These complications can lead to significant impairment

in quality of life (QOL) as well as numerous hospitalizations. Anorectal manometry (ARM) is a technique to measure function in the distal colon, rectum, and anal sphincter in humans. Initial data collected in human patients shows that locomotor training improves bowel function as shown by reduced amount of time to complete their bowel program. ARM techniques have not seen widespread use in animal models of SCI. Therefore, the goal of this preliminary study was to develop an animal model that included ARM and motility measurements for a comprehensive picture of bowel function after SCI in Wistar rats. This model can then be used to elucidate the underlying mechanisms of dysfunction after SCI and test possible therapeutic interventions. The current study has utilized weekly motility measures (24 hour collections from metabolic and home cages) and terminal procedures (rectal and bowel ARM, anal sphincter electromyography (EMG), EMG response to balloon distension of the sphincter, and responses to balloon inflation at many levels of the rectum/distal colon) to investigate bowel function after a T9 contusion (210 kDyne) injury in male Wistar rats (n=36). Food and water intake, urine volume, and animal weight were also recorded. Initial analysis (repeated measures ANOVA) found that there is a significant increase in fecal pellet production in the first week after contusion. After the first week there is a decline in pellet production that becomes significantly reduced from baseline after 35 days postinjury and continues up to 12 weeks post-injury. This decrease is not due to reduced food intake or weight loss. ARM recordings show feasibility of the procedure to detect ongoing bowel activity and responses to stimulation in a chronic SCI model. These data are in line with bowel symptoms in humans and support the development of this animal model for use in mechanistic and translational studies.

Disclosures: R.F. Hoey: None. **C. Hubscher:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.18/R14

Topic: C.11. Spinal Cord Injury and Plasticity

Support: MSFHR
Craig Nielsen Foundation
Rick Hansen Institute
ICORD

Title: The impact of high-thoracic spinal cord injury on cardiac contractility in a porcine model

Authors: *N. MANOUCHEHRI[1], A. M. WILLIAMS[1], K. TAUH[1], M.-S. POORMASJEDI-MEIBOD[1], R. BOUSHEL[1,2], K. SO[1], K. SHORTT[1], K.-T. KIM[3,5], M. WEBSTER[1], F. STREIJGER[1], B. K. KWON[1,4], C. R. WEST[1,2]
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Abstract: Introduction: High-thoracic spinal cord injury (SCI) is a devastating condition characterized by a loss of descending sympathetic input to the heart and vasculature. Chronically, high-thoracic SCI leads to cardiac unloading, myocardial dysfunction and atrophy; however, the immediate or acute cardiac consequences of SCI are not understood. Therefore, this study therefore examined the impact of acute T2 SCI on left ventricular (LV) function using a pig model of SCI. **Method:** Seven Yucatan mini-pigs (24.2±2.5 kg) were instrumented with a LV pressure-volume admittance catheter to assess LV load-dependent function (i.e. stroke volume, SV; maximal rate of pressure generation, dp/dt_{max}), load-independent contractile function (i.e. slope of end-systolic pressure-volume relationship, $ESPVR_{slope}$) and arterial elastance (E_a), and a Swan-Ganz catheter advanced to the pulmonary artery for thermodilution measurements of cardiac output ($\dot{Q}$). Animals received a T2 contusion SCI with compression that was removed 2hrs post-SCI. Measurements were taken at baseline (pre-SCI) and hourly up to 4hrs post-SCI. Data are presented as mean±SD, pre-SCI versus 4hrs post-SCI. **Results:** Mean arterial pressure (87±15 vs. 77±12mmHg, p=0.047) and E_a (3.71±1.20 vs. 2.85±1.08mmHg·ml^{-1}, p=0.001) were lower post-SCI. SV was increased (28±7 vs. 36±11ml, p=0.04) while $\dot{Q}$ was not significantly altered post-SCI (2.73±0.62 vs. 2.98±0.36L·min^{-1}). Although load-dependent dp/dt_{max} was unchanged following SCI (1628±218 vs. 1698±238mmHg·s^{-1}), the $ESPVR_{slope}$ was reduced by 24% post-SCI (2.52±0.80 vs. 1.90±0.66mmHg·ml^{-1}, p=0.03). **Conclusion:** These data are the first to demonstrate impaired LV contractility immediately following high-thoracic SCI, providing novel evidence that sympathetic decentralization is a key contributor to LV systolic dysfunction after SCI.

Disclosures: N. Manouchehri: None. **A.M. Williams:** None. **K. Tauh:** None. **M. Poormasjedi-Meibod:** None. **R. Boushel:** None. **K. So:** None. **K. Shortt:** None. **K. Kim:** None. **M. Webster:** None. **F. Streijger:** None. **B.K. Kwon:** None. **C.R. West:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.19/R15

Topic: C.11. Spinal Cord Injury and Plasticity

Support: ICORD Seed Grant
VGH & UBC Hospital Foundation

Title: Quantitative 7T magnetic resonance imaging and histologic analysis of post-mortem human spinal cord injury specimens

Authors: *C. LAULE[1,2,3,4], H. LIU[1,3], P. KOZLOWSKI[1,3,4,5], A. YUNG[1,3,5], A. T. BAUMAN[1,3,5], F. SAMADI[2,3], A. ALUDINO[6], L. PARKER[8], K. DONG[3], F. STREIJGER[3], W. MOORE[2,3,7], B. K. KWON[3,6]
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Abstract: Introduction: While animal studies have populated much of our knowledge about the histopathologic sequelae of spinal cord injury (SCI), empirical studies of human spinal cords after traumatic injury are comparatively rare. Furthermore, past human studies have largely described histopathologic findings without correlation to detailed clinical information regarding the nature and severity of neurologic impairment or to advanced imaging findings. Magnetic resonance imaging (MRI) has been playing an increasingly important role in spinal trauma patients due to high sensitivity for detection of acute soft tissue damage and cord injuries. In order to provide more quantitative and specific information about spinal cord tissue structure and health, new advanced MRI techniques are being developed. To-date, no study has examined the correlation between clinical findings, advanced MRI metrics and histology using human post-mortem spinal cord injury tissue after traumatic SCI. **Objective:** Our goal is to perform a detailed analysis of the relationships between severity of neurologic impairment, mechanisms of injury, in vivo MRI, ex vivo MRI, and histopathology in human spinal cord tissue obtained post-mortem after traumatic SCI. Such spinal cord tissue has been acquired within our locally established SCI Biobank from individuals who have died after suffering an acute SCI. **Hypothesis:** Severity of neurologic impairment, the mechanism of injury, and quantitative MRI markers for specific aspects of tissue will correlate with histological staining for those same tissue markers. **Methods:** Archived formalin-fixed spinal cord tissue will be used. Neurologic assessments and in vivo spinal cord MRIs acquired prior to death will be reviewed. Spinal cord samples will undergo quantitative MRI at 7 Tesla (T_1, T_2, diffusion, phase, myelin water, inhomogeneous magnetization transfer). Spinal cord tissue will then undergo histological analysis for comparison with the quantitative MRI metrics. The degree and extent of MRI and histology abnormalities will be compared to neurologic scores and injury mechanisms. **Significance:** Our research will shed novel insights into the relationships between neurologic impairment, MRI characteristics, and underlying histopathology in human SCI. We will validate how accurate new MRI methods are at measuring different kinds of damage to the spinal cord. Validating new MRI techniques, and understanding their limitations, is an important next step in moving these more sophisticated and quantitative MR methods towards every-day use in the clinic, and for testing new treatments.

Disclosures: C. Laule: None. **H. Liu:** None. **P. Kozlowski:** None. **A. Yung:** None. **A.T. Bauman:** None. **F. Samadi:** None. **A. Aludino:** None. **L. Parker:** None. **K. Dong:** None. **F. Streijger:** None. **W. Moore:** None. **B.K. Kwon:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.20/R16

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Urodynamics and histological evaluation of the bladder in a porcine model of spinal cord injury

Authors: *M. S. KEUNG[1], E. G. DEEGAN[1,2], F. STREIJGER[1], M. WEBSTER[1], C. MORRISON[1], E. B. OKON[1], N. MANOUCHEHRI[1], K. SHORTT[1], K. SO[1], K.-T. KIM[1,5], L. C. SHERWOOD[6], A. HERRITY[6], C. HUBSCHER[6], D. R. HOWLAND[6], M. BOAKYE[6], L. STOTHERS[1,3], B. K. KWON[1,4]
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Abstract: Introduction: One of the most disabling, impactful, and overlooked consequences of spinal cord injury (SCI) is bladder dysfunction. Aside from the enormous impact it has on the day-to-day function of SCI individuals and their overall quality of life, the costs associated with managing related secondary complications (e.g. urinary tract infections, renal stones, renal failure, and even exacerbation of pressure ulcers) are massive. While the rodent model of SCI has been traditionally used for the development of pharmacologic agents aimed at improving bladder function, such small animal models of SCI are not suitable for the development and translation of novel human-sized devices. As such, larger animal species such as dogs, pigs, and goats have been utilized for the evaluation of bladder devices, albeit not in the context of SCI with a neurogenic bladder. Hence, in this study we investigated the features of morphological and functional changes occurring in the urinary bladder of spinal cord injured pigs. **Method:** SCI was induced by weight-drop contusion at T10 in female Yucatan pigs. Urodynamics and external urethral sphincter (EUS)-electromyography (EMG) assessments were performed simultaneously in awake, slightly restrained (using a sling) pigs on various days post-SCI. In addition, volume and flow rate during voiding and bladder histology was assessed. Voiding efficiency was also calculated as the ratio between voided and infused volume. **Results:** During voiding bladder contractions, pre-SCI pigs exhibited voiding with simultaneous increases in Pdet (detrusor pressure) and Pves (bladder pressure), which occurred during periods of reduced EUS-EMG activity. While fluid elimination from the urethra in SCI pigs coincided with a similar increase in Pdet and Pves, EUS-EMG bursting activity was sustained during these changes. SCI pigs also showed reduced voiding efficiency by almost 10-fold compared to pre-SCI pigs. Hematoxylin

and eosin staining of bladder tissue showed detrusor muscle hypertrophy. Moreover, the urinary bladder of SCI animals showed a 1.5-fold increase in wet weight. **Conclusion:** Spinal cord contused pigs demonstrated detrusor over-activity; which, may have obstructed micturition resulting in incomplete bladder emptying and high residual urine volumes. Our pig model of SCI allows for repetitive measurements of both bladder and EUS function at different time points in the same animal under fully awake conditions and opens promising avenues to investigate lower urinary tract dysfunction in a translational approach.

Disclosures: M.S. Keung: None. **E.G. Deegan:** None. **F. Streijger:** None. **M. Webster:** None. **C. Morrison:** None. **E.B. Okon:** None. **N. Manouchehri:** None. **K. Shortt:** None. **K. So:** None. **K. Kim:** None. **L.C. Sherwood:** None. **A. Herrity:** None. **C. Hubscher:** None. **D.R. Howland:** None. **M. Boakye:** None. **L. Stothers:** None. **B.K. Kwon:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.21/R17

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Rick Hansen Institute
Brain Canada Foundation
Craig H. Neilsen Foundation

Title: MicroRNA biomarkers in CSF and serum reflect injury severity in human acute traumatic spinal cord injury

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Abstract: Introduction: Spinal cord injury (SCI) is a devastating condition with high variability in injury mechanisms and neurologic recovery. Neurologic impairment following SCI is measured and classified by functional examination, and is extremely challenging in the acute setting, lacking sensitivity and having poor prognostic capacity. The lack of objective tools to

classify injury severity and predict outcome impedes the success of clinical trials and therapeutic development for spinal cord injury. Biological markers (biomarkers) that accurately classify injury severity and predict neurologic outcome would represent a paradigm shift in the diagnosis of patients, and in the way clinical trials are conducted. MicroRNA have emerged as attractive biomarker candidates in neurological disorders due to their stability in biological fluids, their phylogenetic similarities, and their tissue specificity.
Method: We have used next-generation sequencing and machine-learning algorithms to identify microRNA associated with injury severity within the CSF and serum of human patients with acute traumatic SCI. Human CSF and serum samples were obtained at five time points (~24, 48, 72, 96 and 120 hours post-injury) from 42 individuals with an acute SCI (22 AIS A, 10 AIS B, 10 AIS C) and 6 non-SCI control patients. Next-generation sequencing data was validated using TaqMan real-time PCR.
Results: We analysed sequencing data from plasma- and CSF derived microRNA from 42 SCI patients and identified over a hundred human extracellular microRNA that are dramatically elevated after SCI. We identified a set of microRNA whose profiles and dynamics can discriminate injury severity between SCI patients.
Conclusion: CSF and serum microRNA have the potential to serve as novel biomarkers for the evaluation of injury severity of SCI or other forms of traumatic, acute, neurologic injury.

Disclosures: S.S. Tigchelaar: None. **R. Gupta:** None. **C. Shannon:** None. **F. Streijger:** None. **S. Sinha:** None. **S. Flibotte:** None. **M.A. Rizzuto:** None. **J. Street:** None. **S. Paquette:** None. **T. Ailon:** None. **N. Dea:** None. **C. Fisher:** None. **M.F. Dvorak:** None. **J. Mac-Thiong:** None. **S. Parent:** None. **C.S. Bailey:** None. **S. Christie:** None. **K.R. Vankeuren-Jensen:** None. **C. Nislow:** None. **B.K. Kwon:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.22/R18

Topic: C.11. Spinal Cord Injury and Plasticity

Support: CDMRP
SCIRP

Title: Measuring the effects of mean arterial pressure changes on spinal cord hemodynamics in a large animal model of acute spinal cord injury, using a novel optical technique

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Abstract: Introduction: Current clinical practice guidelines for acute SCI patients recommend that the mean arterial pressure (MAP) be augmented to 85-90 mmHg to increase spinal cord (SC) perfusion and potentially improve neurologic function. However, clinicians who hemodynamically manage acute SCI patients with MAP augmentation must do so without any real-time physiologic information about what the MAP augmentation is actually doing within the injured cord. A non-invasive method for measuring these parameters inside the injured SC would greatly enhance the ability of clinicians to wisely optimize the hemodynamic management of acute SCI. The purpose of this study was therefore to develop an implantable optical sensor, based on Near Infra-Red Spectroscopy (NIRS), for non-invasive real-time monitoring of regional SC tissue oxygenation and hemodynamics after SCI. **Methods:** Nine Yorkshire pigs weighing between 25-30 kg underwent a dorsal laminectomy at T10 and received a contusion/compression weight-drop injury. A multi-wavelength NIRS system with a custom-made miniaturized optical sensor was applied directly onto the dura at T9 to non-invasively measure tissue oxygenation and hemodynamics within the SC. To validate the NIRS measures, an invasive Intraparenchymal (IP) combined O_2/blood flow sensor was inserted directly into the SC adjacent to the NIRS probe at T11. Using NIRS, the SC tissue oxygenation percentage (TOI%) as well as concentrations of oxygenated, deoxygenated and total hemoglobin were monitored after SCI and during episodes of MAP alterations. Using norepinephrine and nitroprusside, MAP was increased and decreased by 20mmHg for 30 min periods, simulating the types of hemodynamic changes that SCI patients experience post-injury. **Results:** Changes in SC hemodynamics and oxygenation levels were detected in all subjects as measured by both the invasive IP and the non-invasive NIRS sensors. Significant changes of TOI% during MAP increase (1.64 ± 1%, $p<0.005$) and decrease (-3.97 ± 2.17%, $p<0.005$) were indicative of a significant effect of MAP alterations on tissue oxygenation within the injured cord. A consistent decrease in TOI (-15.94 ± 12.14%, $p<0.005$) was observed following SCI, indicating SC tissue hypoxia at the injury site. **Conclusions:** We have demonstrated that our novel NIRS sensor has the potential to monitor real-time post-SCI changes in SC oxygenation and hemodynamics. This pre-clinical demonstration of the ability of NIRS to achieve this is the first step in developing a clinically applicable device that spine surgeons could potentially place on the dura at the time of surgical decompression to monitor SC tissue hemodynamics post-injury.

Disclosures: B. Shadgan: None. **N. Manouchehri:** None. **K. So:** None. **K. Shortt:** None. **M. Webster:** None. **K. Kim:** None. **A. Fong:** None. **F. Streijger:** None. **A. Macnab:** None. **B.K. Kwon:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.23/S1

Topic: C.11. Spinal Cord Injury and Plasticity

Support: CDMRP Log Number: SC130008

Title: Differences in morphometric measures of the uninjured porcine spinal cord and dural sac predict histological and behavioral outcomes after traumatic sci

Authors: *K.-T. KIM[1,3], F. STREIJGER[1], K. SO[1], N. MANOUCHEHRI[1], K. SHORTT[1], E. B. OKON[1], S. TIGCHELAAR[1], C. MORRISON[1], A. FONG[1], M. S. KEUNG[1], J. SUN[1], E. LIU[1], B. K. KWON[1,2]
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Abstract: Introduction: One of the challenges associated with conducting experiments in animal models of traumatic SCI is inducing a consistent injury with minimal variability in the degree of tissue damage and resultant outcomes. In this study we evaluated how the variability in morphometry of the spinal cord and surrounding cerebrospinal fluid (CSF) contributes to the variability in behavioral and histologic outcomes in our porcine model of SCI. **Methods:** Using intra-operative ultrasound imaging, the morphometry of the spinal cord and surrounding CSF compartment at the impact site were assessed in 7 Yucatan mini-pigs undergoing a weight-drop T10 contusion-compression injury. Bivariate and multivariate *analysis and modeling* were used to identify native morphometric determinants of the inter-animal variability in histological and behavioral outcomes. **Results:** The measured biomechanical impact parameters (force, impulse, velocity, displacement) did not correlate with the histologic measures or hindlimb walking behavior (Porcine Thoracic Injury Behavior Scale, PTIBS). In contrast, clear associations were revealed between pre-SCI cord morphometry and the amount of white matter and tissue sparing. Specifically, the dorso-ventral diameter of the dural sac and ventral CSF space were strong predictors of behavioral and histologic outcome and together explained ≥95.0% of the variance. Additionally, a dorso-ventral diameter of the spinal cord less than 5.331 mm was a strong contributing factor to poor behavioral recovery over 12 weeks. **Conclusion:** These results indicate that inter-animal variability in cord morphometry provides a potential biologic explanation for the observed heterogeneity in histological and behavioral *outcomes*. Such knowledge is helpful for appropriately balancing experimental groups for future studies.

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Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.24/S2

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Diagnostic and prognostic potential of serum and CSF UCHL-1 in acute traumatic spinal cord injury

Authors: *A. CHEUNG[1], S. STUKAS[2], J. GILL[2], K. DONG[1], F. STREIJGER[1], C. WELLINGTON[1,2], B. K. KWON[1,3]
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Abstract: Introduction: Currently there are few treatment options for patients with acute SCI, and clinical trials of novel therapies for acute SCI are exceedingly difficult to conduct. A major obstacle for translational research in acute SCI is the lack of biomarkers that can be utilized to objectively stratify injury severity and predict outcome. Research in neurochemical biomarkers for acute SCI is facilitated by the availability of both blood and cerebrospinal fluid (CSF) samples. Many biomarkers that have been shown to be associated with neurological conditions, however, are not present in high enough concentrations in the blood to be detected with today's standard assay technology. Here, we will evaluate CSF and serum samples obtained from patients with acute SCI for the protein Ubiquitin C-Terminal Hydrolase L1 (UCH-L1) using the ultra-sensitive Quanterix Simoa assay platform. **Method:** CSF and serum samples were collected as part of an ongoing clinical initiative in which acute SCI patients have had lumbar intrathecal catheters inserted for the collection of CSF over the first 5 days post-injury. This multicenter clinical initiative has amassed CSF and blood from acute SCI patients with prospectively collected neurologic outcomes at 6 months post-injury (ClinicalTrials.gov NCT01279811). UCH-L1 concentrations and time-course was measured using the Quanterix Simoa platform, and correlated to injury severity and neurologic recovery. **Results:** Our data suggest that UCH-L1 levels in CSF are increased in SCI patients compared with non-SCI controls, with levels being significantly different between AIS grades and over the course of the 5 days examined. Conversely, there was no significantly difference in serum UCHL-1 between control and SCI subjects. While initial levels of CSF UCH-L1 were not significantly different between those SCI patients who improved an AIS grade over 6 months versus those who did not improve,

categorizing subjects based on the trajectory of CSF UCHL-1 over the first 5 days post injury was 80% accurate in predicting AIS conversion in AIS A subjects. Further, 24-h post-injury CSF UCH-L1 concentrations were negative correlated with motor score change over 6 months. **Conclusion:** Our first evaluation of UCH-L1 in acute SCI shows promise as a biomarker to reflect injury severity and predict outcome in acute SCI. *Further* studies are *currently underway* to evaluate UCH-L1 in serum samples of individuals with SCI and add more CSF samples to our current data set.

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Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.25/S3

Topic: C.11. Spinal Cord Injury and Plasticity

Support: 07-3063-SCR-E-0 New Jersey Commission on Spinal Cord
191152 Craig H Neilson Foundation

Title: Muscle and stepping response with electrical stimulation

Authors: *G. F. FORREST[1], A. RAMANUJAM[2], K. MOMENI[2], E. GARBARINI,[2], C. ANGELI[3], S. J. HARKEMA[3]
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Abstract: Acute spinal cord injury (SCI) leads to unloading, immobilization and induces rapid muscle atrophy and accelerated bone loss in the paralyzed limbs, limiting the ability to stand or walk. Some improvements in muscle and bone have been reported with electrical stimulation (ES) or neuromuscular electrical stimulation (NMES) in SCI, but improvements in standing and walking for given changes in shank muscle volume have not been studied. In this study our aim is to present results for bilateral shank volume, for anterior and posterior compartments and for different stimulation parameters, before and after training. In addition, we will incorporate the effects of ES of multiple leg muscles, especially anterior and posterior shank muscles and weight bearing, on the neural control during standing and stepping in individuals with clinically motor complete SCI. For motor complete SCI, the ES alone group does not improve motor pool activation in the flexors and extensors of the lower limbs during standing or stepping. Motor pool activation during standing does not improve after stand training alone and is lower during stepping (without ES) following all training paradigms. Motor pool activation during stepping (without stimulation) increases only after ES and stand training combined. Data suggests that

gains in neural activation and alterations in neural circuitry after severe human spinal cord injury may require both, repetitive task specific training and sufficient muscle activation.

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Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.26/S4

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NJCSCR CSCR14ERG007

Title: Neuromuscular responses to electrical stimulation ramping profiles

Authors: *R. PILKAR, K. MOMENI, A. RAMANUJAM, E. GARBARINI, G. F. FORREST
Human Performance and Engin. Res., Kessler Fndn., West Orange, NJ

Abstract: During neuromuscular electrical stimulation (NMES), electrical current is applied to a nerve to elicit action potentials in denervated, peripheral muscles. NMES has been used to assist or restore neuromuscular function to paralyzed muscle after spinal cord injury (SCI). Further, chronic application of electrical stimulation (ES) has been shown to have a therapeutic effect on tissue health and voluntary function. Surface electromyography (EMG) provides an effective way to analyze underlying muscle activity. However, the presence of overpowering electrical stimulus artifact limits the assessment of the direct effect of ES on a muscle or nerve. Recent advances in biomedical signal processing have yielded algorithms that show significant success in removing ES artifacts from EMG signals recorded from the electrically stimulated muscle. Previously, we showed the effectiveness of a custom-developed computational algorithm, utilizing empirical mode decomposition (EMD) and notch filtering, to remove the ES artifact from EMG recordings of the electrically stimulated (35 Hz, 300μs) rectus femoris muscle (RF). We showed distinguishable, artifact-free muscle activations during two conditions of "ES-alone" and "ES+VOL," which is ES combined with volitional effort to contract the muscle, in SCI (n=5) and able-bodied (n=5) participants. In this investigation, we extend our analysis to examine the neuromuscular responses to linear increases in ES intensity by studying the artifact-free EMG activities in SCI (n=5) and able-bodied (n=5) participants. We confirm our findings by assessing concurrent torque profiles, measured using an isokinetic dynamometer. The results of this investigation can help to establish a relationship between ES intensity and its resultant neuromuscular response of a targeted muscle group. This could be significant as such relationships have only been established using mechanical outputs such as torque which could be the result of contributions from multiple muscle groups.

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Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.27/S5

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation
Advanced Rehabilitation Research and Training (ARRT) Fellowship

Title: Mechanical measurement of muscle contraction for individuals with spinal cord injury

Authors: *K. MOMENI, A. RAMANUJAM, E. L. GARBARINI, G. F. FORREST
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Abstract: Prolonged immobilization after complete spinal cord injury (SCI) produces a rapid denervation of muscles below the level of injury. To minimize muscle deterioration, mechanical loading interventions have previously examined weight bearing and muscle contractions elicited by neuromuscular electrical stimulation (NMES).
In this work, we employed a novel, non-invasive, tensiomyographic measuring technique (i.e., MC sensor), shown to be accurate and reproducible in measuring muscle tension, to mechanically quantify muscle contraction in able-bodied individuals and those individuals with an SCI. Ramping protocol experiments involving volitional and NMES-induced contractions of the lower limbs established a series of recruitment curves for a given set of stimulation parameters. Muscle tension indices (MC sensor values) were compared to joint torque (Biodex values).
Results indicate a strong correlation for normalized muscle tension indices and joint torque values at each contraction and stimulation intensity. The findings of this preliminary study establishes the reliability of using MC sensors during volitional and NMES-induced isometric contractions.

Disclosures: K. Momeni: None. **A. Ramanujam:** None. **E.L. Garbarini:** None. **G.F. Forrest:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.28/S6

Topic: C.11. Spinal Cord Injury and Plasticity

Support: DOD Grant W81XWH-17-1-0413

Title: Identifying dorsal root ganglion subtype specific molecular changes following spinal nerve ligation in rat

Authors: *M. J. GIACOBASSI[1], S. RAGHURAMAN[5], J. Y. XIE[6], K. CHASE[2], L. S. LEAVITT[3], R. W. TEICHERT[2], F. PORRECA[7], B. M. OLIVERA[4]
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Abstract: Previous work uncovered broad changes in rat DRG (Dorsal Root Ganglion) neurons in receptor and ion channel expression following spinal nerve ligation, notably a massive up-regulation of Bradykinin and ATP receptor expression and a down-regulation of cholinergic and TrpV1 expression 14 days post injury (Raghuraman et. al., manuscript in preparation). We have extended this work by identifying the individual DRG cell types that are undergoing these receptor changes and tracking the progression of their transformation. The dorsal root ganglion (DRG) contains a diversity of somatosensory neurons, which can be clustered into broad cell classes based on a number of morphological and functional characteristics. To differentiate between these cell classes, we dissociate the neurons in culture, and assay between 800-1500 neurons in a single experiment. Using CGRP-GFP antibody staining to identify peptidergic neurons and isolectin B4 for nonpeptidergic nocioceptors, we then apply a host of TRP channel agonists, ATP, ACh, and bradykinin while measuring calcium influx to establish consistent "constellations" allowing further cell type classification. The changes in receptor expression observed after spinal nerve ligation have distinct time courses in the different DRG neuronal subclasses.

Disclosures: M.J. Giacobassi: None. **S. Raghuraman:** None. **J.Y. Xie:** None. **K. Chase:** None. **L.S. Leavitt:** None. **R.W. Teichert:** None. **F. Porreca:** None. **B.M. Olivera:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.29/S7

Topic: C.11. Spinal Cord Injury and Plasticity

Title: A translational assessment of adult human and rat spinal cord neural stem/progenitor cell behaviour

Authors: *A. GALUTA[1,2], C. D. GHINDA[4], M. BEDAIWY[4], M. S. TACCONE[4], M. ALSHARDAN[4], C. LAI[4], J. RABSKI[4], S. CHEN[3], E. C. TSAI[4,1,3]
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Abstract: Rationale: The mammalian spinal cord harbors neural stem and progenitor cells (NSPCs) that are recruited following traumatic injury. NSPCs can be utilized to promote regeneration in animal models through the regulation of their proliferation and differentiation behaviour. However, it is unclear how efficiently adult human SC NSPCs can be modulated towards similarly beneficial fates.
Objectives: To compare the *in vitro* proliferation and differentiation tendencies of adult human and rat spinal cord NSPCs under identical conditions and to direct their fate using signaling factors.
Methods: Thoracic spinal cord was obtained from adult humans (n=15) and rats (n=10) and cultured using the neurosphere assay to expand NSPCs. Primary derived NSPCs (pdNSPCs) were assessed for spontaneous differentiation with serum supplementation (1%) and for proliferation with mitogen treatment (epidermal growth factor, basic fibroblast growth factor2). To direct cell fate, pdNSPCs were treated with retinoic acid (RA), platelet derived growth factor (PDGFα), and bone morphogenic protein-(BMP4) to direct differentiation into neurons, oligodendrocytes and astrocytes, respectively. pdNSPCs were treated for 7 or 14 days, fixed, and characterized by immunocytochemistry (β-III tubulin, GFAP, O4, Sox2, BrdU). BrdU was added 24 hours prior to fixation to track proliferation.
Results: Upon spontaneous differentiation, rat pdNSPCs favored a glial phenotype (74.6±6.7%) consisting mostly of astrocytes (71.0±4.2%) while human pdNSPCs formed mostly neurons (68.5±16.9% for pdNSPCs) with little gliogenesis (<2%). Mitogen stimulation increased proliferation of rat pdNSPCs more than in humans (3.7±0.7 fold and 5.5±0.4 fold, respectively, after a 14 day treatment). Neuronal differentiation of human and rat NSPCs could be enhanced with RA treatment, PDGFα only increased oligodenrocyte differentiation of rat pdNSPCs, and BMP4 only increased astrocyte differentiation of human pdNSPCs.
Conclusion: When cultured identically, adult human and rat spinal cord NSPCs possess distinct differentiation profiles and respond differently to external cues relevant to regeneration. This

information is important for the translation of regenerative strategies targeting endogenous human spinal cord NSPCs.

Disclosures: A. Galuta: None. **C.D. Ghinda:** None. **M. Bedaiwy:** None. **M.S. Taccone:** None. **M. Alshardan:** None. **C. Lai:** None. **J. Rabski:** None. **S. Chen:** None. **E.C. Tsai:** None.

Poster

568. Spinal Cord Injury and Plasticity: Animal Models and Human Studies

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 568.30/S8

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Effect of patient-safety oriented enhanced recovery after surgery (pso-eras) on hospital stays of patients undergoing anterior cervical discectomy

Authors: *J. C.-C. WU[1], Y.-H. CHIANG[2], W.-C. LO[3], J.-H. LIN[4], Y.-S. YANG[5], Y.-S. TSOU[3], K.-Y. CHEN[2]
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Abstract: Introduction: Anterior cervical discectomy is an effective method of treating cervical spondylolisthesis and cervical spine degenerative changes, and patients have excellent outcomes with minimal side effects after surgery. The implementation of ERAS had been beneficial in other fields of surgery, and its benefits in anterior cervical discectomy had not been reported. While the benefits of ERAS are desired, patient safety is paramount. In this study, we implement PSO-ERAS in anterior cervical discectomy, and investigate the effects on post-operative recovery, complications and hospital stays.
Materials and Methods: From July 2016 to June 2017, 214 cases were enrolled into our study. 119 cases from July to December of 2016 received anterior cervical discectomy without PSO-ERAS, 95 cases from January to July of 2017 received the same surgery with implementation of PSO-ERAS. PSO-ERAS included explicit implementation of bed rest 4 hours after surgery, and post-operative surgical radiography of cervical spine. Factors of Age, Gender, underlying diseases, levels of surgery, blood loss, surgery time, length of hospital stay, recovery and complications were recorded. Pre-operative pain scores and Japanese Orthopedic Association (JOA) scores were compared to post-operative pain scores and Japanese Orthopedic Association (JOA) scores.
Results: The age for the patients receiving anterior cervical discectomy ranged from 26 to 80 years old, and 108 males versus 107 females. There are a total of 118 patients with diabetes mellitus, coronary artery disease, and hypertension, 58 in the group without PSO-ERAS, and 60 in the groups with PSO-ERAS. Also, average operating time between the 2 groups was

indifferent, averaging at 173.5 minutes and 184.0 min (p=0.142). The length of hospital stays were significantly different (p=0.027), the group receiving PSO-ERAS was 0.42 days shorter than the group without PSO-ERAS.
Conclusion: We observed shortening of hospital stays with implementation of patient-safety oriented enhanced recovery after surgery (PSO-ERAS). While the shortening of hospital stay requires a larger clinical trial to confirm, the concept of patient safety is important and should be paramount in post-operative patient recovery.

Disclosures: J.C. Wu: None. **Y. Chiang:** None. **W. Lo:** None. **J. Lin:** None. **Y. Yang:** None. **Y. Tsou:** None. **K. Chen:** None.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.01/S9

Topic: C.11. Spinal Cord Injury and Plasticity

Support: MWU intramural grant

Title: Endothelin B receptor agonist, IRL-1620, significantly improves motor functions in an adult rat model of spinal cord injury

Authors: *M. FORNARO[1], H. SHARTHIYA[2], K. RINEHART[3], J. RIDGEWAY[4], M. HORNICK[5], S. BRIYAL[5], A. GULATI[5]
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Abstract: Spinal cord injury (SCI) is a major health issue worldwide which impacts those involved both economically and physiologically. Sci is often mentioned among the first conditions for which stem cells may provide a new therapeutic approach. In fact, the recent discovery of niches of endogenous multipotent stem cells within the adult spinal cord has shed light on stem cell therapies to promote a self-healing mechanism within damaged spinal cord. Endothelin B receptor (ET_B) agonist, IRL-1620 has demonstrated neurogenesis by stimulating neuronal stem cells in several models of CNS disease and injury. Endogenous neuronal stem cells have been shown to be present in the spinal cord. However, the effect of utilizing IRL-1620 in a traumatic SCI model has not been previously investigated. The present study was conducted to determine the therapeutic effects of IRL-1620 on functional motor recovery following experimental SCI in rats. Male Sprague-Dawley rats were randomly divided into 5 groups (n=7-10/group): 1 - Sham surgery, 2 - SCI + vehicle (saline), 3 - SCI + IRL-1620 (1 µg/kg, low dose),

4 - SCI + IRL-1620 (3 µg/kg, mid dose), and 5 - SCI + IRL-1620 (5 µg/kg, high dose). Following a T10 bilateral laminectomy, the Infinite Horizons impactor device was used to create a reproducible, moderate concussive injury of 150kdyn. Treatments of vehicle or IRL-1620 were administered intravenously at 2, 4, and 6 hours on days 1, 3, and 6 post-surgery. Function of each hind limb was evaluated using the Basso, Beattie, Bresnahan (BBB) scale preoperatively and on days 3, 5, 7, 10, 14, 21, 30, 60 post-surgery.
To verify that the extent of the injury was consistent, only animals with significant motor impairment (BBB score 0-7) on day 3 post-surgery were included. For the right hind limb, while not statistically significant, locomotor scores for the low dose IRL-1620 group indicated improved recovery as compared to vehicle at days 14 (+50.2%, p=0.2497), 21 (+34.9%, p=0.4349), 30 (+27.2%, p=0.5099), and 60 (+26.8%, p=0.4882). Motor function in the left hind limb of rats treated with the low dose of IRL-1620, however, was significantly improved as compared to that of vehicle-treated animals at post-surgery day 14 (+63.2%, p=0.023), day 21 (+62.5%, p=0.0171), day 30 (+55.2%, p=0.0284), and day 60 (+45.8%, p=0.0652). Mid and high doses of IRL-1620 improved motor function following SCI but did not reach statistical significance. These results for the first time indicate that IRL-1620 significantly improves hind limb locomotor function following SCI. Further study to determine the mechanism of action of IRL-1620 in SCI repair and the morphological changes at the site of the lesion is in progress.

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Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.02/S10

Topic: C.11. Spinal Cord Injury and Plasticity

Support: The United States Department of Defense (USAMRAH #SC140038

Title: The role of fast inhibition in facilitation of phrenic nerve and diaphragm activity during epidural stimulation following complete cervical spinal cord injury in rats

Authors: *V. MARCHENKO, T. BEZDUDNAYA, M. A. LANE
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Abstract: Spinal cord injury (SCI) at mid- to high-cervical spinal levels often results in life-threatening respiratory complications and requires long-term mechanical ventilator assistance. Thus, restoring diaphragm activity and regaining voluntary control of breathing are the primary clinical goals for patients with respiratory dysfunction following cervical SCI. Epidural stimulation (EDS) is a promising strategy that has been explored extensively for non-respiratory

functions, and to a limited extent within the respiratory system. The goal of the present study was to test the efficacy for EDS applied to the center of phrenic nucleus location (C4 cervical segment, C4-EDS) in combination with intrathecal GABAa and glycine inhibitory receptors antagonists (GABAzine and strychnine) administration on paced breathing following complete C1 cervical transection (C1Tx). To avoid the suppressive effect of anesthesia all experiments were performed in decerebrate, unanesthetized, non-paralyzed or paralyzed animals. High-frequency C4-EDS (100-400 Hz) (240-350 μA, 0.2 ms of biphasic pulse duration, stimulation during 0.3 s, one train per sec) was able to maintain breathing with stable diaphragm EMG (DiEMG), normal end-tidal CO_2 level and raise blood pressure. In addition, 100-300 Hz of C4-EDS showed time- and -frequency dependent changes (short-term facilitation) of evoked phrenic nerve (PN) and DiEMG responses during of each stimulus train that may serve as a target mechanism for pacing of phrenic motor circuits. C4-EDS applied with frequencies 350-400 Hz causes depression of PN and DiEMG responses. Ten minutes after intrathecal application of GABAzine and strychnine, (GABAz+STR, 25 μMol - 30 μl) over C3-C5 segments and C4-EDS (200-300Hz), respiratory flow was increased by 26±7.3% ($p<0.05$), DiEMG - by 19±5.6%, and PN responses by 354±42.9%. Based on these results, we conclude that respiratory circuits at the level of phrenic nucleus are tonically inhibited after C1Tx and their pharmacological modulation has the potential to enhance efficacy of EDS in people with SCI.

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Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.03/S11

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH NINDS NSR01NS089313

Title: Exploration of mechanisms of electrical stimulation of spinal cord microcircuits

Authors: *M. K. CHARDON, M. D. JOHNSON, J. F. MILLER, C. J. HECKMAN
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Abstract: Reflex circuits are well defined and repeated segmentally throughout the spinal cord. For example the canonical motor microcircuit (CMM), comprised of Ia sensory axons that connect monosynaptically to homonymous motoneurons (MN) and to the inhibitory interneurons (IN) of their antagonists, forms the basis of reciprocal inhibition throughout the limb and is critical for inter-joint coordination. Because these circuits are repeated throughout the spinal cord, they are an attractive target for electrical stimulation in the context of spinal cord injury (SCI) as CMMs far from the injury site should remain intact and findings on one CMM should

be generalizable. Practically, these circuits are also easily accessible via the Ia afferents found in the dorsal roots and should have the lowest threshold to electrical stimulation. A clear relationship between electrical stimulation and CMM behavior is still misunderstood. For instance, proper CMM functioning is dependent on descending neuromodulation (PICs) which alters the excitability of its neuronal elements and can be disrupted by SCI. This interplay between electrical stimulation and neuron excitability is not known. In addition, the interplay between agonist/antagonist CMM pairs with respect to electrical stimulation is also unknown. Here we tested in the decerebrate cat (n=2), the effects of surface electrical stimulation on sensory inflow to CMMs of an ankle joint agonist/antagonist motor pair, the soleus (Sol) and tibialis anterior (TA). We varied the location of an electrode along the rostral-caudal axis at the dorsal root entry zones favoring the Sol and TA motor pools (L6-S1). We delivered a 5 s pulse train of varying intensities and frequencies. To mimic sensory inflow we superimposed a 3 s Sol TVR. Excitation and inhibition was effectively controlled in a progressive and location dependent manner by electrical stimulation as measured by force production in Sol in response to tendon vibration. Stimulation modulated the tendon vibratory reflex response over regions where sensory input activated the antagonist . This effect was changed as the stimulating electrode was moved away from the antagonist muscle region. Additionally as stimulus intensity and frequency was increased, a post-inhibitory rebound emerged resulting in activation of the agonist muscle. These results suggest that the excitatory/inhibitory balance between motor pools can be effectively modulated by electrical stimulation of the CMM via dorsal spinal stimulation. Activating these circuits to restore proper balance after SCI may aid in post injury therapies aimed at regaining simple motor behaviors such as standing and weight bearing.

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Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.04/S12

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Hale Brain Tumor Research Fund

Title: Intravenous delivery of miR133b along with Argonaute-2 24hrs post-injury enhances spinal cord recovery following cervical contusion in mice

Authors: *C. A. DANILOV[1], Y. GU[5], V. PUNJ[2], Z. WU[6], S. TAHARA[3], F. M. HOFMAN[4], T. C. CHEN[1]
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of Pathology, USC, Los Angeles, CA; [5]Dept. of Spine Surgery, Second Military Med. Univ., Shanghai, China; [6]Dept. of Orthopedics, Tongji Univ., Shanghai, China

Abstract: Axon regeneration of the corticospinal tract (CST) and functional motor recovery are both limited following spinal cord injury (SCI). As a result, spinal cord trauma leads to paralysis or other related conditions that involve axon disconnections. Previous studies in zebrafish, a model of spontaneous nerve regeneration, show elevated levels of microRNA133b (miR133b) in regenerating neurons following a spinal cord injury. Similarly, lentiviral delivery of miR133b at the time of injury after a controlled compression at the thoracic level shows improved functional recovery in mice. In this study, we investigated whether intravenous delivery of miR133b is reliable and efficient at enhancing spinal cord recovery when administered 24hrs after a unilateral cervical contusion in C57Bl/6 mice. Here, we used a system that targets fibrous scar formation at the lesion site by intravenous co-injection of miR133b with Argonaute 2 (Ago2), a protein that participates in miRNA processing and has been found to protect miRNA degradation. The treated group received miR133b/Ago2 and the control group miR-Negative control/Ago2 via tail-vein injection for 3consecutive days starting1day postinjury.We found that intravenous delivery of miR133b/Ago2 strongly inhibited key extracellular matrix (ECM) genes and reduced microglia/macrophage mobilization to the lesion scar. Forelimb function was assessed for 8 weeks post-injury using a grip strength meter task. While a poor recovery of forelimb gripping function was observed in control group, mice receiving miR133b/Ago2 treatment showed the first sign of recovery at about two weeks post-injury and that was improved over time. Our findings show that corticospinal tract (CST) axon re-growth was enhanced in miR133b group as more BDA (biotinylated dextran amine) labeled axons could be found at the injury site and caudal to the lesion, when compared to control group mice. In summary, our results provide an insight regarding a potential miR133b/Ago2 therapy targeting the microenvironment of the contused spinal cord, that can be used within 24hrs of injury.

Disclosures: C.A. Danilov: None. **Y. Gu:** None. **V. Punj:** None. **Z. Wu:** None. **S. Tahara:** None. **F.M. Hofman:** None. **T.C. Chen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeOnc Technologies.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.05/S13

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Funds of Leading Talents of Guangdong Province(87014002)

Title: Controlled-releasing of epothilone B from functional self-assembling peptide nanohydrogel to improve neural regeneration after spinal cord injury

Authors: *C. LI, S. RAMAKRISHNA, L. HE
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Abstract: Fibrous scar is a major obstacle for neural regeneration after spinal cord injury. Microtubule dynamics regulate the scarring. Moderate microtubule stabilization can reduce scarring and promote axon regeneration. Epothilone B, a microtubule stabilizing drug, can promote axon regeneration. Here, we have examined the effects of different concentrations of Epothilone B on differentiation of neural stem cells and show that Epothilone B has double-sided role on neural stem cell. It is show that high concentrations of Epothilone B harm to the cells. Low concentrations of Epothilone B favor the differentiation of neural stem cells into neurons and low concentrations of Epothilone B are more conducive to axon extension. Together, nanohydrogel, releasing Epothilone B, was transplanted to the rats after spinal cord injury. Nerve regeneration can be observed by immunofluorescence staining. The behavior recovery was studied by BBB locomotion assessment. Axon regeneration can be observed after two weeks. Therefore, Epothilone B has application prospects in spinal cord injury repairing.

Disclosures: S. Ramakrishna: None. **L. He:** None.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.06/S14

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Reactive astrocytes inhibit neuronal regeneration after spinal cord injury

Authors: *H. LEE[1,2], H.-L. LEE[3], M. NAM[4], J. LEE[4], K. KIM[2], K. PARK[4], J. C. LEE[4], Y. HA[2]
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Abstract: The spinal cord is a central nervous system that functions primarily in the transmission of neural signals between the brain and the rest of the body by both integrating and transmitting signals. Therefore damage to the spinal cord brings about degeneration of motor capacity, sensory defect, and disability of autonomic nervous system depending on the level of seriousness of the damage done. Histologically, inflammatory damage to the spinal cord can occur in the result of the damage due to decrease in the blood flow and hypoxia following the primary injury. Secondary damages such as nerve apoptosis, neuropathic pain, and formation of

glia scar only exacerbates the symptoms. Despite the countless numbers of breakthroughs and innovation in medical technologies, there has been no absolute cure for spinal cord injuries, and development of drugs to control neuropathic pain accompanied by spinal cord injury has not yet been showing great results throughout the world. Various drugs are currently being used to treat these spinal cord injury related diseases. Among those drugs, gavapentin and pregabaline are widely used. They primarily functions as glutamate inhibitor, designed as agonist of GABA, blocking the voltage gated calcium channel. However, these drugs are only effective in limited period of time, and it only shows significant enough effectiveness in 50 percent of the treated patients. Due to such drawbacks of these drugs, it is critically essential that researchers come up with an alternative drug.
MaoB participates as an enzyme in the metabolism of a dopamine, and it is know to impact numerous neurological disorders such as Parkinson's disease, Alzheimer disease, and multiple sclerosis. There also has been increasing numbers of research findings asserting a prominent correlation between this enzyme and astrocytes. Our reseach focuses on the increased glial scar, which is known to be the aftermath of spinal cord injury. Here, we strive to induce nerve regeneration and rescue neuropathic pain through control of active oxygen and GABA, by specifically inhibiting the metabolism of MaoB in the mitochondria of reactive astrocyte.

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Poster

569. Spinal Cord Injury IV

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.07/S15

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Brain Korea 21 PLUS Project for Medical Science, Yonsei University

Title: Development of advanced-*in vivo* reprogramming system for spinal cord injury therapy

Authors: *H.-L. LEE[1], H. LEE[3], K. KIM[2], Y. HA[4]
[1]Yonsei Univ., Seodaemun-Gu, Seoul, Korea, Republic of; [2]Yonsei Univ., Seoul, Korea, Republic of; [3]Brain Korea 21 Plus Project For Med. Science, Y, Seodaemun-Gu, Seoul, Korea, Republic of; [4]Yonsei Unuversity, Seoul, Korea, Republic of

Abstract: Spinal cord injury is induced by trauma or compression that often leads to blocking the stimulus, and disorder to function of motor system, sensory and autonomic nervous system. The fundamental treatment of spinal cord injury is not yet developed. Generally, it has been applied that decompression therapy, drug treatment and rehabilitation for spinal cord injury. However, the treatments have shown that limited effects. Because of that reason, new treatments

are in the studying including gene therapy and stem cell therapy. Among them, in vivo reprogramming is regarded as promising futuristic technology. In this study, we develop the in vivo reprogramming system for treatment of spinal cord injury by reprogramming the astrocytes to neurons.
In this study, we used two kinds of astrocytes that human astrocytes and primary astrocytes from mouse. These cells were direct reprogramming by specific vectors for 6 weeks. After then reprogramming cells verify by immunocytochemistry. At the In vivo study, male C57BL/6 mice (n=10, postnatal 7 weeks) were randomized into three groups: Group 1= EMEM group and Group 2= reprogrammed group. Genetic transduction was conducted 2 weeks after spinal cord injury and subjects were observed for 6 weeks. To evaluate functional behavior, each group was examined with basso mouse scale (open field test). Also, immunohistochemistry proceed for reveal in vivo direct reprogramming and neuronal regeneration.
At the in vitro study, the both kinds of astrocytes were reprogrammed from astrocyte to neurons by reprogramming. Also, we verified the behavior was improved and astrocytes were reprogrammed to neurons, when we applied in vivo reprogramming technology to spinal cord injury animal models.
In this research, we developed the new type of In vivo reprogramming system for progress of in vivo reprogramming technic. For check the system working, new system was transduced to astrocytes in vitro. The astrocytes were induced to neurons and GFP was positively expressed. As well as, new system induced neurons from astrocytes in spinal cord injured animals. The reprogrammed neurons improved the animal behavior. From these results, we can confirm the new system works as new in vivo reprogramming system. Also they have the potential to new treatment for spinal cord injury.

Disclosures: H. Lee: None. **K. kim:** None. **Y. Ha:** None.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.08/S16

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Drexel Start Up

Title: Biomaterials-based drug delivery systems for promoting recovery after spinal cord injury

Authors: *B. SHULTZ, J. NONG, Z. WANG, Z. ZHANG, Y. ZHONG
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Abstract: Spinal cord injury (SCI) results in partial to complete loss of motor and sensory function below the level of injury. Generally, efforts to improve functional recovery aim to

attenuate secondary injury progression and/or promote tissue regeneration. While stem cell transplantation has yielded mixed to beneficial outcomes following SCI, ethical and safety concerns may hinder rapid translation of stem cell therapies from the lab to the clinic. To date, a large and growing number of drugs have been shown to promote functional recovery in animal models of SCI, including small molecules, proteins and proteoglycans. Administration of these drugs, however, remains a major limitation, as many pharmaceuticals exhibit poor blood-brain barrier permeability. To achieve sufficient doses within the central nervous system, researchers must administer high systemic doses of drugs or implant osmotic pump-driven intrathecal catheters. Because high systemic doses often induce deleterious off-target effects, and indwelling catheterization greatly increases risks of further injury and infection, neither of these approaches are clinically viable. In this study, we describe several drug delivery systems capable of providing sustained, localized drug release to the injured spinal cord. First, we developed and characterized a hydrogel-based system for delivery of triiodothyronine (T3), a poorly soluble small molecule and potent inducer of oligodendrocyte differentiation/myelin production. This system was found to induce modest improvements in oligodendrogenesis and myelination following injury in rats. Because post-SCI chronic inflammatory signaling including tumor necrosis factor-alpha (TNF) have been shown to hinder oligodendrocyte maturation and remyelination, we next sought to deliver an anti-TNF synthetic peptide, XPro1595. To this end, we developed a novel, injectable microparticle-embedded hydrogel system capable of delivering proteins and peptides. All delivery systems were fabricated from naturally occurring polymeric biomaterials, without the use of harsh, environmentally taxing organic solvents or processing methods. Collectively, these systems can serve to bridge the gap between benchtop innovation and clinical utility for therapeutic interventions following SCI.

Disclosures: B. Shultz: None. **J. Nong:** None. **Z. Wang:** None. **Z. Zhang:** None. **Y. Zhong:** None.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.09/S17

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Sodium butyrate exerts neuroprotective effects in spinal cord injury

Authors: *M. CAMPOLO, M. LANZA, G. CASILI, A. FILIPPONE, S. CUZZOCREA, E. ESPOSITO
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Abstract: Sodium butyrate (SB) is a dietary microbial fermentation product of dietary fiber and serves as an important neuromodulator in the central nervous system. Recent experimental

evidence has suggested potential therapeutic applications for butyrate, including its utility in treating metabolic and inflammatory diseases. The aim of the present study was to evaluate the potential beneficial effects of SB in a mouse model of spinal cord injury (SCI) and its possible mechanism of action. SCI was produced by extradural compression for 1 min of the spinal cord at the T6-7 level using an aneurysm clip, and SB (10-30-100mg/kg) were administered by oral gavage 1 and 6 h after SCI. For locomotor activity, study mice were treated with SB once daily for 10 days. Morphological examination was performed by light microscopy through hematoxylin-eosin (H&E) staining. In addition, NF-kB, IkB-alfa, COX2 and iNOS expression were assayed by western blot analysis and IL-1beta and TNF-alfa levels by immunohistochemistry analysis. The results showed that SB treatment significantly ameliorated histopathology changes and improved recovery of motor function changes in spinal cord injury in dose-dependet manner. Moreover, from the results obtained, SB modulated NF-kB pathway showing a reduction in cytokines expression. This study showed that SB exerts neuroprotective effects on spinal cord injury and anti-inflammatory properties. The observed neuroprotective action suggests that SB may serve as a potential candidate for future treatment of spinal cord injury.

Disclosures: M. Campolo: None. **M. Lanza:** None. **G. Casili:** None. **A. Filippone:** None. **S. Cuzzocrea:** None. **E. Esposito:** None.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.10/S18

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH grant P20GM103444
SC-SCIRF grant 2014 I-02

Title: Combinatorial treatment of rolipram and rhoa sirna delivered by pgp nanoparticle increases functional recovery in a rat contusion spinal cord injury model

Authors: *J. LEE[1], S.-J. GWAK[1], J. YU[2], C. MACKS[1], H. ZHU[2], M. LYNN[3], K. WEBB[1], K. MARK[2]
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Abstract: Spontaneous recovery of sensory and motor function following spinal cord injury (SCI) is inhibited by multiple pathophysiological mechanisms including progressive secondary injury, extrinsic growth inhibitors, and intrinsic deficiencies in neuronal biochemistry. Combination therapies using treatment modalities targeting two or more of these barriers have

achieved promising preclinical results, but their application is complex and often requires multiple interventions. We have developed a cationic, amphiphilic copolymer, poly (lactide-co-glycolide)-graft-polyethylenimine (PgP) as a carrier for combinatorial therapy. Previously, we demonstrated that PgP/siRhoA nanoparticles injected in the SCI lesion can achieve RhoA knockdown for up to 4 weeks accompanied by a reduction in apoptosis, cavity size, and astrogliosis [1] and an increase in axonal regrowth/sparing and Rm-PgP nanoparticles injected at the SCI lesion can restore cAMP levels to sham level and reduce inflammation and apoptosis in rat compression SCI model [2]. In this study, we evaluated the effect of Rm-PgP and PgP/siRhoA co-administration on motor functional recovery in rat contusion SCI model. Moderate contusion injury model was created at T9-T10 spinal cord of female SD rats (200-250 g) using the impactor (IH-0400, PSI) with a force of 150 kdyn. Rm-PgP (10 µg Rm) and PgP/siRhoA (20 µg siRhoA) were co-administered immediately after injury by intraspinal injection**.** Rm-PgP (10 µg Rm) only and PgP/siRhoA (20 µg siRhoA) only were used for comparison and untreated SCI and sham (laminectomy only) were used as controls. Motor functional recovery of the hindlimb was evaluated using BBB locomotor rating scale once per week until 4 weeks after SCI. We observed that BBB score of rats treated with co-administration was significantly higher than that of Rm-PgP only, PgP/siRhoA, and untreated SCI rats, at all time points. At 4 weeks, rats were perfused with 4% paraformaldehyde, spinal cords embedded in OCT, sectioned, and stained for histological analysis. We observed that lesion volume was significantly smaller in rats treated with co-administration than that in Rm-PgP only, PgP/siRhoA only, and untreated SCI. In conclusion, these results demonstrate that combinatorial treatment of Rm-PgP and PgP/siRhoA can synergistically improve motor funcitonal recovery and reduce necrotic cavity formation.

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References: 1. Gwak et al., Biomaterials, 121(2017), 155-166. 2. Macks et al., Journal of Neurotrauma, 35 (2018), 582–592

Disclosures: J. Lee: None. **S. Gwak:** None. **J. Yu:** None. **C. Macks:** None. **H. Zhu:** None. **M. Lynn:** None. **K. Webb:** None. **K. Mark:** None.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.11/T1

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH R01 NS085426

Title: Repeated activation of adult dorsal root ganglion neurons using designer receptors exclusively activated by designer drugs (DREADDs) enhances functional sensory axon regeneration after dorsal root crush injury

Authors: *D. WU, T. SALTOS, V. J. TOM
Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Short-term neuronal activity activates growth programs and synaptogenesis programs. However, whether repeated bouts of activation of adult neurons with injured axons enhance axon outgrowth or their integration back into a circuit is not known. We took advantage of DREADDs to remotely alter neuron activity in a spatially and temporally-specific manner. We hypothesized that activating adult DRG neurons expressing the excitatory DREADD hM3Dq with CNO combined with modulation of inhibitory chondroitin sulfate proteoglycans (CSPGs) at the dorsal root entry zone (DREZ) with chondroitinase ABC (ChABC) will promote functional regeneration of primary afferents after a dorsal root crush. We first tested our hypothesis in vitro using adult DRGs transduced with AAV-hM3Dq or -mCherry plated on a spot of CSPG, an established model of the inhibitory environment after injury. With ChABC digestion of CSPG, CNO-activated, $hM3Dq^+$ DRGs grew more axons across the inhibitory substrate than CNO-treated, $mCherry^+$ DRGs. We then assessed if this strategy enhanced regeneration through the DREZ after a dorsal root crush in adult rats. Three weeks after injecting AAV-hM3Dq or -mCherry into C6-C8 DRGs unilaterally, we crushed the ipsilateral C5-T1 dorsal roots. After the crushes, ChABC was injected into the C5-C8 dorsal horn. In all animals, CNO was injected subcutaneously daily starting the day of the root crush for the duration of the experiment (3 months). Sensory and sensorimotor function was assessed weekly. Histological analysis revealed that AAV-DREADD or -mCherry primarily transduced large caliber DRGs. There was no difference between groups in the von Frey or Hargreaves' tests. However, while $hM3Dq^+$ and $mCherry^+$ animals had comparable numbers of foot slips while walking on the grid early on, $hM3Dq^+$ animals had more correct paw placements than $mCherry^+$ animals starting 6 weeks post-crush, suggestive of better proprioceptive, sensorimotor function. This behavioral improvement was associated with increased axon regeneration in $hM3Dq^+$ animals. We saw no difference in the total number of axons that penetrated the DREZ between groups, but $hM3Dq^+$ animals had more axon regrowth into the gray matter. To determine if axons that regenerated established synapse with spinal neurons, we stimulated the ipsilateral median and ulnar nerves to transsynaptically induce c-Fos expression in deafferented dorsal horn. More c-Fos+ neurons were observed in $hM3Dq^+$ animals than in $mCherry^+$ animals. Thus, modulating neuronal activity intermittently for a prolonged period of time is a strategy to promote functional axonal regeneration beyond a ChABC-treated DREZ after dorsal root crush.

Disclosures: D. Wu: None. **T. Saltos:** None. **V.J. Tom:** None.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.12/T2

Topic: C.11. Spinal Cord Injury and Plasticity

Support: UW Department of Neurological Surgery
Royalty Research Fund
Washington State Spinal Cord Injury Consortium
Craig H. Neilsen Foundation

Title: Hemostatic nanoparticles to enhance local blood clotting and limit secondary injury after a moderate contusion spinal cord injury in rodents

Authors: C. P. HOFSTETTER[1], *L. N. CATES[1], J. E. HYDE[1], R. L. HAMMOND[1], N. M. CHAKRAVARTY[1], N. MAISHA[2], J. SILVER[3], E. B. LAVIK[2], M. F. BRUCE[1], Z. Z. KHAING[1]
[1]Dept. of Neurolog. Surgery, Univ. of Washington, Seattle, WA; [2]Chemical, Biochemical, and Envrn. Engin., Univ. of Maryland, Baltimore, MD; [3]Dept Neurosci, Case Western Reserve Univ., Cleveland, OH

Abstract: Traumatic spinal cord injury (tSCI) often leads to a debilitating loss of sensory, motor, and autonomic function and chronic pain. Immediately following the initial trauma, microvessels in the spinal cord rupture leading to hemorrhaging. Bleeding is a major contributor to a cascade of subsequent changes defined as the secondary injury, which includes swelling, inflammation and oxidative stress. Hemorrhage enlarges progressively over time after the primary injury, and the extent of bleeding has been shown to correlate with injury severity and functional deficits. We hypothesize that limiting bleeding would limit secondary injury, and subsequently lead to better functional outcomes. Here we employed newly developed hemostatic nanoparticles (hNPs), which have been shown to localize to the injury site and reduce bleeding after liver resection injury, in a contusion type tSCI in a rodent model. The hNPs (9.0 mg/mL in saline; 0.5 mL injection) or control particles were introduced intravenously within 3 minutes of the tSCI (Infinite Horizon device). Using our unique ultrafast contrast enhanced ultrasound imaging (CEUS), we visualized local spinal blood perfusion, including blood flow in the microcirculation, in real-time before injury, then at one hour and at 4 hours following injury. Preliminary data show that at one hour after injury, area of perfusion deficits seen using CEUS appeared smaller in hNP treated animals to controls (~15% smaller area of perfusion deficit). Interestingly, there was a 15% reduction in swelling of the spinal cord associated with injury in the presence of hNPs compared to control animals. At the end of the study, animals were injected intravenously with tomato lectin (0.2 mg injection) to label all patent blood vessels. Clusters of

hNPs were found within areas of hemorrhage and blood clot within the injury epicenter. Interestingly, hNPs were never seen co-labeled with tomato lectin, suggesting that hNPs were only within spinal parenchyma in areas of active bleeding. Our results suggest that hNPs are localized to the areas of active bleeding exclusively, presumably involved in blood clotting acutely after a contusion type tSCI. Current studies are underway to 1) analyze real-time hemodynamic data obtained from ultrafast CEUS imaging and 2) evaluate the chronic 3D blood flow imaging, functional and histological outcomes from hNP treatment after tSCI.

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Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.13/T3

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Rick Hansen Foundation through the ICORD-Rick Hansen Institute – Blusson Integrated Cure Partnership

Title: Testing robustness of promising FDA approved neuro-protective drug candidates in a cervical hemi-contusion model of rats

Authors: *W. T. PLUNET, N. JANZEN, J. LIU, E. RAFFAELE, S. KAMAKARI, O. SEIRA, K. KOLE, Y. JIANG, L. MCPHAIL, W. TETZLAFF
ICORD, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: A significant number of FDA approved drugs have demonstrated efficacy in preclinical spinal cord injury (SCI). These studies predominantly used a moderate thoracic contusion model yet less than 5% of human SCI are incomplete thoracic injuries. Most human injuries occur at the cervical levels (>65%), half of these are incomplete, and this group should benefit the most from neuroprotective treatments. Moreover, the acute time-period of intervention used in animal studies (often immediate or less than one hour after injury) are difficult to translate in human trials. In addition, many preclinical studies are underpowered, subject to experimenter biases or conflict of interest, which significantly reduces their value as predictors of success in a human trial. We therefore created a team of research staff to assess the effects on functional recovery of the most promising FDA approved drugs when these are administered 3 hours after a cervical spinal cord hemi-contusion injury using group sizes of n = 16-21. Previously, we tested 9 different FDA approved drugs (riluzole, valproic acid, fluoxetine, metformin, inosine, rosuvastatin, acetyl-l-carnitine, glibenclamide, tamoxifen) that had been

reported to improve functional recovery in SCI models. In our experiments none of the 9 treatments improved recovery compared to control groups, and only glidenclamide improved the amount of spared spinal cord tissue. RT-PCR measurements of mRNA expression changes of injured spinal cord tissue in the five drugs we have done short-term studies for indicate appropriate changes in gene expression for all treatments indicating the drugs are biologically active at the injury site. This year we tested 4-Aminopyridine (started 3 hours after injury) both in a cervical hemi-contusion model and the more widely used T9/10 thoracic contusion injury model. In neither of these two injury models did we see better recovery in the treated animals compared to the control groups, and there were no differences in amount of spared tissue area between groups in either injury model. Having poor success with these 10 individual treatments we decided to test combination treatments. We compared a control group against a group treated with glibenclamide plus taxmoxifen, or a group treated with glibenclamide, tamoxifen plus inosine. We are still analysing this study and will report the behavioral and histology results on the poster. As in previous replication studies, establishing robustness in preclinical models is challenging and possible reasons will be discussed. Supported by the Rick Hansen Foundation through the ICORD-Rick Hansen Institute - Blusson Integrated Cure Partnership.

Disclosures: W.T. Plunet: None. **N. Janzen:** None. **J. Liu:** None. **E. Raffaele:** None. **S. Kamakari:** None. **O. Seira:** None. **K. Kole:** None. **Y. Jiang:** None. **L. McPhail:** None. **W. Tetzlaff:** None.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.14/T4

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Charles H. Skip Smith Endowment Fund

Title: Regeneration of dorsal horn spinal cord neurons after injury via *in situ* NeuroD1-mediated astrocyte-to-neuron conversion

Authors: *B. PULS, H. LI, M. METZGER, T. RANA, Y. DING, M. PAN, G. CHEN
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Abstract: Spinal cord injury (SCI) is an acute trauma to the central nervous system that can leave patients with deficits in sensation, movement, and bodily function, including paralysis of the limbs. These deficits result not only from the initial tissue damage, but also from secondary damage caused by the release of toxins, inflammation, oxygen and nutrient restriction, and reactive oxygen species. To protect healthy tissue from this toxic environment, local astrocytes proliferate and form a glial scar which, while limiting the short-term effects of the injury,

prevents the tissue from recovering into functional neural tissue in the long-term. Our lab has pioneered a new technology to convert glial cells into neurons after injury or diseases (Guo et al., 2014). In this study, we use both retroviral and adeno-associated viral (serotype 9) vectors carrying the neurogenic transcription factor NeuroD1 to directly convert injury-induced reactive astrocytes into mature neurons in the dorsal horn of the mouse spinal cord in vivo at high efficiency. We show that these neurons obtain neuronal subtypes specific to the dorsal horn of the spinal cord, consistent with other experiments in the brain where NeuroD1-converted neurons obtain subtypes relevant to their local environment. We also show that these neurons are functional and can re-integrate into local networks. Our future work includes targeting other proliferative cell types including oligodendrocyte precursor cells (OPCs) to generate other subtypes of neurons, and further investigating the mechanisms of NeuroD1-mediated conversion in the spinal cord. This work is supported by the Charles H. Skip Smith Endowment Fund to Gong Chen (Principal Investigator).

Disclosures: B. Puls: None. **H. Li:** None. **M. Metzger:** None. **T. Rana:** None. **Y. Ding:** None. **M. Pan:** None. **G. Chen:** None.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.15/T5

Topic: B.05. Neurotransmitter Release

Support: NRF Grant No. 2017M3C1B2085310

Title: Motor neuron regeneration study on microfluidic platform incorporating microelectrode array

Authors: *S. JUN[1,2], H. JEONG[1], Y. A. CHO[1], H. YOO[1]
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Abstract: Motor neurons of mammalian spinal cord hardly regenerate from damage or lost that are caused by injury or disease. Pharmacologic approach is popular treatment for neuron regeneration. In this study, we propose to combine the pharmacological method with electrical stimulation to enhance the axonal regrowth of motor neurons. From previous study, we confirmed that the axons of motor neurons grow directionally through the micro-grooves in the microfluidic platform and the cell body and axon can be distinguished. The microfluidic platform is attached on the planar-type microelectrode array (MEA) in order to monitor and/or modulate the activity in the growing axons. The micro-grooves of the microfluidic platform were aligned with the electrodes of the MEA. Based on this platform, it is available to apply electric

stimulation to a desired target position of growing axons. Therefore, it is possible to study axonal regeneration by monitoring the morphological change of motor neurons.

Disclosures: S. Jun: None. **H. Jeong:** None. **Y.A. Cho:** None. **H. Yoo:** None.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.16/T6

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH R01 NS081112
CHN #338432

Title: Intermittent hypoxia enhances connectivity between neuronal progenitors and injured cervical spinal cord

Authors: *L. ZHOLUDEVA, M. L. RANDELMAN, R. DILBAROVA, L. QIANG, I. FISCHER, M. A. LANE
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Abstract: There is a growing interest in the use of neural progenitor cells (NPCs) to treat spinal cord injury (SCI). Despite extensive preclinical research, it remains unclear as to how donor cells develop, differentiate and integrate with host injured circuitry, and if integration can be enhanced and/or guided using noninvasive means such as activity based therapy. With a focus on the phrenic circuit and respiratory dysfunction after cervical SCI, the present work tests the hypothesis that pairing cellular transplantation with a rehabilitation strategy (daily acute intermittent hypoxia, dAIH) will enhance neuroplasticity and promote donor-host connectivity. Cultured NPCs (neuronal and glial restricted progenitor cells) isolated from GFP rats were transplanted into a cervical (C3/4) contusion injury in adult Sprague Dawley rats, one week after injury. Animals received 4 weeks of dAIH (10-5minute exposures to 10% oxygen intermittent with normoxia, 5 days a week), beginning one week post-transplantation. Donor cells survive, differentiate, and integrate with the host spinal cord as assessed with transynaptic pseudorabies virus tracing (PRV) and immunohistochemistry. Respiratory training resulted in significantly enhanced donor-host connectivity, compared to untrained transplant recipients. Preliminary data suggests the underlying mechanism for directing donor-cell outgrowth towards phrenic inter- and motoneurons is in part mediated via BDNF expression within the cervical spinal cord. Transplant recipients, with and without dAIH training, showed greater muscle (diaphragm) recovery than vehicle-controls, as measured by terminal electromyography. Transplant and dAIH training recipients demonstrated greater ability to respond to hypoxic but not hypercapnic respiratory

challenge. These ongoing experiments suggest that rehabilitative strategies such as dAIH may be an effective way for enhancing donor cell outgrowth and connectivity.

Disclosures: L. Zholudeva: None. **M.L. Randelman:** None. **R. Dilbarova:** None. **L. Qiang:** None. **I. Fischer:** None. **M.A. Lane:** None.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.17/T7

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH NS096514
NYS DOH SCIRB C32088GG

Title: Moderate hypoxia produces voiding in rats with spinal cord injury-induced severe urinary dysfunction

Authors: *W. F. COLLINS, III[1], M. E. TORPIE[2], C. WANG[2], I. C. SOLOMON[2]
[1]Neurobio. and Behavior, [2]Physiol. & Biophysics, Stony Brook Univ., Stony Brook, NY

Abstract: In humans, spinal cord injury (SCI) results in sustained lower urinary tract (LUT) dysfunction with reduced voiding efficiency and urine retention that are associated with a high degree of morbidity and mortality. Therefore, interventions that improve voiding in individuals with SCI are needed. To this end, we investigated the potential therapeutic benefit of acute exposure to moderate hypoxia (10-12% O_2) to improve LUT function in a rat model of SCI. Adult female Sprague Dawley rats (225-250g) received thoracic (T8 vertebra) SCI consisting of either a moderate contusion (200 kilodynes) or a complete spinal cord transection. Four weeks following SCI, bladder intravesical pressure and external urethral sphincter (EUS) EMG activity were recorded in spontaneously breathing, vagus nerves intact rats under urethane anesthesia (1.4 g/kg) during continuous infusion of saline (0.04-0.07 ml/min) into the bladder. The four-week survival time was chosen because initial recovery from SCI has stabilized (e.g., rats are able to spontaneously void) but functional motor deficits remain. Under these conditions, the degree of LUT dysfunction varied between rats, and the present report focuses on a subset of rats (in both moderate contusion (n=7) and transection SCI (n=7) subjects) that exhibited a severe LUT dysfunction phenotype characterized by rhythmic non-voiding contractions leading to bladder distension and sustained elevated bladder pressure >20 mmHg. Baseline LUT activity was continuously recorded for >60 min, after which the rats were exposed to an acute episode of moderate hypoxia (5 minute duration; 10-12% O_2, balance N_2). In each case, exposure to moderate hypoxia produced an immediate void and an associated transient decrease in bladder pressure. The hypoxia-induced voids occurred in the absence of well-defined bladder

contractions although bladder contractions during hypoxia were observed in some cases. These observations suggest that a single acute exposure to moderate hypoxia is effective in producing voiding in subjects with severe LUT dysfunction.

Disclosures: W.F. Collins: None. **M.E. Torpie:** None. **C. Wang:** None. **I.C. Solomon:** None.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.18/T8

Topic: C.11. Spinal Cord Injury and Plasticity

Support: CIHR grant 14697
NIH grant R01NS047567

Title: Chronic hypoxia induced by pericytes contributes to hypersensitivity and allodynia after spinal cord injury

Authors: A. M. LUCAS-OSMA[1], Y. LI[1], K. HOLYK[1], S. LIN[1], L. SANELLI[1], K. FOUAD[2], *D. J. BENNETT[3]
[1]Neurosci. and Mental Hlth. Inst., [2]Physical Therapy, [3]Univ. of Alberta, Edmonton, AB, Canada

Abstract: The spinal cord is one of the most metabolically active systems, and thus it is critical that blood flow adapts to the demands for oxygen and glucose. Recently, we have shown that pericytes play a key role in regulating blood flow in the spinal cord, by constricting in response to monoamines (Li et al. 2017). Furthermore, we found that after chronic spinal cord injury (SCI) pericytes excessively constrict capillaries, leading to chronic hypoxia in the entire spinal cord below the injury. This hypoxia is produced by a paradoxical excess activity in monoamine receptors (5-HT1), despite the absence of monoamines. These receptors activate pericytes that locally constrict capillaries, reducing blood flow to ischemic levels. The paradoxical receptor activity results from trace amines (tryptamine) produced by pericytes that ectopically express the enzyme aromatic-L-amino-acid-decarboxylase (AADC). Importantly, improving blood flow by blocking AADC, or briefly inhaling pure oxygen, produces substantial relief from hypoxia and improves locomotor function. While these studies focused on motor function, dysfunctions in the sensory systems, including allodynia and pain, are hallmarks of SCI. Thus, we examined here whether improving blood flow normalizes sensory transmission after SCI. Adult rats with a chronic sacral spinal cord transection (2 months prior) were evaluated for the sensitivity to light touch (von Frey hair threshold for tail flick) and noxious heat (tail flick latency on hot plate). We found that initially rats were hypersensitive to light cutaneous stimuli, like allodynia seen in humans with SCI. However, we found that restoring blood flow below the SCI with an AADC blocker reduced the cutaneous hypersensitivity, increasing the von Frey threshold to a level near

that in uninjured rats. Likewise, increasing spinal cord oxygenation by inhalation of 95% oxygen also reduced the hypersensitivity to light touch. Interestingly, the same treatments increased the sensitivity to heat, consistent with a low threshold afferent gating of pain fibers. Our results suggest that a lack of adequate blood flow contributes to hypersensitivity and allodynia after chronic SCI and improving spinal cord blood flow offers a promising new strategy to treat sensorimotor dysfunction.

References

Li Y, Lucas-Osma AM, Black S, Bandet MV, Stephens MJ, Vavrek R, Sanelli L, Fenrich KK, Di Narzo AF, Dracheva S, Winship IR, Fouad K, and Bennett DJ. Pericytes impair capillary blood flow and motor function after chronic spinal cord injury. *Nat Med* 23: 733-741, 2017.

Disclosures: A.M. Lucas-Osma: None. **Y. Li:** None. **K. Holyk:** None. **S. Lin:** None. **L. Sanelli:** None. **K. Fouad:** None. **D.J. Bennett:** None.

Poster

569. Spinal Cord Injury IV

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.19/T9

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life Grant (WFL -US -22 /17)
The U.K. Medical Research Council
The International Spinal Research Trust

Title: Overcoming anddegrading inhibitory proteoglycans globally to promote axonal regeneration andfunctional recovery after a chronic thoracic spinal cord injury

Authors: *A. MILTON[1], M. A. DEPAUL[2], J. VERHAAGEN[4], E. J. BRADBURY[5], J. SILVER[3]
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Abstract: The potently inhibitory environment that surrounds axons three months after a thoracic-level eight (T8) contusive spinal cord injury (SCI) significantly contributes to failured axon regeneration, leading to impaired functional recovery of the lower body. In this chronic model of SCI, the glial scar and perineuronal net (PNN) around as well as far distal the injury site each display a profound upregulation of growth restricting chondroitin sulfate proteoglycans (CSPGs). CSPGs can be stripped of their inhibitory glycosaminoglycan (GAG) chains locally, by enzymatic removal through direct administration of Chondroitinase ABC (ChABC). However,

focal administration of ChABC in the vicinity of the lesion has not been especially effective after a contusive SCI. Impaired fiber regeneration and sprouting is also mediated by GAG binding to the leukocyte common antigen-related family receptor protein tyrosine phosphatase sigma (RPTPσ). This interaction entraps axon terminal in both the scar and at synapses throughout the central nervous system. Systemic administration of synthetic PTPσ receptor blocker, Intracellular Sigma Peptide (ISP), acutely after SCI promotes robust recovery of axon sprouting via the inhibitory PNN. However, ISP alone has shown only minimal therapeutic effects chronically. Thus, our goal was to more expansively degrade and broadly overcome CSPG mediated inhibition to foster both regeneration and sprouting of regenerating axons over long distances. We accomplished this via the use of a far ranging CSPG digestion strategy using lentiviral delivery of ChABC (Lenti-ChABC) combined with ISP treatment. We now show that this combination treatment (but not when either strategy is used alone) significantly improves locomotion after chronic T8 contusive SCI in adult rats. Furthermore, chronically injured animals treated with either ISP, Lenti-ChABC or both (but not saline) had increases in serotonergic fiber sprouting caudal to the injury. However, an obvious correlation between highest 5-HT^{+} fiber density in animals with the most robust functional recovery was not found, suggesting improved behaviors may be attributed only in part to increased serotonergic innervation onto motor targets. These findings demonstrate that manipulating the glial scar and PNN using a minimally invasive enzyme and peptide therapy three months after contusive SCI facilitates nerve growth and recovery of some critical functions. This injury model is clinically relevant and supports a path for a translatable treatment paradigm for individuals suffering from paralysis long after spinal cord trauma.

Disclosures: M.A. DePaul: None. **J. Verhaagen:** None. **E.J. Bradbury:** None. **J. Silver:** None.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.20/T10

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life
Leverhulme Trust
Hertie Foundation
International Spinal Research Trust
Henry Smith Charity
Miami Project to Cure Paralysis
The Walter G. Ross Foundation

Title: Targeting the acetyltransferase with a small-molecule activator to enhance axon regeneration and functional recoveryafter spinal cord injury

Authors: *T. H. HUTSON[1], L. ZHOU[6], I. PALMISANO[2], F. DE VIRGILIIS[3], E. MCLACHLAN[4], C. KATHE[7], K. BARTHOLDI[8], Q. BARRAUD[9], M. C. DANZI[10], A. MEDRANO-FERNÁNDEZ[12], J. P. LOPEZ-ATALAYA[13], A.-L. BOUTILLIER[14], S. HALDER SINHA[15], L. D. MOON[16], T. KUNDU[15], J. L. BIXBY[17], V. LEMMON[11], A. BARCO[18], G. COURTINE[8], S. DI GIOVANNI[5]
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Abstract: Injured axons fail to regenerate in the adult mammalian central nervous system (CNS) leading to permanent deficits in sensorimotor function. Recent work in our lab has shown that increasing the activity of proprioceptive dorsal root ganglion (DRG) neurons using an enriched environment induces a long-lasting increase in their regenerative potential that is dependent on CREB Binding Protein (CBP) mediated histone acetylation (Hutson *et al.* under revision). Pharmacological activation of the acetyltransferase CBP/p300 using a small-molecule activator (CSP-TTK21) which can pass the blood brain barrier, enhanced histone acetylation and neurite outgrowth of DRG neurons. Delivery of CSP-TTK21 within a clinically relevant time frame after a dorsal column injury promoted regeneration of sensory axons, enhanced conduction through the lesion and significantly increased sensorimotor recovery. CSP-TTK21 treatment also promoted sprouting of afferents below the level of the lesion, facilitating spinal circuitry re-organisation that may contribute to behavioural recovery. These findings demonstrate the importance of the chromatin environment to the regenerative capacity of DRG neurons. Identifying and manipulating key histone modifiers that can orchestrate broad changes in gene transcription may lead to significant improvements in axon regeneration and functional recovery.

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Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.21/T11

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig Neilsen Grant 381793

Title: *In vivo* cellular reprogramming to restore respiratory function after SCI

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Abstract: The injured adult mammalian spinal cord is incapable of significant repair. This limitation is due in part to two major neuropathological consequences of spinal cord injury (SCI): i) *the formation of growth-inhibitory glial scars*, of which activated astrocytes are a key component, and ii) *the destruction of intraspinal neuronal connectivity*, contributed to by the loss of the interneurons in the spinal circuitry. Previous cell-based strategies have traditionally been focused on transplantation of various neural stem cells into the injury site to replace lost neurons, improve the inhibitory environment and modulate inflammation; however, there are significant hurdles to their applications. For example, obtaining sufficient amounts of purified cells for transplantation may be difficult; the procedure often requires the use of immuno-suppression, which has detrimental effects on the host; and successful implementation of such a strategy needs to address the challenges of cell survival and appropriate cell differentiation without formation of tumors. Our *in vitro* data validated that Ascl1 and mir124+mir9/9*+NeuroD1 are both potent reprogramming factors that were able to convert the activated astrocytes into functional glutamatergic neurons. However, the mir124+mir9/9*+NeuroD1 group had a higher conversion efficiency than Ascl1 group. The converted neuronal cells not only were stained positive for pan-neuronal markers, but also were mature enough to bear typical neuronal electrophysiological properties. Therefore, using SD rats with cervical contusive injuries as our in vivo models, we applied both strategies to reprogram resident reactive astrocytes to potential functional interneurons. The *in vivo* conversions were also achieved using both strategies injured in cervical spinal cord contu, yet with a much more diminished yield. The reprogrammed interneurons were able to integrate into the phrenic circuit validated by PRV tracing studies. As we expected, functional behavior examinations such as plethysmography showed no significant improvement. However, terminal diaphragm electromyography (tEMG) studies indicated that the animals treated with mir124+mir9/9*+NeuroD1 show a modest recovery. In all, our pilot study serves as a proof-of-concept for its potential translational applications in SCI repair.

Disclosures: S. Fernandes: None. **L.V. Zholudeva:** None. **Y. Li:** None. **P.W. Baas:** None. **M.A. Lane:** None. **L. Qiang:** None.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.22/T12

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life
International Spinal Research Trust
endParalysis

Title: A “stealth” gene switch for GDNF to define the therapeutic time window for motor neuron regeneration following ventral root avulsion/reimplantation

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Abstract: Gene therapy is a powerful strategy to promote spinal cord regeneration. It is essential to restrict transgene expression to the appropriate therapeutic time window. The doxycycline (dox)-inducible system is the most used regulatable gene expression platform, but this system depends on a foreign, immunogenic transactivator (TA). This precludes reliable regulation of the therapeutic gene and limits clinical translation. The glycine-alanine repeat (GAr) of Epstein-Barr virus nuclear antigen-1 inhibits its presentation to cytotoxic T cells, allowing virus-infected cells to evade the host immune system. We generated a chimeric transactivator (GArTA) and show that GArTA has an immune-evading advantage over TA in a bioassay for human antigen presentation. A comparative study of lentiviral vectors expressing the TA and GArTA in the spinal cord shows that the GArTA system is inducible for 6 dox-cycles over a 47 week period, whereas with the TA-based system luciferase expression declines during the 3rd cycle and is not reinducible, indicating that GArTA provides an immune-advantage over TA. Timed expression of GDNF with the “stealth” gene switch significantly increased spinal motor neuron survival and prevented axon trapping in a ventral root avulsion/reimplantion model. Compound muscle action potentials (CMAP) revealed that 4 week GDNF expression led to an earlier recovery of CMAP responses compared to animals with 24 weeks GDNF expression. Although time-restricted GDNF expression in a long distance regeneration model is beneficial, axon growth into the chronically denervated distal nerve stump is still only 10% of the original number of motor axons present in the intact nerve. This may be due to remodeling of the extracellular matrix in

the chronically injured nerve rendering the cellular environment less permissive for axon growth. To overcome this we have applied a lentiviral vector for the enzyme Chondroitinase ABC (ChABC) to render the matrix of the denervated nerve more permissive for axon regeneration. Currently we are investigating whether combinatorial gene therapy for GDNF and ChABC will promote more distal axon regeneration and functional recovery.

Disclosures: F. De Winter: None. **R. Eggers:** None. **E.R. Burnside:** None. **B. Hobo:** None. **S. Hoyng:** None. **M.R. Tannemaat:** None. **M.J.A. Malessy:** None. **E. Muir:** None. **E. Bradbury:** None. **J. Verhaagen:** None.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.23/T13

Topic: C.11. Spinal Cord Injury and Plasticity

Support: SANPORC

Title: Descending motor tracts synapse formation with spinally-grafted porcine iPSC-NPCs: A systematic study using a novel subpial vector-labeling technique in the rat

Authors: *Y. KOBAYASHI, M. SHIGYO, T. TADOKORO, S. MARSALA, M. MARSALA
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Abstract: (Purpose) In our recent study, we have generated and extensively characterized porcine iPSC-derived neural precursors both in vitro and after in vivo brain and spinal grafting. We have demonstrated that such iPSC-NPCs acquire a differentiation profile, which is consistent with a mature porcine CNS tissue including excitatory and inhibitory neurons at 7 -10 months after grafting. The development of synaptic contacts with the host neurons, however, is not defined at present. The goal of our current study was two fold: i) to define the development of regional spinal inter-neuronal synaptic contacts between grafted porcine iPSC-NPCs and neurons of the host, and ii) to study descending motor tracts sprouting and synapse formation with spinally grafted iPSC-NPCs.**(Methods)** Previously established and characterized porcine iPSC-NPC expressing GFP under ubiquitin or synapsin promoter were used for in vivo grafting in immunodeficient or immunocompetent-FK-506 immunosuppressed (3mg/kg/day) naïve rats. Two weeks prior lumbar spinal cord cell grafting, animals received a cervical subpial injection of AAV9-UBI-RFP (10ul) to label descending motor tracts. After cell grafting, animals survived between 2- 6 months and the presence of grafted GFP+ cells and formation of synaptic contacts with labeled RFP+ motor axons studied using immunofluorescence and confocal microscopy.**(Results)** i) In vitro differentiated iPSC-NPCs showed presence of neurons (Tuj1) astrocytes (GFAP) and oligodendrocytes (Olig2). ii) At 2-6 months after in vivo spinal grafting**,**

an extensive GFP+ graftswith a high density of GFP+ neurons (NeuN+) and terminals co-expressing synaptophysin+ puncta on the host neurons was seen. iii) RFP+ descending motor axon terminals were seen throughout GFP+ grafted regions. Some terminals appeared to develop a putative synaptic contacts with grafted GFP+ neurons. **(Discussion)** i) These data demonstrate that iPSC-derived neurons can effectively establish synaptic contact with the regional spinal host interneurons as well as with a long descending motor tracts. ii) The properties of iPSC-derived neurons appear to be similar as seen for fetal tissue or embryonic stem cell-derived neural precursors. iii) Accordingly**,** the use of iPSC-NPCs can represent an alternative cell source to be used in cell-replacement therapies aimed at restoring local synaptic circuitry.

Disclosures: M. Shigyo: None. **T. Tadokoro:** None. **S. Marsala:** None. **M. Marsala:** None.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

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Program #/Poster #: 569.24/T14

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Research Center Network for Realization of Regenerative Medicine
18bm0204001h0006
Research Project forPractical Applications of Regenerative Medicine
18bk010405h003

Title: Three dimensional quantitative evaluation of descending tracts after spinal cord injury

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Abstract: For many researches of spinal cord injury (SCI), evaluations of motor neural circuits are performed. Corticospinal tract is one of the main descending tracts of the motor circuits, and it predominantly works for upper extremities, especially in digit dexterity. In lower extremities of rodents, other descending tracts such as reticulospinal tract, rubrospinal tract, vestibulospinal tract, and monoamine-dependent tracts takes large part of its motor function. Therefore, it is indispensable to evaluate multiple descending axons for SCI researches with thoracic injury model animals which have impairments in lower extremities. Three dimensional evaluation of spinal cord tissue using transparent technique has developed in recent years. When tracer-injected central nervous tissue are transparently cleared and observed with microscopies with long working distance, it is possible to visualize three dimensional neural circuits. There are several reports which utilize the transparent spinal cords, but few of them address the precise

construction of neural circuits like fiber angle or number of branches. We made thoracic SCI model of mice, and injected fluorescent tracers into multiple descending tracts. We had transparent spinal cords with techniques of passive CLARITY technique and ScaleS, and observed them with lightsheet microscopy and multiphoton microscopy. We made an original algorism and used it to evaluate neural fibers, and quantified the outcome. Our findings demonstrate that transparent techniques can be more quantitative evaluation, which are necessary for scientific studies.

Disclosures: M. Shinozaki: None. **N. Nagoshi:** None. **K. Nakanishi:** None. **O. Tsuji:** None. **F.M. Renault:** None. **M. Nakamura:** None. **H. Okano:** None.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

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Program #/Poster #: 569.25/T15

Topic: C.11. Spinal Cord Injury and Plasticity

Support: 2018R1A2A1A0502020292
2014M3A9B6034224
2017R1D1A1B03035100

Title: Suppression of PTEN expression in neural stem cells enhances neurite growth from grafts-derived neurons in the injured spinal cord

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Abstract: Spinal cord injury causes a permanent loss of neurological functions by disrupting neural network connecting above and below the injury site. Transplantation of neural stem cells (NSCs) into the injured spinal cord holds promise to repair the disrupted neural connections by providing new neurons. However, poor capacity of axon growth from NSC-derived neurons would diminish the ability of NSCs to rebuild neural circuits. It has been reported that deletion of phosphatase and tensin homolog (PTEN), a well-known tumor suppressor gene, promotes robust axonal regeneration after CNS injury. We hypothesized that PTEN suppression in NSCs could promote axonal growth from NSC-derived neurons and that transplantation of NSCs with suppressed PTEN would lead to an improved functional recovery by allowing more frequent formation of neural connections with host neurons. NSCs obtained from fetal rat spinal cord at the 14th embryonic day were transduced with AAV2-shPTEN. NSCs with AAV2-shPTEN considerably increased the extent of neurite outgrowth *in vitro* either on permissive or inhibitory substrate. Transplantation of NSCs with AAV2-shPTEN into injured spinal cord resulted in a

significant increase in graft survival. Grafted NSCs with PTEN suppressed exhibited highly elongated morphology compared to those transduced with control AAV2-GFP. Most of the elongated neurites were positive with neurofilament immunoreactivity. Furthermore, we observed frequent synaptic contacts between grafts-derived neurons and host axons, indicating formation of new neural circuits. We are now evaluating functional recovery of injured rats with transplantation of NSCs with AAV2-shPTEN. Our results suggest that suppression of PTEN expression could improve therapeutic value of NSCs in future regenerative strategy for regaining lost neural functions following spinal cord injury.

Disclosures: H. Park: None. **D. Hwang:** None. **H. Kim:** None. **Y. Oh:** None. **B. Kim:** None.

Poster

569. Spinal Cord Injury IV

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 569.26/T16

Topic: C.11. Spinal Cord Injury and Plasticity

Support: PMU-FFF: R-14/03/060-BIE
PMU-FFF: E-15/21/109-COU
PMU-FFF: S-15/05/008-VOG
PMU-FFF: D-16/02/004-TEV
PMU-FFF: E-16/23/117-FEA

Title: ENDF1, a hops-derived neuroregenerative flavonoid to enhance neurite regrowth

Authors: *L. BIELER[1,2], M. VOGL[1,2], M. KIRCHINGER[6], C. URMANN[6], J. TEVINI[3], T. K. FELDER[3,4], L. AIGNER[2,5], H. RIEPL[6], S. COUILLARD-DESPRÉS[1]
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Abstract: Restoration of function after a lesion of the central nervous system, such as spinal cord injury (SCI), is one of the biggest challenges modern medicine is currently facing. The complex pathophysiology of SCI, and especially the accumulation of axon-growth inhibitors, presents a major obstacle to structural and functional repair. To promote regeneration in the CNS, we focused on a group of prenylflavonoids derived from hops. Recently, we identified a flavonoid called “Enhancement of Neuronal Differentiation Factor 1” (ENDF1) presenting great neuroregenerative potential. We showed that ENDF1 acts neuroprotective, promotes neuronal differentiation and enhances regrowth and branching of neurites in sensory neurons. The neuroregenerative activity of ENDF1 was further investigated on rat dorsal root ganglion (DRG)

neurons and compared to NGF, factor known to stimulate neurite outgrowth. DRG neurons were either seeded on pro-regenerative laminin or one of the following extracellular matrix (ECM) derived inhibitors: Semaphorine3A, EphrinA4 or a mix of chondroitin sulphate proteoglycans. Our assays showed that ENDF1 was as efficient as NGF to enhance regrowth and branching of neurites in rat (P2) DRG neurons. Furthermore, ENDF1 neutralised the growth inhibitory effects of the ECM inhibitors tested. To enable *in vivo* applications, we developed a method to encapsulated ENDF1 in beta-cyclodextrin complexes retaining the biological activities and providing solubility and stability under physiological conditions. Mass-spectrometry demonstrated the bioavailability of ENDF1 complexes following intravenous and intraperitoneal applications in rats, thus opening the door for investigation of the regenerative activity of ENDF1 treatments following injury of the nervous system.

Disclosures: L. Bieler: None. **M. Vogl:** None. **M. Kirchinger:** None. **C. Urmann:** None. **J. Tevini:** None. **T.K. Felder:** None. **L. Aigner:** None. **H. Riepl:** None. **S. Couillard-Després:** None.

Poster

570. Sensory Disorders: Visual and Auditory

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 570.01/T17

Topic: D.01. Sensory Disorders

Title: Clinical use of optical density ratio in determining the prognosis of central serous chorioretinopathy

Authors: *J. WON[1], Y. PARK[2]
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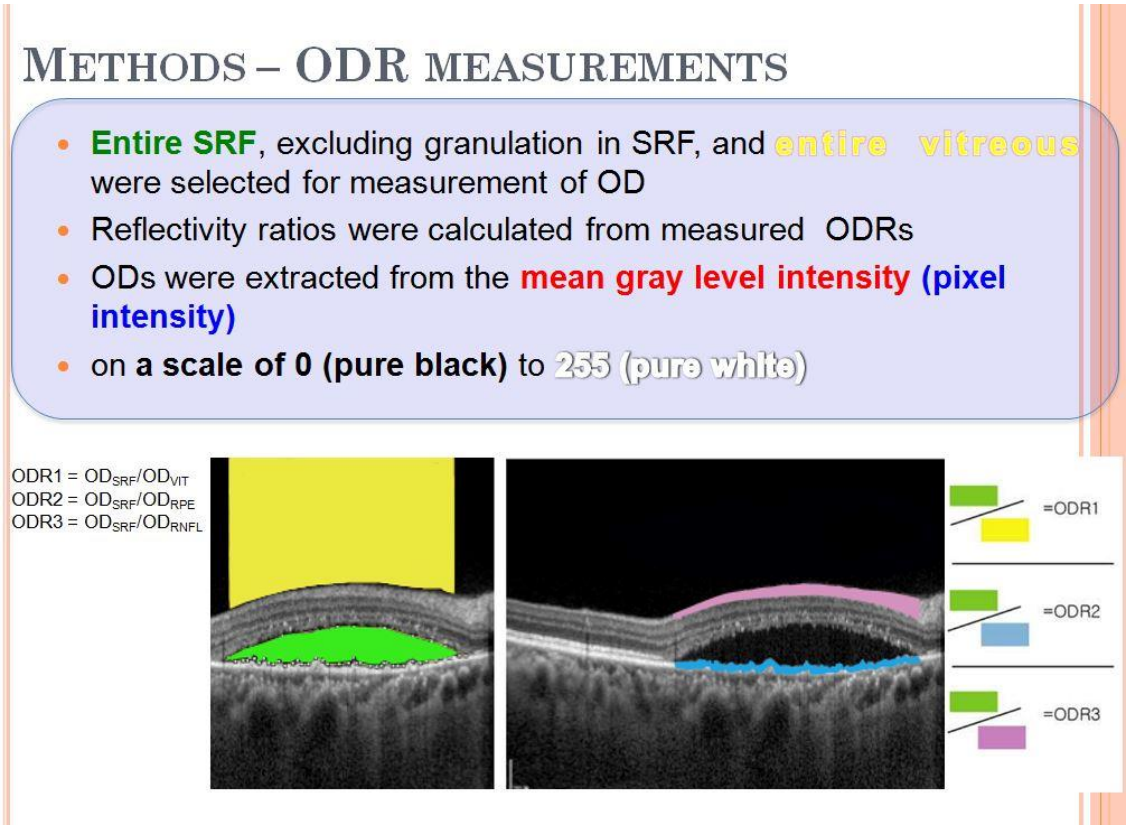
Abstract: Central serous chorioretinopathy (CSCR) is the fourth most common retinopathy after age-related macular degeneration, diabetic retinopathy and branch retinal vein occlusion. Foveal attenuation, chronic macular oedema, and damage of the foveal photoreceptor layer have been reported as causes of visual loss in CSC. Photoreceptor degeneration in the fovea, despite successful retinal reattachment, typically occurs after a duration of symptoms of approximately 4 months. Acute CSCR is a self-limited condition with resolution of neurosensory retinal detachment and generally good recovery of visual acuity within three months. However, recurrences of CSCR have been documented in up to 50% of patients within one year. Treatment should therefore be considered after 3 months if there is angiographic evidence of ongoing foveal leakage in recurrent chronic CSC or in a single CSC episode accompanied by signs of chronic CSC alterations. The prognosis of patients with chronic CSCR is poor partially because of late treatments. If we could predict the chronic nature of CSCR, we could treat the CSCR earlier and get the good prognosis of CSCR. Thus the purpose of our study is to evaluate prognostic factors

of CSCR by using initial Optical Density Ratio (ODR). A total of 87 paticipants with new onset central serous chorioretinopathy was included in the study. The optical density ratio of these eyes was evaluated by Spectralis Domain OCT (Heidelberg Spectralis OCT) at initial and 3months follow-up. The visual outcomes were measured at initial and final visit. The ODR of acute CSC was 2.09±1.36 and the ODR of chronic CSC was 7.07±3.39. It's is showed statistically significant difference (*P*= 0.0001). In ROC Curve, if the ODR of CSC is more than 3.687, CSC tends to be chronic. In conclusion, by using initial OCT findings, we could predict the prognosis (chronification) of CSC, but further evaluation is needed.



Disclosures: J. Won: None. **Y. Park:** None.

Poster

570. Sensory Disorders: Visual and Auditory

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 570.02/T18

Topic: D.01. Sensory Disorders

Support: National Council for Scientific and Technological Development (CNPq) - 202404/2015-3

Title: System x_C^- expression during diabetic retinopathy development: Modulation by Nrf2

Authors: *R. C. SANTOS[1], K. D. CALAZA[2]
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Abstract: Introduction: Diabetic retinopathy is one of the main causes of blindness in young adults, and increased oxidative stress is related with its development. System xc-, a glutamate/cystine exchanger, facilitates cystine uptake. In the intracellular medium, cystine is converted to cysteine, which is used for glutathione (GSH) synthesis, an important antioxidant molecule. System xc- is composed by 4FC and xCT proteins, and xCT, the functional subunit of this system, is under regulation of Nrf-2 due to binding at the antioxidant responsive element (ARE) region and its activity is decreased in the retina in diabetic condition. Objective: to investigate the temporal relationship between xCT levels and Nrf2 activity during the progression of diabetic retinopathy. Methods: Diabetes was induced in male Wistar rats weighting 200 g by a streptozotocin injection, and their retinas were collected after 15 days, 1, 2 and 6 months of diabetes induction. Expression of xCT was analyzed by qPCR and by western blot. Reactive oxygen species (ROS) were quantified using DCFH-DA and GSH levels were measured by a commercial kit. Nrf-2 activity was determined by a commercial kit. Nrf2 binding to ARE region was measured by ChIP protocol. Results: xCT expression (mRNA and protein) in the retina were significantly decreased in PCR (46 ± 15%, N=4) and western blot (38 ± 25%, N=5) analyses after 1 month of diabetes. At 2 months, xCT expression return to normal levels, however, at 6 months of diabetes xCT was again reduced (PCR: 46 ± 15%, N=6 / Western blot: 39 ± 11%, N=6). Activity of Nrf-2, an inducer of xCT, was impaired within 15 days of diabetes (39 ± 15%, N=4) and 1 month (30 ± 15%, N=4). At 2 months, Nrf2 activity came back to normal levels whereas after 6 months, Nrf-2 activity was decreased (37 ± 14%, N=6). Confirming the causal relation between Nrf-2 activity and xCT expression, Nrf-2 binding to xCT ARE region was reduced after 1 month (63 ± 12%, N=4) and 6 months (73 ± 7%, N=4). Consistent with the role of xc- in protection against oxidative stress due to GSH production, after 1 month, GSH levels were reduced (33 ± 12%, N=5) and continued to be subnormal until 6 months of diabetes. Also, ROS is increased after 15 days (205 ± 94%, N=4) and remained altered until later stages. Conclusion: These data show a temporal relationship between xc-, Nrf2, and other parameters implicated in the maintenance of oxidative stress, and suggest that reduced Nrf-2 activity could play a role in impairing proper function of the system xc- during the progression of diabetic retinopathy.

Disclosures: R.C. Santos: None. **K.D. Calaza:** None.

Poster

570. Sensory Disorders: Visual and Auditory

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 570.03/U1

Topic: D.01. Sensory Disorders

Support: Heinsius-Houbolt Foundation

Title: The medial geniculate body as a target for deep brain stimulation to treat tinnitus: A rodent study

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Abstract: Tinnitus is a debilitating phenomenon and remains a therapeutic challenge. Neuromodulation is a promising treatment modality for tinnitus. The medial geniculate body (MGB) of the thalamus plays a key role in the pathophysiology of tinnitus, as it integrates and processes auditory and limbic information. We already showed that deep brain stimulation of the inferior colliculus and dorsal cochlear nucleus, both nuclei of the central auditory pathway, suppressed tinnitus-like behavior in rats. Compared to these candidate targets for deep brain stimulation (DBS), the MGB is more easily accessible using stereotaxy in human. This experiment assessed the effect of high frequency stimulation and low frequency stimulation of the medial geniculate bodies on tinnitus in a noise-induced tinnitus rat model. Anxiety-related side-effects were evaluated in the elevated zero maze and open field. Eleven subjects were included and a repeated measures design was used. Presence of tinnitus was verified using the gap induced pre-pulse inhibition of the acoustic startle response paradigm. Hearing thresholds were determined before and after noise trauma with auditory brainstem responses. Results show tinnitus development after noise-trauma and preserved hearing thresholds of the ear that was protected from noise trauma. We found that high frequency stimulation of the medial geniculate bodies suppressed tinnitus-like behavior. This effect maintained directly after stimulation when the stimulation was turned off. Low frequency stimulation did not have any effects on the gap:no-gap ratio of the acoustic startle response. No anxiety or locomotion related side-effects were found in the elevated zero maze and open field. Thus, high frequency DBS of the MGB might be a promising treatment option for patients with severe, refractory tinnitus.

Disclosures: G. Van Zwieten: A. Employment/Salary (full or part-time):; Maastricht University Medical Center. **M.L.F. Janssen:** None. **J.V. Smit:** None. **A.M.L. Janssen:** None. **M. Roet:** None. **A. Jahanshahi:** None. **R.J. Stokroos:** None. **Y. Temel:** None.

Poster

570. Sensory Disorders: Visual and Auditory

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 570.04/U2

Topic: D.01. Sensory Disorders

Support: PO1-GM118269
TR01-GM104948

Title: Analysis of ketamine-induced gamma burst pattern in rats using k-means clustering of continuous wavelet transform power-frequency estimates

Authors: *J. A. GUIDERA[1], N. E. TAYLOR[2], J. T. LEE[4], K. VLASOV[5], J. PEI[5], E. N. BROWN[6], K. SOLT[3]
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Abstract: INTRODUCTION: Ketamine is a general anesthetic that induces distinct frontal electroencephalogram (EEG) oscillations in humans, including a "gamma burst" pattern characterized by periods of elevated delta (1 - 4 Hz) power alternating with periods of elevated gamma (25 - 80 Hz) power (Akeju et al. 2016). Whether the ketamine-induced gamma burst pattern extends beyond the prefrontal cortex (PFC) to subcortical or other cortical structures is not known. In addition, an objective method for identifying the distinct delta dominant and gamma dominant states to enable their separate analysis is lacking. In this study, we conducted simultaneous cortical and subcortical local field potential (LFP) recordings in rats under ketamine anesthesia. We used k-means clustering, which has been used to identify sevoflurane-induced LFP states (Hudson et al. 2014), and the continuous wavelet transform (CWT) to objectively identify the gamma and delta dominant states of the ketamine-induced gamma burst pattern.
METHODS: Five anesthetized male Sprague-Dawley rats were implanted with intracranial electrodes in the PFC, parietal cortex (PC), and central thalamus (CT). In a separate surgery, a femoral central venous catheter was placed. After full recovery from surgery, the rats underwent an infusion of ketamine (2 $mg{\cdot}kg^{-1}min^{-1}$ iv). LFP power and coherence were estimated using wavelet methods. K-means clustering of PFC LFP power-frequency estimates was used to identify delta dominant and gamma dominant states.
RESULTS: Ketamine induced a gamma burst pattern in PFC, PC, and CT LFPs that was most prominent in the PFC LFP. K-means clustering of CWT PFC LFP power-frequency estimates effectively identified delta dominant and gamma dominant states. At the group level, the gamma and delta dominant states were characterized by peak increases in gamma and delta PFC, PC,

and CT LFP power, respectively. During the delta dominant state, cortical (PFC-PFC and PFC-PC) and thalamo-cortical (PFC-CT and PC-CT) LFP delta coherence were increased. During the delta dominant and gamma dominant states, prefrontal (PFC-PFC) and thalamo-parietal (PC-CT) LFP gamma coherence were increased.
CONCLUSIONS: These results suggest that the ketamine-induced gamma burst pattern extends beyond PFC to subcortical and other cortical structures. The delta dominant state of the gamma burst pattern is accompanied by increased low-frequency connectivity between distant regions. K-means clustering of CWT power-frequency estimates enables characterization of anesthetic-induced oscillatory dynamics that consist of more than one state.

Disclosures: J.A. Guidera: None. **N.E. Taylor:** None. **J.T. Lee:** None. **K. Vlasov:** None. **J. Pei:** None. **E.N. Brown:** None. **K. Solt:** None.

Poster

570. Sensory Disorders: Visual and Auditory

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Program #/Poster #: 570.05/U3

Topic: D.01. Sensory Disorders

Support: Swiss National Science Foundation (168587)
Swiss National Science Foundation (177744)
Tinnitus Research Consortium (GR409411)

Title: Detecting tinnitus in nonhuman primates by using a non-acoustic startle paradigm

Authors: *L. ROGENMOSER, P. KUSMIEREK, D. ARCHAKOV, J. RAUSCHECKER
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Abstract: Tinnitus impairs the quality of life of millions of Americans, making it a current concern for Public Health in an aging population. Animal models are indispensable for the development of evidence-based therapy. Existing rodent models of tinnitus have been criticized because their results failed to translate to human patients. The reason for this may be that rodents lack a brain area in medial prefrontal cortex (vmPFC) that human imaging studies have shown to be causally related to the gating of tinnitus. Since vmPFC is highly developed in nonhuman primates, we aimed to establish a tinnitus model in rhesus monkeys.Tinnitus was determined by using a non-acoustic startle paradigm. In contrast to most animal studies in which the startle response is recorded by an accelerometer, we measured the eye blink, as commonly done in human startle experiments. Eye blinks were monitored by recording electromyographic (EMG) activity in response to air-puffs as startle stimuli, preceded by short auditory stimuli varying in frequency and intensity. The tones were adjusted according to the hearing thresholds, which were determined by frequency-specific Auditory Brainstem Response recordings. The threshold-

adjusted intensity levels were: +30dB SL,+6dB SL and -6dB SL. Since tinnitus loudness is known to range between 6-30 dB SL, we expected tinnitus to mask the mimicking frequency at the +6dB SL level, revealing the tinnitus frequency by an altered startle response. In this pilot study, one monkey was tested at its baseline, at a reversible tinnitus level (after administration of salicylate, 200mg/kg), and at a follow-up level. In order to ensure translation of the results to humans, a sample of human tinnitus patients and of matched control subjects without tinnitus underwent the same testing paradigm.The peaks of the EMG activity were extracted and subjected to inferential statistics. Unlike previous studies on tinnitus that make use of the Prepulse Inhibition phenomenon for tinnitus detection, our preliminary data strongly suggest the opposite, namely a Prepulse Facilitation. Our preliminary results suggest that the preceding tone facilitates the eye blink response as long as it is reliably perceived. In both species, the +6dB adjustment revealed the tinnitus frequency by a lack of facilitation.
The use of a non-acoustic startle stimulus is advantageous since it is free from acoustic interference and less aversive (especially for patients with hearing issues like tinnitus and hyperacusis). Since startle paradigms do not require instrumental conditioning or training, this set-up could easily be applied to a larger population, such as geriatric monkeys in a primate center.

Disclosures: L. Rogenmoser: None. **P. Kusmierek:** None. **D. Archakov:** None. **J. Rauschecker:** None.

Poster

570. Sensory Disorders: Visual and Auditory

Location: SDCC Halls B-H

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Program #/Poster #: 570.06/U4

Topic: D.01. Sensory Disorders

Support: 1U54NS083924-01 NINDS-NIH
USDE Title V-P031S130068
UCC seed fund

Title: Retinal albinism and abnormal electroretinograms in VAMP7 null

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Abstract: Throughout evolution, the visual systems have preserved photoreceptor cells for phototransduction and chemical synapses for downstream neuronal connectivity. The composed signal of an electroretinogram (ERG) records the electrical activity of phototransduction and

synaptic transmission evoked by light stimulation. In *Drosophila* ERGs, light stimulation induces a sustained depolarization associated with phototransduction with characteristic On and Off responses associated with synaptic transmission at the afferent and efferent visual inputs. Nevertheless, the molecular mechanisms that underlie these components and their relevance in image acquisition are not completely elucidated. Light-evoked responses are transient when the TRPC channels are reduced, and severely diminished when the phototransduction pathway is impaired. In turn, a reduction in the endocytic capacity for activated photoreceptors extends the prolonged depolarization after light (PDA), while both alterations can modify the On/Off components. Proteins required for membrane fusion and recycling like non-canonical SNAREs can potentially modify the On/Off and/or the phototransduction components. However, the role of non-canonical SNAREs in the visual system is not clear. VAMP7 is a non-canonical SNARE involved in membrane recycling and fusion with lysosomes. Here we scrutinize the role of VAMP7 in the visual system of *Drosophila* by using a VAMP7 null model. VAMP7 null ERGs display overall biphasic characteristics like WHITE null animals. A detailed ERG analysis indicates that VAMP7 null displays abnormal Off components. Additionally, VAMP7 null presented diminished and slower phototransduction kinetics with an extended PDA. A slower recovery of the fast ERG component was also observed. Consistent with the role of lysosomes in the accumulation of visual pigments in the retina, *Drosophila* VAMP7 null adults display retinal albinism. The same TRPC mediated phototransduction pathway has been observed in vertebrate photosensitive pigment cells of the iris. Our data, suggest that similar visual pathophysiology may be present in patients with abnormal lysosomal function, as in Hermansky-Pudlak Syndrome, a condition with retinal albinism and visual impairment.

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Poster

570. Sensory Disorders: Visual and Auditory

Location: SDCC Halls B-H

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Program #/Poster #: 570.07/U5

Topic: D.01. Sensory Disorders

Support: Agencia Espacial Mexicana-CONACyT grant 275058
BUAP-DITCO2016-09

Title: Effect of Vestibular Galvanic Stimulation on eye movements and orientation of the head and body

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Abstract: Vestibular alterations have a high prevalence in the population in Mexico that varies from 1.8% in adults to more than 30% in old age. This work provides data for the design of a prosthetic device based on galvanic vestibular stimulation (GVS). We used three arrays of peri auricular electrodes for the bilateral bipolar GVS, arranged parallel to the semicircular canals (CSS, anterior, posterior and horizontal) with the polarity inverted on each side of the head. Twenty voluntary subjects, 7 women and 13 men (age 20 to 30 years) were recruited. For the experiments subjects were informed of the GVS character and consent informed was signed according to the Helsinki declaration of Ethical Principles for Medical Research Involving Human Subjects. The direct current injection had a mean of 1.7 ± 0.2 mA and duration of 10 s. The inclination of the head was measured by means of a triaxial accelerometer system (3DM-GX3-15, MicroStrain), the distribution of body weight (CBP-center of pressure) was measured using a stabilometric platform, and eye movements were studied by means of a video nystagmograph (Micromedical Technologies). The distance traveled (sum of the trajectories of displacements), magnitude and direction of the displacement vector was analyzed. The GVS in parallel to the anterior SCC, modulated gaze control by modifying the magnitude of the horizontal travel path and its direction and decreased the magnitude of the CBP tilt vector as the path traveled by the inclination of the head in the roll plane increased. The GVS in the direction of the posterior SCC, produced head tilt in the roll plane, decreased the path traveled from the antero-posterior CBP, and changed the direction of inclination of the CBP. The GVS in the direction of the horizontal SCC, decreased the path of the eyes vertically. The path traveled by the inclination of the head increased in the planes of the roll and pitch and the direction of inclination of the head changed from left and front to the left and back. Results showed that GVS had an effect on the parameters studied with periauricular electrode arrays aimed to specifically stimulating the three SCC. Funded by AEM-CONACYT (275058) and BUAP-DITCO2016-09

Disclosures: O. González: None. **R. Vega:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent. **E. Soto:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent.

Poster

570. Sensory Disorders: Visual and Auditory

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 570.08/U6

Topic: D.01. Sensory Disorders

Support: NSERC PGS D
Jonathan & Joshua Memorial Graduate Scholarship
CIHR

Title: Involvement of pedunculopontine tegmental nuclei in sensorimotor gating: Chemogenetic-induced inhibition of general, cholinergic and glutamatergic PPTg neurons

Authors: *N. FULCHER[1], E. AZZOPARDI[2], C. DE OLIVEIRA[2], S. SCHMID[1,2]
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Abstract: The human brain persistently receives sensory inputs from the environment. An innate process that filters out redundant stimuli is called sensorimotor gating, which can be quantified through prepulse inhibition (PPI) of the acoustic startle response (ASR). Deficits of PPI are seen in a host of psychiatric illnesses, such as schizophrenia, autism spectrum disorder, Tourette's syndrome, etc. Chronic lesions of the midbrain pedunculopontine tegmental nucleus (PPTg) have shown to disrupt PPI. Albeit underlying mechanisms of PPI remain unclear, cholinergic PPTg projections to the startle-mediating giant neurons in the brainstem have been suggested as an integral part of the PPI pathway. Importantly, the PPTg is comprised of three distinct neuron types: cholinergic, glutamatergic and GABAergic neurons. Our work revisits the hypothesis that PPTg projections in general, and PPTg cholinergic projections specifically, mediate PPI. We intracranially delivered bilaterally a general-, a cholinergic- or a glutamatergic cell-specific inhibiting DREADD (designer receptors exclusively activated by designer drugs), or a control vector, into the rat PPTg. Three weeks later, animals were tested for startle, PPI of ASR, open-field and morphine-induced conditioned place preference (CPP) deficits after receiving an i.p. injection of the DREADD ligand clozapine-n-oxide (CNO) in Dimethyl Sulfoxide (DMSO) to activate the virus, or vehicle. In order to enhance area specificity, general DREADD expression was combined with local CNO micro-infusions into the PPTg through chronically implanted bilateral cannula After behavioural testing, animals were perfused and immunohistochemistry or FISH (fluorescence *in situ* hybridization) was performed to ensure successful expression and location of the respective DREADDs. Results suggest that transient DREADD inhibition of all PPTg neurons disrupts PPI, while cholinergic inhibition does not significantly alter PPI. Moreover, data suggests that glutamatergic silencing disrupts PPI. These data highlight the important role of the PPTg in sensorimotor gating and its deficits, but suggest that glutamatergic and not cholinergic PPTg neurons mediate PPI.

Disclosures: E. Azzopardi: None. **C. De Oliveira:** None. **S. Schmid:** None.

Poster

570. Sensory Disorders: Visual and Auditory

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Program #/Poster #: 570.09/U7

Topic: D.01. Sensory Disorders

Support: W81XWH-15-2-0024

Title: Blast-induced structural and transcriptome changes in the ear lead to hearing impairments in rats

Authors: *Y. WANG, R. URIOSTE, Y. WEI, D. WILDER, Y. CHENG, S. SAJJA, I. GIST, P. ARUN, J. LONG
Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: Auditory dysfunction is one of the most common disabilities in military personnel and civilian exposed to blast shockwaves. To better understand the pathological processes underlying this injury, we have evaluated damages to ear structures and carried out global gene expression profiling of the cochlea at acute and chronic stages after blast shockwave exposure of rats in an advanced blast simulator using RNA-Seq. Auditory dysfunction was verified by DPOAE and ABR assessments which revealed significant changes in the ABR waveforms and elevations of threshold after blast exposure. These changes were observed over the entire acoustic frequency spectrum and persisted over several months. Compared to high frequency (40 kHz) hearing loss after blast exposure, low frequency (8 kHz) hearing recovered relatively early after insult. Cochlear RNA-seq identified (according to an FDR-corrected p-value 0.05) 1158 differentially expressed genes (DEGs) which represent 3.98% of the total, at 1 day post-injury, of which 462 were up-regulated and 696 were down-regulated. At 28 days post-injury, the data showed 48 DEGs (0.16% of the total), of which 28 were up-regulated and 20 were down-regulated. The DEGs were categorized according to gene ontology (GO) annotation. The top categories in biological processes which include localization, regulation of cation channel activity, transport, nervous system development, neurotransmitter levels and cell-cell signaling were significantly altered at 1 day post-injury, while a category in antigen processing and presentation was significantly changed at 28 days post-injury. Seven DEGs were found in the acute and chronic phases that associate with inner ear mechanotransduction, cytoskeletal reorganization, myelin development and axon survival. Further studies on altered gene expression may provide insights into new therapeutic targets and methods for treating or preventing blast-induced auditory deficits.

Disclosures: Y. Wang: None. **R. Urioste:** None. **Y. Wei:** None. **D. Wilder:** None. **Y. Cheng:** None. **S. Sajja:** None. **I. Gist:** None. **P. Arun:** None. **J. Long:** None.

Poster

570. Sensory Disorders: Visual and Auditory

Location: SDCC Halls B-H

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Program #/Poster #: 570.10/U8

Topic: D.01. Sensory Disorders

Support: MOST-GACR: 105- 2923-B-009 -001 -MY3

Title: Salicylate enhanced the neural synchrony at the auditory cortex in behaving rats

Authors: *T.-W. CHIU[1], S.-T. HSU[2], D. SUTA[3]
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Abstract: High dose salicylate (SS) is well known to induce temporal tinnitus in human and animals and the SS-treated animal model has widely used for exploring how the temporal tinnitus can be generated. Here we tried to determine the neural mechanisms of SS-induced tinnitus by assessing the changes in auditory evoked potentials (AEPs) recorded from the auditory cortex in awake rats after systemic injection of SS. AEPs were recorded from awake rats (n=6) with electrodes surgically implanted at the auditory cortex a. A week after surgery, we recorded cortical AEPs to clicks and tone pips with frequencies at 1, 10 and16 kHz at random intensity steps from 0 to 75 dB SPL. Control data were first collected. Animals were then given daily injection of SS (250 mg/kg, i.p.) for 5 consecutive days. Sound evoked responses were collected every day within 2-6 hrs post- injection. Average EPI (EP integral) was first extracted and amplitude-intensity-functions were obtained to evaluate the SS effects. Then, the single trial AEPs were analyzed by level-response probability function, inter-trial correlation and inter-trial coherence (with EEG lab) to evaluate the SS-effects on neural synchrony of cortical activities. Comparing to the control, SS significantly increased the average EPI, response probability, inter-trial correlation coefficients and inter-trial trial coherence to the middle to high intensity sounds. Results suggested that SS enhanced AEPs may relate to an elevation of the central gain manifested by enhanced neural synchrony.

Disclosures: T. Chiu: None. **S. Hsu:** None. **D. Suta:** None.

Poster

570. Sensory Disorders: Visual and Auditory

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 570.11/U9

Topic: D.01. Sensory Disorders

Support: CIHR MOP-125962
FRQS-AMD program

Title: Anti-VEGF antibody and kinin B1 receptor blockade differently impact laser-induced choroidal neovascularization

Authors: *E. H. VAUCHER[1], S. HACHANA[1], O. FONTAINE[1], R. COUTURE[2]
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Abstract: The neovascular aged-related macular degeneration (AMD) causes severe vision loss due to neuronal death in retina consecutive to inflammation, breakdown of blood retinal barrier and choroidal neovascularization. In seeking of efficient treatments to prevent retinal damage, the kallikrein-kinin system (KKS), a key player in inflammation, has been tested in the present study. Particularly, the role of kinin B1 receptor (B1R) has been examined since this receptor plays a crucial role in retina inflammation in diabetic retinopathy. Moreover, the interaction of B1R with the vascular endothelial growth factor VEGF is investigated in relation to the central role of this factor in AMD. The choroidal neovascularization (CNV) was induced in the left eye of Long-Evans rat. Treatments were initiated immediately with a single intravitreal injection of B1R siRNA (10 nmol/5µL) or anti-VEGF (125µg/5µL), or after one week with an eye-drop application of the B1R antagonist R-954 (≈100 µg/10µL bid) or their respective controls. The impact of those treatments was measured on vascular permeability, leukostasis and on gene expression of retinal inflammatory mediators (qRT-PCR). The distribution of B1R on retinal cell types was investigated by immunocytochemistry. The B1R was found overexpressed on endothelial and glial cells in retinas with CNV. Anti-VEGF and B1R blockade/deletion significantly reduced CNV lesions and the inflammatory response (adherent leukocytes and enhanced vascular permeability). Whereas anti-VEGF blunted the overexpression of most markers (B1R, B2R, VEGF, VEGF-R2, HIF-1α, TNF-α, MCP-1, ICAM-1 and VCAM), R-954 had no significant impact on the VEGF system, HIF-1α, MCP-1, VCAM-1. However, the overexpression of both kinin receptors, IL-1β, TNF-α and ICAM-1 was prevented by R-954. Our data suggest that VEGF and B1R pathways have different effects on retina damage in a model of AMD and that blockade of B1R by eye-drops application of R-954 may represent a less invasive therapy in the treatment of AMD than anti-VEGF therapy.

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Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 571.01/U10

Topic: D.03. Somatosensation: Pain

Support: SAF2017-83674-C2-1-R and SAF2017-83674-C2-2-R, MINECO, Spain, and ERDF, European Commission

Title: Photomodulation of spontaneous electrical activity in guinea pig corneal cold nerve terminals by means of a p2x channel -permeant photoswitch

Authors: *V. MESEGUER, D. ARES, E. VELASCO, S. QUIRCE, M. ACOSTA, C. BELMONTE, J. GALLAR
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Abstract: Photo-isomerizable small molecules (photoswitches) allow modulation of neural activity by acting on native ion channels without requiring exogenous gene expression. DENAQ, a synthetic photoswitch, confers light-induced firing to retinal ganglion cells (RGCs) in a mouse model of retinitis pigmentosa; DENAQ entry into RGCs depends on functional upregulation of P2X receptors. We explored here: first , whether photoswitches modulate the electrical activity of peripheral sensory nerve terminals of the cornea. Secondly, whether this modulation depends on functional expression of P2X and/or TRPV1 channels at corneal nerve terminals. We recorded nerve terminal impulse (NTI) activity in cold terminals of excised guinea-pig corneas pre-incubated with 2 mM DENAQ for 40 min at 34ºC. In a separate set of experiments, DENAQ was co-applied with either the P2 receptor antagonist suramine at 1mM or the TRPV1 channel antagonist capsazepine at 10 µM performed 15 minutes before DENAQ. Afterwards, extracellular electrophysiological recording of NTI activity was initiated in the dark. Cold-thermoreceptor terminals fired spontaneous NTIs at 34ºC; this frequency increased markedly in response to a cooling ramp down to 15°C. A Light-Emitting Diode was used to deliver 125 mW/cm^2 of blue (460 nm) light in 5 cycles of alternating 15-sec light/dark intervals. In DENAQ-treated corneas, ongoing activity at 34ºC in the dark was higher (5.16 ± 0.66 $imp*s^{-1}$) than in the light (3.28± 0.62 $imp*s^{-1}$, $p=2.96 \times 10^{-14}$, Bonferroni post-hoc pair test, n=25). Contrarily, in non-treated corneas, ongoing activity was not affected by exposure to the light (7.37 ± 0.81 vs 7.55 ± 0.83 $imp*s^{-1}$, p=0.318, n=24). Prior application of the P2 receptor antagonist suramine significantly reduced the photo-modulation mediated by DENAQ (9.01 ± 1.04 in dark vs 8.69 ± 0.97 $imp*s^{-1}$ in light, p=0.331, n=8). The possibility of TRPV1 mediated DENAQ entry was excluded by application of the TRPV1 antagonist capsazepine, which did not modify NTI ongoing activity (4.08 ± 0.96 in dark vs 3.33 ± 0.89 $imp*s^{-1}$ in light, p=0.015, n=7). Taken together, these results suggest that DENAQ enter to corneal cold sensory nerve endings primarily through P2X channels and produces a robust decrease of the cold thermoreceptors spontaneous electrical activity in the presence of blue light.

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Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 571.02/U11

Topic: D.03. Somatosensation: Pain

Title: Spiperone produces nociception in rats by activation of calcium-activated chloride channels

Authors: *A. PLUMA[1], I. VELAZQUEZ-LAGUNAS[2], J. MURBARTIAN[3], V. GRANADOS-SOTO[4]
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Abstract: Previous studies have suggested that spiperone activates Ca^{2+}-activated Cl^- channels (CaCCs) in cell culture. Since stimulation of CaCCs in vivo leads to nociception, it is likely that spiperone would produce nociception in naïve rats and would increase formalin-induced nociception. The aim of this investigation was to assess the participation of CaCCs in the pronociceptive effect of spiperone in rats. In addition, we compared the effects of spiperone with that of the CaCCs activator Eact. Local peripheral injection of spiperone (1-10 μg) or Eact (1-30 μg) induced nociception in naïve rats in a dose-dependent manner. Furthermore, local peripheral injection of spiperone (3-10 μg) or Eact (10-30 μg) enhanced 0.5% formalin-induced nociception. Local peripheral administration of selective CaCCs inhibitors ($T16A_{inh}$-A01 and $CaCC_{inh}$-A01, 0.1-1 μg) diminished spiperone (10 μg)- and Eact (30 μg)-induced nociception. Moreover, CaCCs inhibitors (0.1-1 μg) dose-dependently reduced the pronociceptive effect of spiperone (10 μg) or Eact (30 μg) and 0.5% formalin. Finally, the TRPV1 channel blocker capsazepine (3-30 μg) reduced in a dose-dependent fashion nociception induced by Eact but not by spiperone. Our results suggest that spiperone and Eact activate CaCCs in vivo to induce nociception and to enhance formalin-induced nociception. The nociceptive effect of Eact, but not spiperone, also depends on activation of TRPV1 channels. Thus, spiperone induces nociception by activation of CaCCs but not TRPV1 channels.

Disclosures: A. Pluma: None. **I. Velazquez-Lagunas:** None. **J. Murbartian:** None. **V. Granados-Soto:** None.

Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 571.03/U12

Topic: D.03. Somatosensation: Pain

Support: SIP IPN Project 20181089

Title: Analgesic and anti-inflammatory screening of two regioisomers of phenylisolin-1,3-dione, thalidomide analogues

Authors: I. M. CUMBRES-VARGAS[1], C. CAMPOS RODRIGUEZ[2], S. R. ZAMUDIO[3], J. G. TRUJILLO-FERRARA[4], *E. RAMIREZ-SAN JUAN[1]
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Abstract: Thalidomide is considered an anti-inflammatory and analgesic drug, that can modulate the production of proinflammatory cytokines such as: TNF-α, interferon ɤ, interleukin 10, interleukin 12 and prostaglandins. However, due to its teratogenic effects, thalidomide has been classified as a restricted drug. Thalidomide is a chiral molecule formed by a phthaloyl and glutarimide moiety: *S*-isomer has been suggested to be responsible of the teratogenic side effect. Recent studies have found that the pharmacological effects such as analgesic and antiinflammatory, induced by thalidomide are consequence to the presence of pthaloyl group. Based on the previous (*o*-phenyl)-isoindolin-1,3-dione (OFI) and (*p*-phenyl)-isoindolin-1,3-dione (PFI), symmetric thalidomide analogues, were synthesized to preserve or improve the analgesic and anti-inflammatory effect of lead compound.
The antinociceptive and anti-inflammatory effects were evaluated by two tests: 1) the formalin test, in which 1% formalin was injected subcutaneously into the plantar surface of the right hindpaw and the number of flinching of the injected paw was measured; 2) the tail-flick test using radiant heat (I=14), in which "tail flick" latency was measured. For each test, 5 groups of male Sprague Dawley rats (n= 8) were employed: 1) indomethacin (5 mg/kg), 2) 0.5% carboxymethylcellulose in phosphate buffer as the control group, three PFI doses 3) 100, 4) 316 and 5) 421.7 mg/kg.
The results have shown that OFI 100 mg/kg and 421.7 mg/kg doses decreased the number of flinches in the formalin, but the effect was not significant. Nevertheless, the same OFI doses caused a significant increase in the withdrawal latency of the tail whereby OFI could be considered as an analgesic drug. On the other hand, PFI presented a significant decrease in the number of flinching with the three tested doses and a significant increase in the withdrawal latency of the tail suggesting that PFI has an analgesic and further anti-inflammatory effect.

Disclosures: I.M. Cumbres-Vargas: None. **C. Campos Rodriguez:** None. **S.R. Zamudio:** None. **J.G. Trujillo-Ferrara:** None. **E. Ramirez-San Juan:** None.

Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 571.04/V1

Topic: D.03. Somatosensation: Pain

Support: Astellas Pharma Inc.

Title: A novel lysophosphatidic acid receptor 5 antagonist, AS2717638, exerts analgesic effects in rodents

Authors: *N. MURAI, H. HIYAMA, T. KISO, T. SEKIZAWA, T. WATABIKI, H. OKA, T. AOKI
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Abstract: Lysophosphatidic acid (LPA) is a bioactive lipid that acts via at least six G protein-coupled receptors, LPA receptors 1-6 (LPA1-6), for various physiological functions. We examined 1) whether LPA5 is involved in pain signaling in the spinal cord; and 2) the pharmacological effects of a novel LPA5 antagonist on synaptic transmission in spinal cord slices, intrathecal prostaglandin (PG)- and (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-induced allodynia, and neuropathic and inflammatory pain in rodents. Intrathecal injection of a selective LPA5 agonist, geranylgeranyl diphosphate, and a non-selective agonist, LPA, induced allodynia in wild type, but not in LPA5 knockout mice. These results suggest that LPA5 is important for pain signal transmission in the spinal cord. AS2717638 (6,7-dimethoxy-2-(5-methyl-1,2-benzoxazol-3-yl)-4-(piperidin-1-ylcarbonyl)isoquinolin-1(2*H*)-one) bound to the LPA-binding site on LPA5 and selectively inhibited LPA-induced cAMP accumulation in human LPA5- but not LPA1-, 2-, or 3-expressing cells. Further, oral administration of AS2717638 inhibited LPA5 agonist-induced allodynia in mice. AS2717638 also significantly improved PGE_2-, $PGF_{2\alpha}$-, and AMPA-induced allodynia, while both pregabalin and duloxetine alleviated only PGE_2-induced allodynia in mice. Similarly, AS2717638 significantly ameliorated static mechanical allodynia and thermal hyperalgesia in a rat model of chronic constriction injury (CCI)-induced neuropathic pain. In addition, AS2717638 reduced miniature EPSC frequency in dorsal horn neurons from CCI rats. AS2717638 also showed analgesic effects in a rat model of inflammatory pain. These findings suggest that LPA5 is involved in broad pain signaling in the spinal cord such as neuropathic and inflammatory pain and that pharmacological antagonism of LPA5 is an attractive novel pain therapy.

Disclosures: **N. Murai:** A. Employment/Salary (full or part-time):; Astellas Pharma Inc. **H. Hiyama:** A. Employment/Salary (full or part-time):; Astellas Pharma Inc. **T. Kiso:** A. Employment/Salary (full or part-time):; Astellas Pharma Inc. **T. Sekizawa:** A. Employment/Salary (full or part-time):; Astellas Pharma Inc. **T. Watabiki:** A. Employment/Salary (full or part-time):; Astellas Pharma Inc. **H. Oka:** A. Employment/Salary (full or part-time):; Astellas Pharma Inc. **T. Aoki:** A. Employment/Salary (full or part-time):; Astellas Pharma Inc..

Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 571.05/V2

Topic: D.03. Somatosensation: Pain

Support: European Union Seventh Framework Programme (FP7/2007 - 2013) under grant agreement no 602919.

Title: Fingolimod does not reduce pain behavior in the spinal nerve ligation model of pain or prevent the development of morphine tolerance in rat

Authors: *V. JOKINEN[1], T. LILIUS[1], P. RAUHALA[1], E. KALSO[1,2]
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Abstract: AIMS OF INVESTIGATION: Neuropathic pain and opioid tolerance remain major clinical challenges. Microglia activation and related neuroinflammation are known to play a significant role in the pathophysiology of both conditions. Interestingly, fingolimod (FTY720), sphingosine-1-phosphate (S1P) receptor agonist, used to treat multiple sclerosis has been recently recognized as a potent microglia modulator. Indeed, fingolimod has been shown to decrease chemotherapy-induced neuropathic pain behavior via microglial S1P receptor, and to reduce pain behavior in a model of central neuropathic pain and a spared nerve injury model for neuropathic pain. The aims of this investigation were to evaluate the efficacy of fingolimod on peripheral nerve injury in the spinal nerve ligation model of pain, but also on the development of morphine tolerance. The research was confirmatory.
METHODS: Effects of fingolimod (0.01 or 1 mg/kg, i.p.) on mechanical and thermal withdrawal thresholds were studied after ligation of lumbar spinal nerve (SNL) on male Wistar-Han rats (120-150 g). Fingolimod was administered daily beginning on the second day after the surgery. Mechanical allodynia was assessed using a force gauge with blunt tip on postoperative days 4, 7, 11, 13, and 27. Cold allodynia was assessed using acetone on days 7, 8, 11, and 13.
Morphine tolerance was induced in male Sprague-Dawley rats (170-250 g) using two different schemes: progressively increasing morphine doses delivered through injections, or constant-releasing morphine pumps. Fingolimod (0.1 or 1 mg/kg, i.p.) was administered daily. The development of morphine tolerance was assessed using thermal antinociceptive tests, tail-flick and hot plate, on days 4, 7, and 8.
N=6-12 per study group. The treatments applied were compared to the relevant drug vehicle controls treated otherwise identically. All behavioral measurements were performed in a blinded fashion.
RESULTS: The SNL caused a robust decrease in withdrawal thresholds for mechanical and thermal stimulus. Fingolimod (0.01 or 1 mg/kg) did not, however, have an effect on either the mechanical or thermal allodynia tested on any of the experiment days. Fingolimod administration with morphine did not prevent the development of morphine tolerance.
CONCLUSION: The results suggest that fingolimod holds no beneficial effects on neuropathic pain related to peripheral nerve injury, but also that concomitant fingolimod treatment does not have an effect on the development of morphine tolerance. The inconsistent results regarding the efficacy of fingolimod in different models of neuropathic pain warrants further research.

Disclosures: V. Jokinen: None. **T. Lilius:** None. **P. Rauhala:** A. Employment/Salary (full or part-time):; part-time medical advisor for Orion Pharma during the experiments. **E. Kalso:** None.

Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 571.06/V3

Topic: D.03. Somatosensation: Pain

Title: Antinociceptive and antipruritic effects of kappa opioid receptor agonists on pain and itch models

Authors: A. ZANON, Jr[1], Y. DARBAKY[1], *L. DIOP[2]
[1]ANS Biotech, Riom, France; [2]ANS Biotech, Riom Cedex, France

Abstract: Itch and pain are unpleasant sensations mediated by nociceptive neurons. These noxious stimuli are perceived as distinct, however, and induce different behavioral responses in rats such as itching or biting. Nalfurafine, a selective κ-opioid receptor (KOR) agonist, has been approved in Japan for the treatment of itch in patients with chronic kidney disease. (-)U-50,488H, another KOR agonist, is frequently used as a pharmacological tool in preclinical and clinical models of pain, notably in neuropathic, visceral and inflammatory pain. The aim of these studies was to compare the effects of Nalfurafine and (-)U-50,488H in pain and itch models.
Methods Nalfurafine and (-)U-50,488H were first evaluated in the ALGOGram™, an *in vivo* High Throughput Screening tool based on a battery of 11 validated animal models/tests covering 5 pain areas. In follow-up studies of inflammatory pain, Nalfurafine (3, 10, 30 µg/kg, s.c.) or NaCl 0.9% (n=10/group) was given 15 min before an intraplantar injection of Capsaicin (0.1%), and mechanical threshold was determined using the electronic Von Frey test. In a model of pruritus, Nalfurafine (3, 10, 30 µg/kg, s.c.), (-)U-50,488H (0.1, 0.3, 1 mg/kg, s.c.) or NaCl 0.9% (n=10/group) was given 30 min before the intradermal injection of Serotonin (5-HT) 2% used here as a pruritic stimulus. The cumulative scratching time was measured during a 45-minute period of observation post dose.
Results: In the screening model (ALGOGram™), Nalfurafine and (-)U-50,488H exhibited potent activity in various pain models. In the inflammatory pain model, Nalfurafine decreased tactile allodynia induced by intraplantar administration of Capsaicin. Within the same dose-range, Nalfurafine was effective in reducing itching behavior induced by intradermal injection of 5-HT. Likewise, (-)U-50,488H was also effective in the 5-HT model of pruritus.
Conclusions: This study demonstrates that both κ-opioid receptor agonists were effective in models of pain and pruritus. In particular, Nalfurafine exhibited equipotent antinociceptive and antipruritic activities in both pain and non-histaminergic pruritus models.

Disclosures: A. Zanon: A. Employment/Salary (full or part-time):; Andrea Zanon. **Y. Darbaky:** A. Employment/Salary (full or part-time):; Yassine Darbaky. **L. Diop:** A. Employment/Salary (full or part-time):; Laurent Diop.

Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

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Program #/Poster #: 571.07/V4

Topic: D.03. Somatosensation: Pain

Support: CIHR Grant FDN148413
QPRN

Title: Anxiety-like behaviors are attenuated by a NTS2-selective analgesic in rats experiencing chronic inflammatory pain

Authors: *M. VIVANCOS[1], R. FANELLI[2], M. ORLIAGUET[1], J.-M. LONGPRÉ[1], J. MARTINEZ[2], F. CAVELIER[2], P. SARRET[1]
[1]Pharmacol. and Physiol., Univ. De Sherbrooke, Sherbrooke, QC, Canada; [2]IBMM, UMR-CNRS-5247, Montpellier, France

Abstract: Chronic pain is commonly associated with affective disorders, such as anxiety and depression and the under-management of pain can have significant impact on the development of comorbidities. In recent years, the tridecapeptide neurotensin (NT) has emerged as an important modulator of nociceptive transmission, exerting its analgesic activity by interacting with class A G protein-coupled receptors, namely NTS1 and NTS2. In addition, the neurotensinergic system is also thought to play a major role in the physiological expression of stress and anxiety. In the present study, our goal was thus to evaluate if a NTS2-selective compound can produce sustained analgesic responses and reduce the anxiety-like behaviors that are associated with chronic pain.
We previously synthesized a series of NT(8-13) analogs harboring site-specifically modified natural or unnatural amino acids. Binding studies demonstrated that incorporation of a reduced amide bond between Lys^8-Lys^9, substitution of the Tyr^{11} by a positively charged amino acid (Lys) and replacement of the Leu^{13} residue with the more hydrophobic (trimethylsilyl)alanine (TMSAla) non-natural amino acid (JMV-5966) improved the selectivity by more than 100-fold toward NTS2 (1.38 nM and 166 nM for NTS2 and NTS1, respectively). Furthermore, the presence of these modifications greatly increased the plasma stability (half-life > 2 hours). Then, JMV-5966 was tested in acute (tail-flick test), tonic (formalin test) and chronic pain models (Chronic Constriction Injury (CCI) and Complete Freund's Adjuvant (CFA)). We found that intrathecal (i.t.) injection of JMV-5966 at 23 nmol/kg produced potent analgesic responses in different pain conditions, compared to saline-treated rats. We next evaluated the anxiolytic potential of JMV-5966 in a rodent model of persistent inflammatory pain (CFA model), in which rats develop hypersensitivity to mechanical stimuli and display anxiety-like behaviors in light/dark and elevated plus maze paradigms. We found that intracerebroventricular injection of

JMV-5966 at 11.4 nmol/rat significantly reduces the anxiety-like behaviors in the light/dark and elevated plus maze tests. Diazepam (1.5 mg/kg, i.p.) was used as a reference anxiolytic drug. Altogether, these results prove that activation of the NTS2 receptor subtype represents a promising avenue to both improve pain control and treat the anxiety-like behaviors associated with chronic pain.

Disclosures: M. Vivancos: None. **R. Fanelli:** None. **M. Orliaguet:** None. **J. Longpré:** None. **J. Martinez:** None. **F. Cavelier:** None. **P. Sarret:** None.

Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 571.08/V5

Topic: D.03. Somatosensation: Pain

Title: Trk inhibitor ameliorates spontaneous pain behaviors in rats treated with complete freund's adjuvant (CFA)

Authors: *S. KOYAMA, Y. OHTSUKA, M. WAKABAYASHI, Y. ENDO, H. ARAI, S. MIHARA, T. KOMATSU, M. MICHISHITA, N. SHINOTSUKA, T. TABATA, K. FUKANO, M. TANAKA, A. KAGEYAMA, T. SHIRAI, K. YAMAMOTO, K. KAWASAKI, S. YOSHIKAWA
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Abstract: Spontaneous behaviors such as rearing and horizontal movements in rodents could be valuable readouts for better understanding painful symptoms, considering that preclinical studies focusing only on evoked pain have resulted in low success rate of clinical trials in pain research area. We have previously validated that rats treated with complete freund’s adjuvant (CFA) into hind paw showed significant decrease in both rearing and horizontal movements. Tropomyosin receptor kinase (Trk) inhibitor has been an attracting analgesic candidate for inflammatory pain including osteoarthritis related pain because of the involvement of nerve growth factor (NGF) in the pathogenesis. However, its efficacy on spontaneous pain in animal models as well as patients has not been investigated yet. Here, we evaluated peripherally selective pan-Trk inhibitor (compound A) on both rearing and horizontal movements in rats treated with CFA. We also investigated the efficacy of compound A in combination with current analgesics (NSAIDs or pregabalin) in this animal model. Our results show that both compound A and NSAIDs treatment significantly ameliorated the deficit in rearing and horizontal movements in rats treated with CFA compared to vehicle treatment. Pregabalin treatment showed the tendency to improve the decreased spontaneous behaviors, however, with no significant difference from vehicle treatment. Interestingly, compound A showed more potent efficacy in some combination therapies. This study strongly supports a therapeutic potential of trk inhibitor in the treatment of

inflammatory pain and also suggests the usefulness of some combination therapies of compound A and other analgesics in inflammatory pain.

Disclosures: **S. Koyama:** A. Employment/Salary (full or part-time):; Asahi Kasei Pharma Corporation. **Y. Ohtsuka:** A. Employment/Salary (full or part-time):; Asahi Kasei Pharma Corporation. **M. Wakabayashi:** A. Employment/Salary (full or part-time):; Asahi Kasei Pharma Corporation. **Y. Endo:** A. Employment/Salary (full or part-time):; Asahi Kasei Pharma Corporation. **H. Arai:** A. Employment/Salary (full or part-time):; Asahi Kasei Pharma Corporation. **S. Mihara:** A. Employment/Salary (full or part-time):; Asahi Kasei Pharma Corporation. **T. Komatsu:** A. Employment/Salary (full or part-time):; Asahi Kasei Pharma Corporation. **M. Michishita:** A. Employment/Salary (full or part-time):; Asahi Kasei Pharma Corporation. **N. Shinotsuka:** A. Employment/Salary (full or part-time):; Asahi Kasei Pharma Corporation. **T. Tabata:** A. Employment/Salary (full or part-time):; Asahi Kasei Pharma Corporation. **K. Fukano:** A. Employment/Salary (full or part-time):; Asahi Kasei Pharma Corporation. **M. Tanaka:** A. Employment/Salary (full or part-time):; Asahi Kasei Pharma Corporation. **A. Kageyama:** A. Employment/Salary (full or part-time):; Asahi Kasei Pharma Corporation. **T. Shirai:** A. Employment/Salary (full or part-time):; Asahi Kasei Pharma Corporation. **K. Yamamoto:** A. Employment/Salary (full or part-time):; Asahi Kasei Pharma Corporation. **K. Kawasaki:** A. Employment/Salary (full or part-time):; Asahi Kasei Pharma Corporation. **S. Yoshikawa:** A. Employment/Salary (full or part-time):; Asahi Kasei Pharma Corporation.

Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

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Program #/Poster #: 571.09/V6

Topic: D.03. Somatosensation: Pain

Support: NRF-2017R1A5A2015391

Title: Combined treatment with low doses of ibprofen and dexamethasone attenuate trigeminal neuropathic pain

Authors: ***D. K. AHN**[1], S.-H. KANG[1], M.-K. PARK[2], J.-Y. SON[1], J.-S. JU[1], M.-K. LEE[3]
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Abstract: The present study investigated anti-nociceptive effects of combined therapy of dexamethasone and ibuprofen on neuropathic mechanical allodynia in rats with inferior alveolar nerve injury. Sprague-Dawley male rats were anesthetized with ketamine (40 mg/kg) and xylazine (4 mg/kg). Under anesthesia, the left lower second molar was extracted, followed by the

placement of a mini-dental implant to intentionally injure the inferior alveolar nerve. Inferior alveolar nerve injury, induced by the mal-positioning of dental implants, produced a significant mechanical allodynia on postoperative day (POD) 1 and persisted until POD 30. Intraperitoneal injection of high doses of ibuprofen (30 mg/kg) or dexamethasone (25, 50 mg/kg) inhibited mechanical allodynia, but low doses of ibuprofen (1, 5, 10 mg/kg) or dexamethasone (2.5 mg/kg) did not attenuate neuropathic mechanical allodynia in rats with inferior alveolar nerve injury. We examined effects of combined treatment with low doses of ibuprofen (5 mg/kg) and dexamethasone (0.01, 0.1, 1 mg/kg) on neuropathic mechanical allodynia on POD 1, 2, 3 (early treatment) respectively. Early combined treatment with ibuprofen (5 mg/kg) and dexamethasone (0.1, 1 mg/kg) significantly inhibited mechanical allodynia. This anti-allodynic effect was recovered within 24 hours after injection. We also examined combined treatment with ibuprofen and dexamethasone on mechanical allodynia on POD 7, 8, 9 (late treatment). Similar to early and late treatment with ibuprofen (5 mg/kg) and dexamethasone (0.1, 1 mg/kg) also significantly inhibited mechanical allodynia. Anti-nociceptive effect of combined treatment of low doses ibuprofen and dexamethasone is compatible to effects of gabapentin treatment. We confirmed anti-nociceptive effects of combined therapy on neuropathic mechanical allodynia by analysis of *c-fos* expression. Inferior alveolar nerve injury produced significantly increases in *c-fos* immuno-positive cells in the medullary dorsal horn on POD 3 and 9. Combined treatment with ibuprofen (5 mg/kg) and dexamethasone (1 mg/kg) significantly inhibited the number of *c-fos* immuno-positive cells on POD 3 and 9, respectively. These results suggest that combined treatment with low dose of ibuprofen and dexamethasone, which inhibited the trigeminal neuropathic pain, is a new potential therapeutic target for neuropathic pain control including the orofacial area pain (supported by NRF-2017R1A5A2015391).

Disclosures: D.K. Ahn: None. **S. Kang:** A. Employment/Salary (full or part-time):; full-time. **M. Park:** A. Employment/Salary (full or part-time):; full-time. **J. Son:** A. Employment/Salary (full or part-time):; full-time. **J. Ju:** A. Employment/Salary (full or part-time):; full-time. **M. Lee:** A. Employment/Salary (full or part-time):; full-time.

Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 571.10/V7

Topic: D.03. Somatosensation: Pain

Title: The antinociceptive effects of low dose morphine but not pregabalin are enhanced by the selective Cav2.2 blocker CNV2197944 in the rat

Authors: N. UPTON[1], *A. S. FISHER[1], C. TAYLOR[1], K. GIBSON[2], Z. ALI[2]
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Abstract: Introduction: Cav2.2 remains a compelling analgesic target but despite 2 decades of intensive research, a selective small molecule blocker efficacious at safe doses in humans remains elusive.
At the spinal level, there is the opportunity to modulate presynaptic release from primary terminals of primary afferents by targeting μ-opioid, $\alpha_2\delta$ pathways as well as Cav2.2.
This study aimed to determine if low doses of the Cav2.2 blocker CNV2197944 could potentiate the effects of low doses of morphine and/ or pregabalin (PGB) preclinically- given the convergent nature of modulation between all three mechanisms.
Results: *Inflammatory pain;* Intraplantar injection of Complete Freund's Adjuvant (CFA) induced hypersensitivity was detected by a shift in weight-bearing between injured and non-injured hind paws at 24 hrs post dose. Both Celebrex (10mg/kg p.o.) and CNV2197944 (30mg/kg p.o.) at all test times significantly reversed the hypersensitivity. Morphine alone, produced a dose-related reversal of the hypersensitivity over the dose-range of 0.3-10mg/kg i.p. From this and previous studies, minimally effective doses of CVN2197944 (3 & 10mg/kg p.o.) and Morphine (0.3mg/kg i.p.) were selected for combined administration to evaluate potential additive/synergistic effects. Overall, the effects of CVN2197944 appeared to be largely additive to those of morphine (0.3mg/kg i.p.) at the time points evaluated.
Neuropathic pain; The chronic constriction injury (CCI) model of neuropathic pain was used to determine the effects of CNV2197944 (1 & 10mg/kg p.o.) alone and in combination with PGB (3mg/kg p.o.) following a sub chronic dosing regimen.
The control dose of PGB (30mg/kg p.o.) produced a clear reversal of the mechanical allodynia that occurs following CCI surgery, equivalent to shams at the 1 hr time point, indicating efficacy. CNV2197944 dose-dependently attenuated CCI-induced mechanical allodynia, with a significant increase in PWT observed at the 1 hr time-point in the 10mg/kg p.o. group.
CNV2197944 (10mg/kg p.o.) in combination with PGB (3mg/kg p.o.) increased PWT at both the 1 hr and 3 hr time-point but, the effect was not greater than that observed with CNV2197944 alone at 10mg/kg p.o. As such, there was no indication of a synergistic effect of these two compounds on PWT.
Conclusion: Using the current repeat-dose protocol PGB and CNV2197944 caused no overt sedative effects on the day of testing either alone or, in combination, compared to vehicle treated CCI animals
CNV2197944 could enhance the effect of morphine in the CFA model but not PGB in the CCI model. Suggestive of a different modulation of opioid versus $\alpha_2\delta$ mechanism and/or inflammatory versus neuropathic pain.

Disclosures: N. Upton: A. Employment/Salary (full or part-time):; Transpharmation Ltd.,. **A.S. Fisher:** A. Employment/Salary (full or part-time):; Transpharmation Ltd.,. **C. Taylor:** A. Employment/Salary (full or part-time):; Transpharmation Ltd.,. **K. Gibson:** A. Employment/Salary (full or part-time):; Calchan. **Z. Ali:** A. Employment/Salary (full or part-time):; Calchan.

Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

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Program #/Poster #: 571.11/V8

Topic: D.03. Somatosensation: Pain

Support: 16J02472
17K15577

Title: Drug induced pain responses in human iPSC derived sensory neurons using MEA system

Authors: *A. ODAWARA[1,2,4,5], N. SHUHEI[2], M. NAOKI[2], I. SUZUKI[3]
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Abstract: Functional evaluation assays using human induced pluripotent stem cell (hiPSC)-derived sensory neurons are expected to predict the pain-related toxicity of drugs and the pharmacological effects. However, evaluation assays in hiPSC-derived sensory neurons has not been established, and electrophysiological response to pain-related molecules are not known. In this study, we aimed to evaluate the physiological responses aginst pain-related molecules including anti-cancer drugs in cultured hiPSC-derived sensory neurons using high-throughput multi-electrode array (MEA) system. Human iPSC-derived sensory neurons were cultured on MEA chips (Presto), and the electrophysiological responses against capsaicin, menthol, allyl isothiocyanate (AITC), anti-cancer drug vincristine and oxaliplatin were measured by the MEA system. We firstly confirmed the expression of typical sensory marker Nav1.7, TRPV1, TRPM8, and TRPA1 using immunostaining in culture hiPSC-derived sensory neurons at 8 weeks culture. Evoked responses against capsaicin, menthol, and AITC administration were detected using MEA system. To confirm the responses depending on each receptor, we examined the responses in presence of each receptor antagonist. As the responses almost disappeared in presence of each channle bloker, these responses were confirmed to be channel specific responses. The evoked responses against anticancer drug vincristine and oxaliplatin administration were also detected. Next, we examined whether the increase of cold sensitivities occur in presence of anticancer drug oxaliplatin in vitro hiPSC-derived sensory neurons. The responses against AITC were increased in presence oxaliplatin and in a concentration-dependent manner. In summary, we have succeeded in detecting the electrophysiological pain reponses against capsaicin, menthol, allyl isothiocyanate (AITC), anti-cancer drug vincristine and oxaliplatin in hiPSC-derived sensory neurons using MEA system. We found that the increase of cold sensitivities in vivo phenomenon was also detected in vitro hiPSC-derived sensory neurons. MEA measurements using hiPSC-derived sensory neurons are useful to pain evaluation assay in human peripheral nervous system.

Disclosures: A. Odawara: None. **N. Shuhei:** None. **M. Naoki:** None. **I. Suzuki:** None.

Poster

571. Pain Models: Pharmacology

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Program #/Poster #: 571.12/V9

Topic: D.03. Somatosensation: Pain

Support: MR157005C

Title: Effect of midazolam on morphine-mediated analgesia, tolerance, and respiration in a rat model

Authors: *B. CHEPPUDIRA, H. KLEMCKE, A. TREVINO, R. CHRISTY, S. CRIMMINS
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Abstract: Introduction: Midazolam and morphine are often used clinically to achieve sedation and analgesia. Development of analgesic tolerance and respiratory depression are some undesirable effects produced by morphine when used repeatedly. In the present study, we examined the role of midazolam on morphine-mediated analgesic tolerance and respiratory parameters in a rat model of opioid tolerance. Methods: Adult male Sprague-Dawley rats received subcutaneous midazolam (**MI**; 2.5 mg/kg, n = 6) or morphine (**MO**; 10 mg/kg, n = 6) or midazolam (2.5 mg/kg) + morphine (10 mg/kg, n = 6; **MM**) or saline (**S**; 0.5 ml, n = 5), twice per day for four days and once on the fifth day. Analgesia was tested by paw withdrawal from a heat stimulus. Respiratory parameters by whole body plethysmography were recorded as well. Results: Co-administration of morphine and midazolam (MM) produced prolonged analgesia compared to morphine alone ($P < 0.001$), and reduced development of analgesic tolerance ().05). Acute and chronic treatment of midazolam had no effect on the nociceptive threshold ($P > 0.05$). When compared with S rats, tidal volume was unaffected by any treatment on days 1-4 ($P > 0.05$). On the contrary compared with S, respiration rate decreased on post-treatment days 2-4 by MM ($P < 0.05$), and on days 2 ($P < 0.05$) and 3 ($P < 0.05$) by MI. MO alone did not affect respiration rate on any day ($P > 0.05$). Conclusions: Our preliminary data indicate that midazolam reduces morphine-mediated tolerance but contributes to respiratory depression. Experiments are underway to further examine midazolam-morphine interactions in opioid tolerance.

Disclosures: B. Cheppudira: None. **H. Klemcke:** None. **A. Trevino:** None. **R. Christy:** None. **S. Crimmins:** None.

Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 571.13/V10

Topic: D.03. Somatosensation: Pain

Support: National Research Foundation of Korea (NRF) grant funded by the Korea government (NRF-2017M3A9E4057926).

Title: Mechanism of the analgesic effect of duloxetine in oxaliplatin-induced neuropathic pain

Authors: *W. KIM[1], J. LEE[2], S. WOO[2], S. KIM[3]
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Abstract: Oxaliplatin is a widely used chemotherapy agent, which also induces serious peripheral neuropathy. Duloxetine is a dual reuptake inhibitor of serotonin and norepinephrine, and is shown to be effective against pain. However, its effect as well as its mechanism of action in oxaliplatin-induced allodynia are not fully understood. A single injection of oxaliplatin (6 mg/kg, intraperitoneal; i.p.) induced cold and mechanical allodynia in rodents. Cold and mechanical allodynia were assessed by acetone and von Frey filament tests, respectively. When significant allodynic signs were observed, three different doses of duloxetine (10, 30, and 60 mg/kg, i.p.) were injected. Administration of 30 and 60 mg/kg of duloxetine significantly reduced both the cold and mechanical allodynia, whereas 10 mg/kg did not. By using an *in vivo* extracellular recording method, we further confirmed that 30 mg/kg of duloxetine could significantly inhibit the hyperexcitability of spinal wide dynamic range (WDR) cells. Furthermore, we conducted experiments to clarify the site of action of duloxetine in the spinal cord. The anti-allodynic effect of duloxetine was completely blocked by an intrathecal injection of phentolamine (non-selective adrenergic receptor antagonist, 20 g), or prazosin (α_1-adrenergic receptor antagonists, 10 g); however, idazoxan (α_2-adrenergic receptor antagonist, 10 g) could not block the anti-allodynic effect of duloxetine. These results suggest that 30 mg/kg of duloxetine treatment alleviates oxaliplatin-induced cold and mechanical allodynia in rodents through the activation of the α_2-adrenegic receptors.
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Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 571.14/V11

Topic: D.03. Somatosensation: Pain

Title: Synergistic effect of treatment with NMDA antagonists and muscarinic M1 positive allosteric modulator in the rat neuropathic pain model

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Abstract: Neuropathic pain is a major therapeutic challenge in the clinical research. Current analgesics are not completely effective and cause serious adverse effects. Literature review suggests that increase in N-methyl-D-aspartate receptor (NMDAR) contributes to central sensitization in neuropathic pain. Recent research showed that NMDA antagonist can reduce hyperalgesia and allodynia condition in animal models of neuropathic pain. However, clinical studies using NMDA antagonists on neuropathic pain suggests minimal therapeutic effects. Muscarinic M1 receptors are involve in the modulation of pain. Therefore we attempted for combination therapy of NMDA antagonist with muscarinic M1 positive allosteric modulator. In the current study we tested BQCA (1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid) (M1 PAM) in combination with NMDA antagonists (Memantine, MK-801 and SDZ-220581) in chronic constricted injury model of neuropathic pain in rats. Paw withdrawal thresholds were evaluated using Von Frey monofilaments. Motor side effects were assessed using open field and rotarod test. Combination therapy showed significant synergistic analgesic effects in all the tested combinations. Moreover no adverse motor side effects was observed in all the tested combinations. These observations recommend further studies in finding out a promising therapy for treating neuropathic pain.

Disclosures: A. Vuyyuru: A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **V. Goura:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Kallepalli:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **P. Jayarajan:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Abraham:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Nirogi:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.

Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 571.15/V12

Topic: D.03. Somatosensation: Pain

Title: Exploring the effects of neurotensin receptor 1 gene (NTSR1) polymorphisms on receptor function

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Abstract: Neurotensin (NT) is a tridecapeptide widely distributed throughout the brain, acting as a neuromodulator of dopaminergic and serotonergic transmission. Among the three NT receptors identified, the high-affinity NT receptor NTS1 which belongs to the G-protein coupled receptor class mediates several of the central and peripheral effects of NT. In Human, the *NTSR1* gene is located on chromosome 20q3 and contains three introns and four exons spanning more than 10 kb. Over the last decades, mapping and sequencing of the human genome have provided detailed information on the function of any gene or protein of interest and have given us important insights into the genetic variations among individuals. Genetic variations in the *NTSR1* gene have previously been associated with disease vulnerability and variation in drug responses. Indeed, single-nucleotide polymorphisms (SNPs) have been reported to be associated with schizophrenia, alcohol dependence and performances of processing speed and working memory in healthy Chinese-Han subjects. Likewise, *NTSR1* gene variants were found to be associated with opiate dependence in population of European ancestry. Importantly, all these SNPs are located in intronic non-coding DNA or untranslated 5'UTR and 3'UTR regions. Polymorphisms in the coding sequence of the *NTSR1* gene have, however, been identified in people from different ethnic origins. These three SNPs result in amino acid substitutions in the gene encoding hNTS1, leading to the replacement of Alanine by Valine at position 72 (A72V), Glutamine to Histidine at position 275 (Q275H) and Valine by Isoleucine at position 304 (V304I). A72V and V304I are respectively located in the first and sixth transmembrane domains whereas Q275H is present in the third intracellular loop. The mutation A72V is found in 1% of the Caucasian population, Q275H at 5% in the African population and V304I at 2% in combined populations. To our knowledge, these mutations discovered by genomic screening have not been associated with genetic susceptibility or phenotypes. The present study was therefore designed to determine whether these SNPs located in the hNTS1 coding sequence result in gain or loss of function. We investigated whether these single mutations affect the NT binding to hNTS1, induce changes in receptor trafficking (i.e. internalization, cell surface expression), modify the G protein-dependent and G protein-independent signaling pathways associated to NTS1 activation, or regulate the

formation of hNTS1 homo-or heteromers. Altogether, these results will provide a better understanding of the impact of these SNPs on NTS1 function and may help to link them to potential phenotypes.

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Poster

571. Pain Models: Pharmacology

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Topic: D.03. Somatosensation: Pain

Support: NIH grant T32 GM008244
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Title: MMG22 efficacy and target receptor expression in the dorsal root ganglia after peripheral nerve injury

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Abstract: The Mu opioid receptor (MOR) and the metabotropic glutamate receptor 5 ($mGluR_5$) are G-protein coupled receptors involved in pain and analgesia. $mGluR_5$ antagonists have been shown to decrease opioid induced analgesic tolerance and self-administration, while increasing opioid analgesic potency. Previous studies have shown that the expression of both MOR/OPRM1 and $mGluR_5$/GRM5 (protein/mRNA respectively) can be differentially regulated in various rodent models of pain. However, how expression levels change during the development and maintenance of neuropathic pain has not been investigated. MMG22 is a novel bivalent ligand made of an $mGluR_5$ antagonist and a MOR agonist. The first objective of these studies was to determine the effectiveness and potency of MMG22 in decreasing mechanical hyperalgesia caused by nerve injury. These studies also examined the co-localization and temporal dynamics of $mGluR_5$/GRM5 and MOR/OPRM1 expression in the lumbar dorsal root ganglia and lumbar dorsal horn after nerve injury. Studies comparing receptor expression patterns to the analgesic efficacy of MMG22 are ongoing. Adult C57BL/6J male and female mice were used for these studies. Mice were subjected to the spared nerve injury (SNI) model of nerve injury induced neuropathic pain. Behavioral testing on mice included von Frey, and conditioned place preference assays. Cumulative dose response curves for subcutaneous morphine and MMG22

were obtained on days 10, 20 and 30 after nerve injury. Mice were sacrificed at 1, 3, 10, 20 and 30 days after surgery. The dorsal root ganglia of L4 - L6 spinal nerves and the corresponding L4 - L6 segments of spinal cord were removed and used for 2 color in situ hybridization using RNAScope® (ACD/Biotechne®) technology, immunohistochemistry, and western blot analysis. The antinociceptive efficacy and potency of MMG22 in reducing mechanical hyperalgesia were greatest 10 days after spared nerve injury. MMG22 (10mg/kg but not a lower dose) was able to induce analgesic conditioned place preference in SNI mice 10 days after, but not 4 weeks after nerve injury. The same dose of MMG22 was unable to induce conditioned place preference in naïve mice or sham mice at any time point. We observed co-localization of OPRM1 and GRM5 mRNAs in some dorsal root ganglia neurons and dorsal horn neurons. We are presently comparing changes in MOR/mGluR$_5$ protein and OMPR1/GRM5 mRNA levels in lumbar dorsal root ganglia as a function of time after peripheral nerve injury.

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Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

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Program #/Poster #: 571.17/V14

Topic: D.03. Somatosensation: Pain

Support: R01 GM12374601

Title: Novel neuroactive steroid with hypnotic properties exerts analgesia in post-surgical pain model

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Abstract: Introduction: We have recently shown that novel neuroactive steroid (3β,5β,17β)-3-hydroxyandrostane-17-carbonitrile (3ß-OH) induces hypnosis in neonatal rats and provides analgesia in chronic pain, likely by blocking T-type calcium channels (T-channels). Several studies implicate T-channels in chronic pain; however, their role in acute pain resulting from surgical tissue injury is yet to be determined. Therefore, the aim of our study was to investigate if novel neurosteroid analogue, 3ß-OH, can also be used as analgesic in an acute post-operative pain model.
Methods: An incisional pain model was developed by performing deep tissue incision of the plantar surface of the hind paw in Sprague-Dawley rats. In order to establish the anesthetic dose

in young adult rats, animals were injected intra-peritoneally (i.p.) with different doses of 3ß-OH and loss of righting reflex (LORR) was monitored. To test spinal, local and systemic analgesic effects of 3ß-OH, the drug was injected intrathecally (i.t.) between L4 and L5 vertebrae, intraplantarly into the plantar surface of the incised paw (i.pl). or i.p.. In vivo assessment of antihyperlagesic effect was measured in tests of paw threshold responses to mechanical or radiant heat stimulus.
Results: After testing the range of doses of 3ß-OH injected i.p., we identified that 60 mg/kg i.p. dose induced LORR in more than 90% of injected rats. The same dose reduced the amount of isoflurane necessary to achieve anesthesia for surgical incision from 2.5 to 1%. Furthermore, animals that underwent surgery under 3ß-OH with 1% isoflurane, exhibited reduction in the response to thermal stimulus as compared to the group anesthetized with 2.5% isoflurane only. After i.t. injection of three different doses in healthy animals, 3ß-OH exerted a significant analgesic effect to mechanical stimulus during 120 minutes post-injection, as compared to the vehicle (VEH) group. Furthermore, when 16 µg dose was injected repeatedly 2 h and 24 h post-surgery, mechanical hypersensitivity was significantly reduced post-injection. Also, single acute i.t. injection of the same dose given either 24 h or 48 h post-injection, exerted significant antihyperagesic effect. After applying repeated i.pl. injections during three consecutive days (2, 24 and 48 h post-surgery), we noticed a significant increase in mechanical hypersensitivity threshold of incised paws vs. VEH, during 7 days of post-operative recovery.
Conclusion: Our study strongly suggests that 3ß-OH, a novel T-channel blocking neuroactive steroid analog, may be a promising general anesthetic with unique analgesic properties following systemic, intrathecal and peripheral delivery.

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Poster

571. Pain Models: Pharmacology

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Program #/Poster #: 571.18/V15

Topic: D.03. Somatosensation: Pain

Support: Supported in part by grant from Saudi Arabian Cultural Mission, USA

Title: Glial glutamate transporter activator attenuates nociception and rescues hippocampal memory deficit in mice

Authors: *G. ALOTAIBI, S. RAHMAN
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Abstract: Previous studies have shown that glial glutamate transporter-1 (GLT-1) in the hippocampus and anterior cingulate cortex (ACC) is critically involved in pain processing and modulation. However, the role of glial GLT-1 in nociceptive pain involving the hippocampus and ACC, important brain regions associated with cognitive and affective modulation of pain remains unknown. The objective of the present study was to investigate the role of LDN-212320, a GLT-1 activator, in nociceptive pain and associated hippocampal cognitive impairments. We evaluated the effects of LDN-212320 in formalin-induced nociceptive pain model. In addition, formalin-induced impaired hippocampal cognitive behaviors were measured using Y-maze, elevated-plus maze (EPM) and object-recognition test. Furthermore, GLT-1 expression was measured in the hippocampus and ACC using Western blot analysis. The LDN-212320 (10 or 20 mg/kg, i.p.) significantly attenuated formalin-evoked nociceptive behavior. The anti-nociceptive effects of LDN-212320 were reversed by systemic administration of DHK (10 mg/kg, i.p.), a GLT-1 antagonist. Moreover, intraperitoneal (i.p) administration of LDN-212320 (20 mg/kg,) significantly increased time spent in novel arm of the Y-maze compared to formalin-injected mice. In addition, treatment with LDN-212320 (20 mg/kg, i.p) significantly reversed the formalin-induced deficits in spontaneous alternation in the Y-maze test. Formalin-injected mice exhibited a reduced preference for the displaced object. However, mice treated with LDN-212320 (10 mg /kg or 20 mg/kg, i.p) significantly increased preference for the displaced object. Administration of LDN-212320 (20 mg/kg) significantly reversed formalin-induced less number of open arm entries of the EPM. Additionally, LDN-212320 (10 or 20 mg/kg, i.p.) increased GLT-1 expressions in the hippocampus and ACC. Taken together, these results suggest that the GLT-1 activator, LDN-212320, prevents nociceptive pain associated with hippocampal memory deficit by upregulating astroglial GLT-1 expression in the hippocampus and ACC. Therefore, GLT-1 activator could be a novel a drug candidate for nociceptive pain.

Disclosures: G. Alotaibi: None. **S. Rahman:** None.

Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 571.19/V16

Topic: D.03. Somatosensation: Pain

Title: Anti-hyperalgesic effect of haloperidol and morphine combined therapy on chronic constriction injury-induced neuropathic pain in rats

Authors: L. MENA-VALDÉS[1], J. V. ESPINOSA-JUÁREZ[1], O. A. JARAMILLO-MORALES[1], A. ALEJO-MARTÍNEZ[1], *F. J. LOPEZ MUNOZ[2]
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Abstract: The prototype butyrophenone, haloperidol, has been widely used as antipsychotic agent. Recently, some authors have described the anti-nociceptive effects of haloperidol mediated by sigma-1 receptors antagonism. Besides, morphine is a prototype analgesic opioid drug currently used in the clinical practice. **Objective.** To determine the type of interaction generated by the combined therapy of haloperidol with morphine in neuropathic pain, induced by chronic constriction injury (CCI). **Methods**. The anti-hyperalgesic effects of haloperidol (0.0178, 0.0316, 0.0562 and 0.1000 mg/kg, s.c.) and morphine (1.0 mg/kg, s.c.) were determined after single-doses, both in monotherapy and combined, using the von Frey test in the CCI model. Evaluations were done until 10 days post-surgery at 30, 60, 90, 120 and 180 minutes after drugs administration. **Results.** Haloperidol showed a dose-dependent anti-hyperalgesic effect on CCI rats, while the assayed dose of morphine achieved a moderate anti-hyperalgesic effect. The analysis of pharmacological potency of tested treatments demonstrated that haloperidol ED_{50} (0.0785) was twice higher than combined therapy ED_{50} (0.0382), being the combination the most potent. Moreover, it was found that among four combinations evaluated two (haloperidol 0.0178 and 0.0316 mg/kg + morphine 0.1 mg/kg) resulted in additive effects and two combinations produced anti-hyperalgesic effect of type potentiation (haloperidol 0.0562 and 0.1000 mg/kg + morphine 0.1 mg/kg). **Conclusions**. These results demonstrate that low doses of morphine significantly enhance anti-hyperalgesic efficacy and potency of haloperidol, suggesting a potential use of this pharmacological combination in neuropathic pain therapy.

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Poster

571. Pain Models: Pharmacology

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Title: Spinal 15-lox-1 contributes to nsaid-unresponsive hyperalgesia

Authors: *A. GREGUS[1], M. W. BUCZYNSKI[1], D. S. DUMLAO[2], P. C. NORRIS[2], G. RAI[5], A. SIMEONOV[5], D. J. MALONEY[5], A. JADHAV[5], Q. XU[3], S. C. WEI[4], B. L. FITZSIMMONS[3], E. A. DENNIS[2], T. L. YAKSH[3]

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Abstract: While nonsteroidal inflammatory drugs (NSAIDs) are the first line of therapeutics for the treatment of mild to moderate somatic pain, they are not generally considered to be effective for neuropathic pain. In the current study, direct activation of spinal Toll-like 4 receptors (TLR4) by the intrathecal (IT) administration of KDO_2 lipid A (KLA), the active component of lipopolysaccharide (LPS), elicits a robust tactile allodynia that is unresponsive to cyclooxygenase (COX) inhibition, despite elevated expression of COX metabolites in the spinal cord. IT KLA increases 12-lipoxygenase-mediated hepoxilin production in the lumbar spinal cord, concurrent with expression of the tactile allodynia. The TLR4-induced hepoxilin production also was observed in primary spinal microglia, but not in astrocytes, and was accompanied by increased microglial expression of the 12/15-lipoxygenase enzyme 15-LOX-1. Finally, the inhibitors ML127 and ML351 both reduced activity of the rat homolog of 15-LOX-1 heterologously expressed in HEK-293T cells and completely abrogated NSAID-unresponsive allodynia *in vivo* following IT KLA. Taken together, these findings suggest that the spinal TLR4-mediated hyperpathic state is mediated at least in part through activation of microglial 15-LOX-1.

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Poster

571. Pain Models: Pharmacology

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Program #/Poster #: 571.21/W2

Topic: D.03. Somatosensation: Pain

Support: NIH/NIGMS P01-GM118629

Title: Analysis of frontal electroencephalogram after fentanyl administration

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Abstract: Fentanyl is frequently used during cardiovascular surgery to induce hemodynamic stability, beneficial for creating a clear operating plane. We retrospectively analyzed the frontal electroencephalogram (EEG) data from patients (n=6) at the Massachusetts General Hospital (MGH) who received fentanyl during induction of general anesthesia for cardiac surgery. After

fentanyl-induced loss of consciousness, but before intubation, an increase in the delta oscillation (1-4 Hz) and profound decrease in their beta and gamma band activity (15-40 Hz) developed. This pattern of EEG activity is distinct from other common forms of anesthetics such as propofol or sevoflurane which develop high power oscillations in the 0.5-1.5Hz and 8-12 Hz bands during sedation. We observed that during fentanyl administration, the patient's EEG changes above some threshold concentration, and thereafter, power within the delta band (1-4Hz) tracks the trajectory of the predicted effect site concentration. This suggests a potential dose-dependent electroencephalogram signature for opioid effect. Additionally, there appears to be a ceiling effect on the EEG power in the delta band suggesting that the maximum effective dose of fentanyl may be achieved with less drug being administered. We hypothesize that, beyond this ceiling, administration of additional fentanyl might not convey significant additional benefit of sedation, analgesia, or hemodynamic stability. Further study will be required to explore these possibilities. These studies also suggest that the EEG could be used to titrate opioid effect, which could help optimize the quantity of opioids delivered during surgery, and reduce potential post-operative complications associated with opiate administration.

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Poster

571. Pain Models: Pharmacology

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Program #/Poster #: 571.22/W3

Topic: D.03. Somatosensation: Pain

Support: R01DA03531
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Title: Loss of GluN2B impacts nociceptive processing of intrathecal NMDA

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Abstract: Glutamatergic signaling within the spinal cord is implicated in the development and maintenance of chronic pain and opioid tolerance. Intrathecally delivered excitatory amino acids (EAAs), including N-methyl-D-aspartate (NMDA), are well characterized as eliciting distinct

behavior profiles: an early-onset behavioral expression of caudally directed grooming and scratching behaviors and a transient thermal hyperalgesia. These behaviors can be inhibited by pre- or co-treatment with NMDA antagonists or opioid agonists, allowing us to interrogate the spinal circuitry underlying the initiation and modification of distinct aspects of nociceptive signaling. We therefore sought to characterize the effect of intrathecally delivered NMDA and the ability of well-characterized NMDA receptor antagonists, including MK-801, ifenprodil, and agmatine, to inhibit these NMDA-elicited behaviors in both wildtype (WT) and GluN2B-knockdown (KD) mice. The GluN2B-knockdown mice were generated by intrathecal injection of AAV9-hSyn-Cre into Grin2B-floxed mice at p21. We observed that an equivalent dose of NMDA elicited significantly fewer nociceptive behaviors in KD mice as compared to WT, and that this decrease in potency could be overcome by increasing the dose of intrathecal NMDA. However, thermal hyperalgesia was unchanged from the WT to KD conditions, suggesting that an alternative NR2 subunit or nitric oxide synthase accounts for that effect. MK-801 was able to inhibit both nociceptive behaviors and thermal hyperalgesia in both WT and KD animals, but neither agmatine nor ifenprodil was effective at inhibiting nociceptive behaviors or thermal hyperalgesia in KD animals. These data suggest that GluN2B receptors contribute to NMDA-evoked nociceptive behaviors but not NMDA-evoked thermal hyperalgesia and that agmatine targets NR2B subunits.

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Poster

571. Pain Models: Pharmacology

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Program #/Poster #: 571.23/W4

Topic: D.03. Somatosensation: Pain

Support: FAPDF
CNPq

Title: Antinociceptive effect of a modified protonectin isolated from parachartergus fraternus wasp

Authors: *P. GALANTE[1], M. MORTARI[2]
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Abstract: According to International Association for the Study of Pain (IASP), pain is an unpleasant sensory or emotional experience associated with actual or potential tissue damage. It is a public health problem that affects about a third of the world population. Therefore,

neuroactive compounds are of great interest for their relevant potential to design novel drugs, important to prevent and/or treat diseases as well as to develop other pharmacological tools to minimize side effects and increase the effectiveness of the treatment. In this context, the arthropods venom presents as a rich and effective platform for the design of new neuroactive compounds. Succeeding this premise, an antinociceptive compound was bioinspired from a peptide isolated from the social wasp Parachartergus fraternus, denominated protonectin-F. Previous study showed that in the hot plate test when administered intracerebroventricular (i.c.v), protonectin-F revealed an antinociceptive activity comparable to morphine sulfate 1µM, presenting a lower motor deficit. The evaluation of the mechanism of action by pharmacological antagonism performed with naloxone hydrochloride (4 mg / kg), presenting the same inhibitory effect of antinociceptive activity during 240min of the test. These data suggest that protonectin-F may act on the opioid pathway directly in the recognition of opioid receptors or indirectly by activation in the release of endogenous opioids.

Disclosures: M. Mortari: None.

Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 571.24/W5

Topic: D.03. Somatosensation: Pain

Title: Comparative analysis of the antinociceptive effect of naproxen-arginine and sodium naproxen

Authors: *N. VEGA CABRERA[1], A. ALEJO-MARTÍNEZ[2]
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Abstract: The main function of pain is to warn about potentially harmful situations or stimuli; however, it becomes a pathological condition when it provokes dysfunction to the sufferer; so it looks like one of the major reasons for medical consultation worldwide. **Objective**: The aim of this study was to compare the antinociceptive effect of the naproxen-arginine versus sodium naproxen administered either separately in male Wistar rats on the “Pain-induced functional impairment model in the rat” (PIFIR model). **Method:** Nociception was induced by the intra-articular injection of uric acid (30%) in the right hind limb producing its dysfunction. Antinociception was determined by evaluating temporal curves and the dose-response curves of each analgesic. **Results:** Naproxen-arginine and sodium naproxen (0.316-177.8 mg/kg p.o.) demonstrated a dose-dependent antinociceptive response with similar efficacy (AUC= 275.1±13.3 au and 240.0±18.0 au, respectively). In the time course, when the maximum doses of both compounds were compared (177.8 mg/kg) it was observed that the administration of

naproxen-arginine obtained a maximum effect of 86.6±8.4% at 1.5 h versus naproxen-sodium (64.2±4.3%). To analyze the pharmacological potency, the ED_{50} were compared, naproxen-arginine was more potent than sodium naproxen. **Conclusions**: The results suggest that naproxen-arginine and sodium naproxen have adequate antinociceptive effects on the PIFIR model, and support the use of these analgesic compounds for the treatment of arthritic pain.

Disclosures: N. Vega Cabrera: None. **A. Alejo-Martínez:** None.

Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 571.25/W6

Topic: D.03. Somatosensation: Pain

Title: Comparative analysis of the antinociceptive effect of naproxen-arginine and sodium naproxen

Authors: *A. ALEJO-MARTÍNEZ[1], O. A. JARAMILLO-MORALES[2], J. V. ESPINOSA-JUÁREZ[2], L. MENA-VALDÉS[2], F. J. LÓPEZ-MUÑOZ[2]
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Abstract: The main function of pain is to warn about potentially harmful situations or stimuli; however, it becomes a pathological condition when it provokes dysfunction to the sufferer; so it looks like one of the major reasons for medical consultation worldwide. **Objective**: The aim of this study was to compare the antinociceptive effect of the naproxen-arginine versus sodium naproxen administered either separately in male Wistar rats on the "Pain-induced functional impairment model in the rat" (PIFIR model). **Method:** Nociception was induced by the intra-articular injection of uric acid (30%) in the right hind limb producing its dysfunction. Antinociception was determined by evaluating temporal curves and the dose-response curves of each analgesic. **Results:** Naproxen-arginine and sodium naproxen (0.316-177.8 mg/kg p.o.) demonstrated a dose-dependent antinociceptive response with similar efficacy (AUC= 275.1±13.3 au and 240.0±18.0 au, respectively). In the time course, when the maximum doses of both compounds were compared (177.8 mg/kg) it was observed that the administration of naproxen-arginine obtained a maximum effect of 86.6±8.4% at 1.5 h versus naproxen-sodium (64.2±4.3%). To analyze the pharmacological potency, the ED_{50} were compared, naproxen-arginine was more potent than sodium naproxen. **Conclusions**: The results suggest that naproxen-arginine and sodium naproxen have adequate antinociceptive effects on the PIFIR model, and support the use of these analgesic compounds for the treatment of arthritic pain.

Disclosures: A. Alejo-Martínez: None. **O.A. Jaramillo-Morales:** None. **J.V. Espinosa-Juárez:** None. **L. Mena-Valdés:** None. **F.J. López-Muñoz:** None.

Poster

571. Pain Models: Pharmacology

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 571.26/W7

Topic: D.03. Somatosensation: Pain

Support: T32NS070201
R01NS026363
R01NS070814

Title: Peripherally-restricted opioids and cannabinoids attenuates neuropathic pain in mice

Authors: *S. GRENALD[1], Z. CHEN[2], Q. HUANG[2], S. HE[2], Y. GUAN[2], S. RAJA[2]
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Abstract: Damage to somatosensory nervous tissue produces pronounced chronic neuropathic pain that is refractory to current therapeutic options due to off-target CNS effects. Moreover, neuropathic pain affects approximately 20 million adults, representing 7-8% of the US population. Previous work has demonstrated the inhibition of tactile and thermal hypersensitivity in rodent models of neuropathic pain with the use of the peripherally acting mu opioid receptor (MOR) agonists, loperamide and DALDA. The potential advantages of the use of peripheral opioids on the inhibition of neuropathic pain is further supported by their lack of CNS penetration, which is thought to prevent some of the deleterious adverse effects. Additionally, cannabinoids have demonstrated efficacy in producing analgesia in rodent and human models of inflammatory and neuropathic pain. We aimed to determine if co-treatment with peripherally restricted opioids and cannabinoids would result in a synergistic antinociceptive effect in the management of neuropathic pain. We utilized various behavioral paradigms as an opportunity to determine potential therapeutic synergy following the co-administration of the peripherally-restricted opioid, DALDA, and a peripherally acting cannabinoid, CB13, in rodent neuropathic pain models. A significant reduction in mechanical hypersensitivity was observed in mice treated with CB13 and DALDA compared to vehicle-treated controls. The mixture of the two agents potentiated this reduction in mechanical allodynia in a synergistic manner. Furthermore, co-treatment with these peripherally acting agents enhanced spontaneous activity in the animals without inducing motor deficits, and resulted in significant preference using the Conditioned Place Preference (CPP) behavioral paradigm. *In vivo* imaging of animals genetically encoded with the calcium indicator GCaMP6, suggests a peripheral site of action. Studies are ongoing in conditional knockout mice to shed mechanistic insight and determine the contribution of each of these receptor systems in the reduction of neuropathic pain-related behavior. Thus, dual targeting

the peripheral opioid and the cannabinoid systems may present a novel avenue to explore for the management of chronic neuropathic pain.

Disclosures: S. Grenald: None. **Z. Chen:** None. **Q. Huang:** None. **S. He:** None. **Y. Guan:** None. **S. Raja:** None.

Poster

572. Peripheral Mechanisms of Persistent Pain

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 572.01/W8

Topic: D.03. Somatosensation: Pain

Support: NIGMS (grant number P20GM103643)

Title: Analysis of 3'UTR isoform diversity in human dorsal root gangla (DRG) neurons using PacBio IsoSeq and CSI-UTR

Authors: *M. MICHAEL[1], E. GRLICKOVA-DUZEVIK[1], J. C. PETRUSKA[2,3], E. C. ROUCHKA[4], B. J. HARRISON[1]
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Abstract: 3’ Untranslated Regions (3’UTRs) influence gene function by controlling transcript stability, translational efficiency and mRNA transport. These functions are coordinated by 3’UTR interaction with specific cis-interacting molecules, including microRNAs and RNA-Binding Proteins (RBPs). The recent surge of high-throughput transcriptome sequencing has revealed a surprising diversity of 3’UTR isoforms. 3’UTR isoforms are regulated in a tissue-type and cell-type specific manner, and the longest 3’UTRs transcripts are expressed in the nervous system. To profile 3’UTR isoform diversity in Human DRG neurons, we performed Pacific Biosciences (PacBio) IsoSeq whole-transcript sequencing of RNA pooled from seven Human DRG samples. Limitations of the IsoSeq methodology are 1) it is cost-prohibitive for use with larger comparative experiments and 2) the resulting data is not quantitative across conditions. Therefore, we developed a pipeline employing an in-house developed algorithm - CSI-UTR - to analyze 3’UTR isoform expression in DRG neurons using standard mRNA libraries sequenced with Illumina technology.

Disclosures: M. Michael: None. **E. Grlickova-Duzevik:** None. **J.C. Petruska:** None. **E.C. Rouchka:** None. **B.J. Harrison:** None.

Poster

572. Peripheral Mechanisms of Persistent Pain

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 572.02/W9

Topic: D.03. Somatosensation: Pain

Support: NIGMS (grant number P20GM103643)

Title: Analysis of 3'UTR-RNA Binding Protein (RBP) interactions in dorsal root ganglia (DRG) neurons

Authors: *E. GRLICKOVA-DUZEVIK, M. MICHAEL, B. J. HARRISON
Dept. of Biomed. Sciences, Col. of Osteo. Med., Univ. of New England, Biddeford, ME

Abstract: RNA binding proteins (RBPs) are required for post-transcriptional control of gene function. RBPs have multiple roles in neurons, and contribute to neuron-type specificity and neuroplasticity. 3' untranslated regions (3'UTRs) of transcripts contain binding sites for RBPs. The majority of protein coding genes have multiple 3'UTR isoforms that interact with specific subsets RBPs thereby conferring transcript-specific functions. Publicly available high-throughput sequencing databases provide the opportunity to reanalyze data using updated/novel algorithms. We developed and employed a novel analysis algorithm called CSI-UTR to characterize 3'UTR isoforms and RBP binding sites in RNA-Seq profiles generated from dorsal root ganglia (DRG) neurons. Using this approach, we assessed strain, sex, age and cell-type specific variation in RBP-3'UTRs interactions in rat, mouse and Human DRG. This approach, applied to the study of somatosensory neurons, is uncovering exciting new insights about the multimodality of touch sensation and is providing novel targets to develop therapeutics for neuropathic pain.

Disclosures: E. Grlickova-Duzevik: None. **M. Michael:** None. **B.J. Harrison:** None.

Poster

572. Peripheral Mechanisms of Persistent Pain

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 572.03/W10

Topic: D.03. Somatosensation: Pain

Support: NIGMS (grant number P20GM103643)

Title: The differential roles of collateral sprouting and regeneration in the development of neuropathic pain following nerve injury

Authors: *S. DINSDALE[1], E. GRLICKOVA-DUZEVIK[2], M. MICHAEL[2], J. C. PETRUSKA[3,4], B. J. HARRISON[2]
[1]Col. of Arts and Sci., [2]Dept. of Biomed. Sciences, Col. of Osteo. Med., Univ. of New England, Biddeford, ME; [3]Dept. of Anatom. Sci. & Neurobio., [4]Kentucky Spinal Cord Injury Res. Ctr., Univ. of Louisville, Louisville, KY

Abstract: Rodent models of nerve injury-induced neuropathic pain (NP) typically employ injury to the sciatic nerve and/or its branches. Studies using these models have provided pioneering mechanistic data about the drivers of peripheral nerve regeneration and the causes of NP. It however remains largely unclear what contribution collateral sprouting of afferents plays to the development of NP. To address this, we have been using an adapted version of the spared dermatome model of collateral sprouting. This model was developed by Jack Diamond and colleagues in the 1980s to induce collateral sprouting of sensory neurons anatomically isolated from the injured neurons (sprouting neurons are in separate ganglia to the injured ones) - an approach that is not feasible using standard sciatic nerve models. Using this model, they discovered that collateral sprouting is neurotrophically distinct to axon regeneration: Collateral sprouting is dependent on NGF, whereas regeneration of injured axons could proceed even in the presence of NGF blockade. We have been assessing the utility of Jack Diamond's spared dermatome model in the study of neuropathic pain. We present data comparing the relative contribution of collateral sprouting and regeneration to hot, cold and mechanical hypersensitivity, hyperalgesia and allodynia.

Disclosures: S. Dinsdale: None. **E. Grlickova-Duzevik:** None. **M. Michael:** None. **J.C. Petruska:** None. **B.J. Harrison:** None.

Poster

572. Peripheral Mechanisms of Persistent Pain

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 572.04/W11

Topic: D.03. Somatosensation: Pain

Support: NIGMS (grant number P20GM103643)

Title: Development of a high-throughput aptamer screen to target the Nerve Growth Factor (NGF) pathway

Authors: *E. D. MCCORMAC[1], E. GRLICKOVA-DUZEVIK[2], M. MICHAEL[2], B. J. HARRISON[2]

[1]Col. of Arts and Sci., [2]Dept. of Biomed. Sciences, Col. of Osteo. Med., Univ. of New England, Biddeford, ME

Abstract: Nerve Growth Factor (NGF)-responsive neuron populations contribute to the etiology of diverse human diseases including neuropathic pain. Therapeutic trials involving NGF have demonstrated that targeting this trophic pathway could be a powerful approach. However, the use of NGF as a pharmacological agent may be unfeasible due to an unacceptable side-effect profile. A long-term goal is to develop therapeutics that regulate the actions of NGF, while minimizing potential side-effects. Towards this end, we are developing a cell culture based assay to quantify the effects of novel NGF modifying peptides conjugated to cell-penetrating peptides (CPPs). To optimize this assay, we have assessed a panel of candidate CPPs for toxicity profiles, cell penetration efficiency and effects on cell morphology and excitability. We now plan to employ this assay in a high-throughput format to screen candidate NGF pathway modifying aptamers for drug development.

Disclosures: E.D. McCormac: None. **E. Grlickova-Duzevik:** None. **M. Michael:** None. **B.J. Harrison:** None.

Poster

572. Peripheral Mechanisms of Persistent Pain

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 572.05/W12

Topic: D.03. Somatosensation: Pain

Support: Cellectricon AB
Swedish Research Council
Knut and Alice Wallenberg Foundation
Family Lundblad Foundation

Title: An *in vitro* approach to investigate excitability differences in DRG neurons from neuropathic and inflammatory pain disease models

Authors: *A. BERSELLINI FARINOTTI[1], D. NASCIMENTO[1], R. RUDJITO[1], K. SANDOR[1], S. LARDELL[2], P. KARILA[2], C. SVENSSON[1]
[1]Karolinska Institutet, Stockholm, Sweden; [2]Cellectricon AB, Göteborg, Sweden

Abstract: In dorsal root ganglion (DRG) neurons, differential expression of e.g. ion channels and markers of nerve stress indicate mechanistic differences in the pain pathophysiology. However, after prolonged peripheral inflammation, changes in DRG neurons resemble changes observed after nerve injury. This suggests that pain of inflammatory origin may evolve into a condition that resembles neuropathic pain. In our previous work we have characterized the

neurochemical profile of DRG neurons in mice from an arthritis model that show this “shift” in pain mechanism over time. The aim of the current study was to explore if changes in neuronal excitability induced by nerve injury or long-term inflammation is still present after establishment of primary cell cultures using a combined electric field stimulation and imaging approach.
BALB/c female mice were subjected to a spared nerve injury (SNI, model of neuropathic pain) or collagen antibody-induced arthritis (CAIA, model of long-lasting joint inflammation with a neuropathic component). Lumbar DRG neurons were collected 6 days after SNI and up to 50 days after induction of CAIA. Primary DRG neuronal cell cultures were prepared in 384 well plates and two days after plating the cells were loaded with a calcium indicator and transient calcium elevations were recorded as the change in the ratio of the fluorescence intensity before and during electric field stimulation. The excitability responses in DRG neurons from the animal models were compared to DRGs from untreated or sham operated control animals.
Results: Mechanical hypersensitivity was observed from day 1-6 in the SNI model and day 3-50 in the CAIA model. A robust joint inflammation was noted from day 7-30 in the CAIA model and even though the inflammation resolved, pain-like behavior persisted for at least an additional 20 days. In vitro, there was a clear difference in the excitability response (approximately 100% as measured as the ratio of the fluorescence intensity; N=6 animals and 18 wells/condition) in DRG neurons from the ipsilateral side compared to the contralateral side in the SNI model. A similar pattern was seen comparing data from experiments in which the neuronal cultures were established from DRGs collected after induction of CAIA compared to saline.
Conclusions: Using a high capacity system, we demonstrate that, in primary cultures from mice subjected to nerve injury, neurons retain differences in excitability in vitro. This opens up opportunities to explore underlying conditions in the excitatory properties of sensory neurons and may open new avenues for utilizing in vitro models to advance our understanding of pain pathophysiology.

Disclosures: A. Bersellini Farinotti: None. **D. Nascimento:** None. **R. Rudjito:** None. **K. Sandor:** None. **S. Lardell:** A. Employment/Salary (full or part-time):; Cellectricon AB. **P. Karila:** A. Employment/Salary (full or part-time):; Cellectricon AB. **C. Svensson:** None.

Poster

572. Peripheral Mechanisms of Persistent Pain

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 572.06/W13

Topic: D.03. Somatosensation: Pain

Support: RGNF-2017-18-SC-PUN-35330

Title: Amelioration of neurogenic and inflammatory hyperalgesia through modulation of iNOS, COX-2 and inflammatory cytokines by bergapten and mixture design in mice

Authors: *G. SINGH, JR[1], R. BHATTI, Jr[1], P. SINGH[2]
[1]Dept. of Pharmaceut. Sci., [2]Dept. of Chemitry, Guru Nanak Dev Univ., Amritsar I, India

Abstract: Background Bergapten is the major bioactive component of medicinal plants such as *Aegle marmelos* documented for analgesic activity. **Aim** To explore the toxicity, median effective dose (ED_{50}) and underlying mechanisms of analgesic effect of bergapten alone and in mixture. **Methods:** The mice were assessed for general behaviour and mortality in varying doses (50, 300, 2000 $mgKg^{-1}$) of bergapten for acute toxicity over 14 days. The analgesic effect was investigated using acetic acid and formalin induced hyperalgesia and anti-inflammatory activity was explored in carrageen induced paw edema. ED_{50} of bergapten was calculated using Design Expert software. Involvement of nitric oxide and cyclooxygenase pathways was investigated by agonist challenges with L-arginine and substance P respectively. The expression of inducible nitric oxide synthase and Cycloxygenase 2 was determined in spinal sections by immunohistochemical analysis. Lipopolysaccharide (LPS) challenge was used to assess in-vivo effect on inflammatory cytokines (TNFα and IL-6). Formation of mixture design of different concentration of different drugs (Bergapten, paracetamol and indomethacin) by using Design Expert software and characterization of mixture design by XRD, LCM and SEM. **Results:** Acute toxicity studies revealed no behavioural abnormality or mortality with bergapten treatment and unremarkable histological findings. Bergapten was found to significantly decrease acetic acid and formalin induced hyperalgesia (ED50=3.102 mg $kg-^{1}$) and carageenan induced paw edema with no toxicity symptoms. Bergapten produced a marked decrease in iNOS and COX-2 expression as well as TNFα and IL-6. The findings corroborate to modulation of iNOS and COX-2 and inflammatory cytokines by bergapten. This study provides promising insights and prospects for application of bergapten in pain management. In mixture design study markedly enhanced the analgesic study on both
neurogenic and inflammatory phase. XRD and SEM data mixture of drugs are crystalline in nature and non-interacting with one another in solid state mixture.

Disclosures: G. Singh: None. **R. Bhatti:** None. **P. Singh:** None.

Poster

572. Peripheral Mechanisms of Persistent Pain

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 572.07/W14

Topic: D.03. Somatosensation: Pain

Support: DE018661
DE023090

Title: Inflammation-induced hyper-excitability and spontaneous activity of trigeminal afferent neurons that innervate subcutaneous orofacial regions: Implications in orofacial pain

Authors: *V. VIATCHENKO-KARPINSKI, F. EROL, J. LING, J. GU
Anesthesiol., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Tissue inflammation results in hyperalgesia and spontaneous pain. It has been proposed that the inflammatory pain is due to the enhanced excitability and spontaneous activity of afferent neurons that innervate the affected tissues. This hypothesis has been tested in isolated dorsal root ganglion neurons of rats following hind paw inflammation. In the present study, we seek to determine whether inflammation in orofacial regions may induce hyper-excitability and spontaneous activity of trigeminal afferent neurons that innervate subcutaneous orofacial regions. Different from previous studies, the present work applied patch-clamp recordings from neurons situated in whole-mount trigeminal ganglions. Orofacial tissue inflammation was induced by subcutaneous injection of Complete Freund's Adjuvant (CFA) and neurons innervate this region was retrogradely labeled by DiI. All patch-clamp recordings were performed from DiI-labeled small-sized (soma diameter from 19 to 28 μm) trigeminal neurons. At room temperatures of 24ºC and under current-clamp configuration, rheobase for evoking action potentials became significantly reduced from 533.3±52.48 pA (n =15) in neurons of control group to 400±34.18 pA (n=18, $p<0.05$) in neurons of CFA-injected group. Spontaneous action potentials were never seen in both control (n=9) and CFA groups (n=18) at 24 ºC However, a small population of neurons (6 out of 18 cells) in CFA group but none in control group showed spontaneous action potentials at the temperature of 34ºC. Under voltage-clamp recordings, we examined currents evoked by voltage steps and we also examined passive membrane properties. We found that input resistance of cells was significantly increased from 368.3±35.7 mΩ (n = 15) in control group to 668.3±62.4 mΩ (n =18) in CFA group. We also observed significant reduction of outward potassium currents following CFA injections. Taken together, orofacial inflammation results in hyper-excitability and spontaneous activity in trigeminal ganglion neurons, which may be due to the changes of potassium channels in trigeminal ganglion neurons following tissue inflammation.

Disclosures: V. Viatchenko-Karpinski: None. **F. Erol:** None. **J. Ling:** None. **J. Gu:** None.

Poster

572. Peripheral Mechanisms of Persistent Pain

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 572.08/W15

Topic: D.03. Somatosensation: Pain

Title: Anaplerosis in the transition of acute pain to chronic

Authors: *O. K. MELEMEDJIAN, T. LUDMAN
Dept. of Neural & Pain Sci., Univ. of Maryland Dent. Sch., Baltimore, MD

Abstract: Acute pain is an essential physiological response to injury, allowing for a quicker recovery by promoting the protection of damaged tissue. In some cases, this acute, protective pain becomes chronic, a debilitating condition which persists after the initial injury has healed. Furthermore, the molecular bases for how chronic pain is initiated and maintained are not well understood. Since metabolism is inextricably linked to every aspect of cellular function and the shift from acute to chronic pain would require metabolic changes that can maintain the chronic pain state, we hypothesized that metabolic reprogramming leads to the transition of acute pain to chronic. Utilizing the nerve growth factor (NGF)-induced hyperalgesic priming model, we tested this hypothesis. Intraplantar injection of NGF evokes tactile hypersensitivity that resolves within 72 hours. However, the animals that received NGF become primed for developing prolonged hypersensitivity following the intraplantar administration of prostaglandin E2. We determined that pain is associated with a distinct metabolic phenotype where sensory neurons display increased glycolysis and reduced pyruvate oxidation. Moreover, during the primed phase the animals do not display tactile hypersensitivity and the sensory neurons exhibit enhanced anaplerotic flux. Crucially, normalizing pyruvate oxidation alleviated pain while inhibiting anaplerosis prevented the resolution of pain following NGF injection. Hence, these findings provide novel insights into the role of anaplerosis in the development and maintenance of chronic pain.

Disclosures: O.K. Melemedjian: None. **T. Ludman:** None.

Poster

572. Peripheral Mechanisms of Persistent Pain

Location: SDCC Halls B-H

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Program #/Poster #: 572.09/W16

Topic: D.03. Somatosensation: Pain

Support: 1R01DE026806-01A1

Title: legumain, a cysteine protease produced by oral cancer, generates trigeminal nociception through PAR_2

Authors: *E. W. CHEN[1], N. H. TU[2], R. KLARES, III[3], D. CHO[3], M. KIM[4], L. EDGINGTON-MITCHELL[5], N. W. BUNNETT[6], B. L. SCHMIDT[7]
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Abstract: Oral squamous cell carcinoma (SCC) patients experience severe, mechanically-induced pain. Oral SCC pain is mediated by the action of proteases in the cancer microenvironment, which cleave protease activated receptor 2 (PAR_2), in turn activating downstream signaling in nociceptor neurons. Our research with oral cancer patients show that the poorly characterized protease legumain is increased in oral cancer patient samples. We therefore investigated whether legumain could induce nociception in a mouse using reflexive and operant nociceptive assays. Mice with conditional knock out of PAR_2 in neurons expressing $Na_v1.8$ (PAR_2-$Na_v1.8$) were used to confirm the role of PAR_2 activation by legumain. Legumain 300 ng induced nociception, as measured by paw withdrawal (percent reduction in nociceptive threshold), in wild-type mice but not PAR_2-$Na_v1.8$ mice at day 0 (93.55 ± 11.36 vs 56.8 ± 23.05, n=5), day 1 (88.8 ± 9.07 vs 58.6 ± 25.88, n=5) and day 4 (85.2 ± 10.83 vs 48 ± 17.89, n=5) ($p<0.01$, $p<0.05$, and $p<0.01$, two-way ANOVA multiple comparisons analysis, respectively). To measure nociception in the trigeminal region legumain 300 ng was injected into the facial region and facial withdrawal was measured. Facial withdrawal following legumain injection was increased in wild-type compared to PAR_2-$Na_v1.8$ mice at day 0 (2.8 ± 0.24 vs 1.4 ± 0.11, n=5), day 1 (2.90 ± 0.15 vs 1.87 ± 0.16 n=5) and day 4 (2.78 ± 0.08 vs 1.8 ± 0.43 n=5) following the injections ($p<0.01$, $p<0.01$, and $p<0.01$, two-way ANOVA multiple comparisons analysis, respectively). We measured the effect of legumain on operant nociceptive behavior with the dolognawmeter, an assay and device that measures gnaw time as a proxy for nociception. Injection of legumain 100 ng into the tongue significantly increased gnaw time in wild-type mice compared to PAR_2-$Na_v1.8$ mice (percent change from baseline, 143.70 ± 169.54 vs 2.57 ± 25.35, n=7, $p<0.05$ two-way ANOVA). A similar nociceptive effect was observed after 300 ng legumain injection (150.9 ± 123.5 vs 4.89 ± 53.15, n=7, $p<0.05$, two-way ANOVA). We measured the effect of legumain on dissociated trigeminal neurons from wild-type mice using whole cell patch clamp recording. Legumain 4.4 ng increased half-width of action potentials (vehicle 1.66 ± 0.32 ms, n=3; legumain 5.45 ± 0.07 ms, n=2), and decreased rheobase (vehicle 60 ± 0 pA, n=3; legumain 35 ± 7.07 pA, n=2) in wild-type mice, indicating that legumain induces neuronal hyperexcitability. Our results suggest that legumain causes nociception through inhibition of potassium channels, which leads to increased neuronal excitability. Legumain-induced nociceptive behavior is dependent on cleavage of PAR_2.

Disclosures: E.W. Chen: None. **N.H. Tu:** None. **R. Klares:** None. **D. Cho:** None. **M. Kim:** None. **L. Edgington-Mitchell:** None. **N.W. Bunnett:** None. **B.L. Schmidt:** None.

Poster

572. Peripheral Mechanisms of Persistent Pain

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 572.10/W17

Topic: D.03. Somatosensation: Pain

Support: R01NS079166
R01NS095747

Title: Cellular and molecular mechanism of HIV-gp120 induced sensory neuropathy

Authors: *S. YUAN, J. DU, Y. SHI, W. RU, M. CABO JAUME, X. LIU, S.-J. TANG
Neurosci. and Cell Biol., Univ. of Texas Med. Br. at Galveston, Galveston, TX

Abstract: Painful sensory neuropathy in HIV patients is often described pathologically as 'dying-back' degeneration of the peripheral free-endings. However, in an HIV pain mouse model, we observed not only the typical 'dying-back' degeneration of $PGP9.5^+$ nociceptors but also the re-innervations of $GAP43^+$ nerve fibers in the hindpaw glabrous skins. The $GAP43^+$ fibers were largely $CGRP^+$, indicating that they were peptidergic nociceptors. Single fiber recording of the skin-nerve preparations revealed that gp120 induced a decrease of c-fibers and an increase of δ fibers. Blockage of axon growth by local application of Sem3a abolished the gp120-induced innervation of $GAP43^+$ fibers and alleviated mechanical allodynia. We further showed that Wnt5a signaling played a critical role in the gp120-induced innervation and allodynia. In particular, the Wnt-planar cell polarity (PCP) pathway mediated both the innervation and allodynia. Our findings reveals novel cellular and molecular mechanisms of HIV-associated sensory neuropathy.

Disclosures: S. Yuan: None. **J. du:** None. **Y. shi:** None. **W. ru:** None. **M. Cabo Jaume:** None. **X. Liu:** None. **S. tang:** None.

Poster

572. Peripheral Mechanisms of Persistent Pain

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 572.11/W18

Topic: D.03. Somatosensation: Pain

Support: TTUHSC Start Up Fund
TTUHSC Seed Grant

Title: Epigenetic changes in DRG neurons under hyperglycemia

Authors: *M. CHATTOPADHYAY, V. S. THAKUR
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Abstract: Abstract
The purpose of this study was to explore how histone deacetylases (HDACs) and histone acetyltransferases (HATs) could influence the expression of voltage gated sodium channel 1.7

($Na_v1.7$), epithelial sodium channels (ENaCs) and high mobility group box 1 protein (HMGB1) under hyperglycemic environment. $Na_v1.7$ is a voltage gated sodium channel that is expressed at high levels in the dorsal root ganglion (DRG). Studies have shown that this channel is an important component in nociception. The mammalian epithelial Na^+ channel (ENaC) family are shown to be the components of a mechano-sensory receptor for touch and are expressed in cervical and lumbar DRG. High mobility group box 1 protein (HMGB1) is a cytokine mediator of inflammation and is known to play a key role in pain. Epigenetic mechanisms such as DNA methylation, histone modifications, or non-coding RNA-mediated pathways, could influence chromatin structure and DNA accessibility, leading to turning 'on' or 'off' our genes. The precise epigenetic mechanisms in hyperglycemic neurons are not fully understood. Our preliminary studies suggest that increase in histone deacetylases (HDAC) levels/activity is associated with diabetic neuropathy. The current studies were conducted in immortalized F11 neuronal cell line, a hybrid cell line of rat embryonic dorsal root ganglion neurons and mouse neuroblastoma cells under normoglycemic and hyperglycemic conditions for 24 hours. Our study demonstrates increases in HDAC3, HMGB1, ENaC and $Na_v1.7$ in hyperglycemic F11 cells after 24 hours as compared to cells propagated in normoglycemic conditions. PCAF (P300/CBP-associated factor), a transcriptional coactivator, was down regulated after 24 hours of high glucose insult compared to normal glucose condition. The results from this study demonstrate that epigenetic regulatory changes affected by HDAC3 and PCAF could play an important role in the upregulation of $Na_v1.7$ and ENaC channels under hyperglycemic conditions in F11 neuronal cell lines.

Disclosures: M. Chattopadhyay: None. **V.S. Thakur:** None.

Poster

572. Peripheral Mechanisms of Persistent Pain

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 572.12/X1

Topic: D.03. Somatosensation: Pain

Support: HEIF proof of concept grant
Orion collaboration grant

Title: How the activation of macrophages by a novel neuropeptide leads to hypersensitivity of peripheral sensory neurons?

Authors: N. DEMCHENKO, B. ABDELKADER, *K. OKUSE
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Abstract: Recent microarray analysis of rat DRG mRNA in different models of neuropathic pain has revealed up-regulation of a common gene, vgf. VGF is 617-amino acid protein, a precursor

for neuropeptides. We have previously found that a VGF-derived peptide, TLQP-21 activates rat bone marrow-derived macrophages, and inoculation of TLQP-21-stimulated macrophages into rat hind paw caused mechanical hypersensitivity. We then successfully identified gC1qR as a receptor for TLQP-21 using affinity chromatography and LC-MS/MS techniques. Application of a neutralizing antibody against gC1qR reduced the number of cells responding to TLQP-21 significantly. Furthermore, application of the gC1qR-neutralizing antibody to rats with partial sciatic nerve ligation resulted in a delayed onset of nerve injury-associated mechanical hypersensitivity. These results indicate that TLQP-21 stimulates macrophages via gC1qR, and the activated macrophages somehow sensitizes sensory neurons. Furthermore, conditioned medium from TLQP-21 activated macrophage culture induced hypersensitivity of DRG neurons. This suggests the potential mechanism of bi-directional crosstalk between sensory neurons and macrophages in eliciting neuropathic pain.

Disclosures: **N. Demchenko:** A. Employment/Salary (full or part-time):; Orion Corporation. **B. Abdelkader:** None. **K. Okuse:** 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.; Orion Corporation.

Poster

572. Peripheral Mechanisms of Persistent Pain

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 572.13/X2

Topic: D.03. Somatosensation: Pain

Support: NIH Grant K99-HD093858 (SM)
NIH Grant RO1-NS11892 (KB)

Title: Endometriosis (ENDO) induced vaginal hyperalgesia in the rat: Influence of the endocannabinoid system on sensory and sympathetic cyst innervation

Authors: *S. L. MCALLISTER[1], N. DMITRIEVA[2]
[1]Dept. of Anesthesia, Perioperative and Pain Med., Stanford Univ. Sch. of Med., Stanford, CA; [2]Florida State Univ., Tallahassee, FL

Abstract: Endometriosis is a painful disorder defined by extrauteral endometrial growths (ectopic growths/cysts). How the growths contribute to painful symptoms such as dyspareunia (vaginal hyperalgesia) is poorly understood. A rat model of endometriosis (ENDO) is created by autotransplanting pieces of uterus onto abdominal arteries (Vernon & Wilson, 85). These ectopic growths form vascularized cysts and are associated with painful symptoms including vaginal hyperalgesia similar to women with endometriosis. In both rats and women, the cysts recruit

their own sensory and sympathetic nerve supply (Berkley et al., 04; 05). Previous studies suggest that the cyst sensory and sympathetic innervation contribute to the development and maintenance of endometriosis-associated vaginal hyperalgesia (McAllister et al, 09; 12; Zhang et al., 08). Further, the cyst sensory and sympathetic innervation express cannabinoid receptors (CB1) and CB1 receptor agonists decrease, whereas CB1 receptor antagonist increase, endometriosis-associated hyperalgesia (Dmitrieva et al, 10; McAllister & Sinharoy, 18). These findings, with the knowledge that the endocannabinoid system (ECS) is involved in uterine function and dysfunction and that exogenous cannabinoids have been used for centuries to alleviate dysmenorrhea, suggest that the ECS is involved in both endometriosis and its associated pain (Dmitrieva & Berkley, 02; Bradshaw & Walker, 04; Russo, 02). To test this hypothesis further, ENDO rats were treated with either (1) URB597, a fatty acid amide hydrolase (FAAH) inhibitor to prevent the degradation of the endocannabinoid AEA, a CB1 receptor agonist or (2) Rimonabant (RIM), a CB1 receptor antagonist, or (3) DMSO (vehicle). Treatment was delivered for 4 weeks (i.p.) during the time period that cyst innervation and vaginal hyperalgesia are known to develop in the rat model of endometriosis (McAllister et al., 2012). Then, cyst sensory and sympathetic innervation were analyzed and quantified in the stage of proestrus. Results show that relative to vehicle, URB597 significantly increased cyst sensory and sympathetic innervation whereas, RIM significantly reduced cyst sensory innervation. No significant differences were found between groups relative to cyst number, size, or burden. Together, these findings provide further support for the involvement of the ECS in mechanisms underlying endometriosis and its associated pain, potentially through ECS effects on cyst innervation, thereby providing a novel approach for the development of badly-needed new treatments.

Disclosures: N. Dmitrieva: None.

Poster

572. Peripheral Mechanisms of Persistent Pain

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 572.14/X3

Topic: D.03. Somatosensation: Pain

Support: NIH grant NS103812

Title: Dorsal root ganglionic field stimulation selectively blocks nociceptive sensory afferents

Authors: *B. PAN[1], D. CHAO[1], Q. H. HOGAN[2]
[1]Anesthesiol., [2]Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Dorsal root ganglion field stimulation (GFS) has been shown to be effective in relieving clinical pain associated with nerve injury and neuropathic pain in nerve injury animal models. However, its mechanism has not been explored. We employed *in vivo* single unit

recording from fibers teased from the 4th lumbar dorsal root. Fiber types (Aβ, Aδ, C) were defined by conduction velocity. Action potentials (APs) generated by GFS (20Hz) in C-type units progressively vanished within 20 seconds, whereas block of GFS-induced Aβ activity persisted, while Aδ showed intermediate stability. Activity generated peripherally by electrical stimulation of the sciatic nerve and punctate mechanical stimulation of the receptive field (glabrous skin) was likewise promptly blocked (within 20 s) by GFS, with a preferential blockade of AP trains in C-type units, whereas Aβ and Aδ units were minimally affected. After tibial nerve injury, punctate mechanical stimulus (von Frey) threshold was reduced from 29.4±5.48 gram (n=10) to 2.71±0.45 gram (n=7), which was reversed to 14.29±2.98 gram (n=7) during GFS. These results suggest that GFS produces use-dependent blocking of afferent AP trains, possibly by inducing enhanced filtering of APs at the sensory neuron T-junction.

Disclosures: B. Pan: None. **D. Chao:** None. **Q.H. Hogan:** None.

Poster

572. Peripheral Mechanisms of Persistent Pain

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 572.15/X4

Topic: D.03. Somatosensation: Pain

Support: R01/NS102432
R01/NS099338

Title: Linking toll like receptor activation to Fcγ receptor mediated pain

Authors: *M. A. HUNT[1], A. B. FARINOTTI[2], D. S. M. NASCIMENTO[2], K. SANDOR[2], T. L. YAKSH[1], C. I. SVENSSON[2]
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Abstract: Fcγ receptors (FcγRs) expressed on dorsal root ganglia (DRG) sensory neurons have recently been implicated as drivers of pain, in the absence of inflammation, in autoimmune disorders such as rheumatoid arthritis (RA). Neuronal FcγRs, activated by antibodies in immune complex (IC), have been shown to directly induce pain like behaviors in mice. The work presented here aimed to explore whether FcγR expression, in DRGs, changes in response to inflammatory stimuli.
Using qPCR, we found that Fcgr1 and Fcgr2b mRNA levels in L3-L5 DRGs increase in response to both intrathecal (IT) injection of endogenous (disulfide HMGB1) and exogenous (LPS) toll like receptor 4 (TLR4) ligands. In order to determine if this occurs also in the absence of immune cells, and thus, is a general feature of TLR ligands, we used neuronal enriched primary DRG cultures. We observed that stimulation with LPS increased Fcgr mRNA levels, but not TLR2

(LTA), TLR5(FLA-ST), or TLR7 (ssRNA40) agonists. Following IT injection of the TLR agonists, we observed similar results with TLR4 acting as the strongest inducer of Fcgr mRNA (assayed with NanoString's nCounter® PanCancer Immune Profiling Panel). The largest increase in Fcgr mRNA in DRGs was observed between 6 and 24 hours following IT LPS, and peak protein expression was observed around 24 hours. While FcγRI was only detected in macrophages in the DRG, FcγRIIb was observed exclusively in neuronal soma. FcγRIIb was primarily present in small and medium sized DRGs (soma area <700μm2). Following IT LPS, both the number of neurons expressing FcγRIIb and intensity of FcγRIIb immunoreactivity increased. Network analysis on the NanoString data indicated interferon regulatory factor (IRF) and Jak/STAT signaling may act as key intermediates/regulators of TLR4 activation induced Fcgr expression changes. Indeed, we observed that IRF7 is present in small/medium sized DRG neurons, and IRF7 expression significantly increases in response to LPS both in vitro and in vivo.
These studies indicate that transient TLR4 activation increases FcγRIIb expression in neurons. It's currently unknown whether neuronal FcγRIIb is associated with excitatory or inhibitory properties in sensory neurons, thus the change in FcγRIIb expression could either potentiate or reduce the pronociceptive actions of antibodies in IC. It is important to further explore the role of FcγRIIb in nociception as this could be clinically relevant to pain in autoimmune disorders such as RA, where patients are often non-responsive to analgesic therapies.

Disclosures: M.A. Hunt: None. **A.B. Farinotti:** None. **D.S.M. Nascimento:** None. **K. Sandor:** None. **T.L. Yaksh:** None. **C.I. Svensson:** None.

Poster

572. Peripheral Mechanisms of Persistent Pain

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 572.16/X5

Topic: D.03. Somatosensation: Pain

Title: Sigma-1 receptor chaperones substance P in the mouse dorsal root ganglions

Authors: *H.-E. WU, T.-P. SU
IRP/NIDA/NIH, Baltimore, MD

Abstract: Synthesized at the somata of sensory neurons in the dorsal root ganglion (DRG), the excitatory neurotransmitter substance P (SP) is transported to central and peripheral sensory nerve terminals, where SP is released upon noxious stimulus to elicit pain signaling/response. The ER chaperone protein sigma-1 receptor (Sig-1R) has been known to play a role in modulating the neuropathic pain and also known to enriched at the DRGs. The study examined the relation between SP and Sig-1R at the DRG within the context of the neuropathic pain. It has been shown that the knockout of the SP encoded tac1 gene potentiates the morphine

antinociception. This in turn is in alignment with the notion that Sig-1R antagonism can increase opioid analgesia. Therefore, we test the hypothesis that the Sig-1R may chaperone the SP, leading to the maintenance of stable SP level that provides excitatory signaling in the DRGs. The SP's tissue expression level, its protein amount, and the mRNA level were compared in DRGs obtained from wild type and Sig-1R knockout mice. The DRG neurons were stratified into different neuronal subpopulations by their function and size in the immunohistochemical experiments. Immunohistochemical staining in Sig-1R knockout DRGs, when compared to those seen from wild type samples, showed a decreased SP expression. Concomitantly, immunoblotting of SP also demonstrates a trend of decreased expression in the Sig-1R knockout mouse DRGs. Intriguingly, the SP mRNA level was increased in the Sig-1R knockout mouse DRG, when compared to those seen in wild type mouse DRGs. Taken together, our results suggest that the endogenous Sig-1R chaperones SP and leads to an increased level of SP. In contrast, in Sig-1R knockout DRGs, the decreased level of SP may call for a transcriptional increase of SP, thus leading to an increase of its mRNA. Our results suggest a key role of Sig-1R on the stability of SP in the DRG and implicate thus the Sig-1R as a target for treating neuropathic pain through the regulation of SP.

Disclosures: H. Wu: None. **T. Su:** None.

Poster

572. Peripheral Mechanisms of Persistent Pain

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 572.17/X6

Topic: D.03. Somatosensation: Pain

Support: NIH Grant R01DE022903
NIH Grant NIDA R031410

Title: Human carbonic anhydrase-8 AAV8 gene therapy produces prolonged analgesia and anti-hyperalgesia in mice by inhibiting nerve growth factor signaling

Authors: *G. Z. ZHUANG[1], U. UPADHYAY[1], X. TONG[1], Y. KANG[1], D. M. ERASSO[1], E. S. FU[1], K. D. SARANTOPOULOS[1], E. R. MARTIN[2], R. C. LEVITT[1]
[1]Dept. of Anesthesiol., [2]John P. Hussman Inst. for Human Genomics, Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: Chronic pain affects more than 100 million US adults according to the IOM report (2011) on Chronic Pain in America. Our understanding of the development of persistent pain and the specific environmental and genetic factors thought to impact susceptibility to chronic pain is lacking. Acatalytic carbonic anhydrase-8 (CA8, protein sign) is an allosteric inhibitor of inositol trisphosphate receptor-1 (ITPR1), which regulates neuronal intracellular calcium release. In our

previous investigation, we demonstrated that murine *Car8* (murine gene symbol) is involved in persistent pain regulation via the ITPR1-intracellular calcium signaling pathway. We showed that murine Car8 overexpression in the dorsal root ganglion (DRG) by injection of the sciatic nerve downregulated pITPR1, inhibited ATP-stimulated intracellular free calcium release, and abolished mechanical allodynia and thermal hyperalgesia. In this study, we assess the human CA8 signaling pathway to show evidence that overexpression of the human wild-type carbonic anhydrase 8 ($CA8^{WT}$) in NBL and HEK293 cultures, but not the reported *CA8* S100P loss-of-function mutation ($CA8^{MT}$), inhibits nerve growth factor (NGF)-induced phosphorylation of ITPR1, TrkA (NGF high-affinity receptor), and ITPR1-mediated cytoplasmic free calcium release *in vitro*. In addition, we show that gene transfer using AAV8-V5-$CA8^{WT}$ viral particles via sciatic nerve injection demonstrates retrograde transport to dorsal root ganglia (DRG) producing prolonged $CA8^{WT}$ expression, inhibition of ITPR1 and TrkA activation by phosphorylation and profound analgesia and anti-hyperalgesia in male C57BL/6J mice. AAV8-V5-$CA8^{WT}$-mediated overexpression prevented and treated allodynia and hyperalgesia associated with chronic neuropathic pain produced by the spinal nerve ligation (SNL) model. These AAV8-V5-CA8 data provide a proof-of-concept for precision medicine through targeted gene therapy of NGF-responsive somatosensory neurons as a long-acting local analgesic able to prevent and treat chronic neuropathic pain through regulating TrkA signaling, ITPR1 activation, and intracellular free calcium release by ITPR1.

Disclosures: G.Z. Zhuang: None. **U. Upadhyay:** None. **X. Tong:** None. **Y. Kang:** None. **D.M. Erasso:** None. **E.S. Fu:** None. **K.D. Sarantopoulos:** None. **E.R. Martin:** None. **R.C. Levitt:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 573.01/X7

Topic: D.06. Auditory & Vestibular Systems

Support: JSPS KAKENHI 16H01501
JSPS KAKENHI 16K07026
JSPS KAKENHI 16H06542
JSPS KAKENHI 17H01769
Takahashi Industrial and Economic Research Foundation

Title: Triadic forebrain structures that directly control the auditory midbrain of echolocating bats

Authors: *T. ITO[1], R. YAMAMOTO[2], T. FURUYAMA[3], K. HASE[3], K. I. KOBAYASHI[3], S. HIRYU[3]

[1]Anat. II, Kanazawa Med. Univ., Ishikawa, Japan; [2]Physiol. I, Kanazawa Med. Univ., Uchinada, Japan; [3]Doshisha Univ., Kyoto, Japan

Abstract: Echolocation bats change spectrotemporal parameters of sonar pulses during navigation. Furthermore, echoes may convey emotional values which interfere or modify the plan of pathways. Since the echoes arrive several milliseconds after the emission of pulses, the inferior colliculus (IC), the midbrain auditory nucleus that analyzes the echoes, must be quickly adjusted for each pulse. Here, we demonstrate that three forebrain structures, namely infralimbic cortex (IL), magnocellular part of the basal nucleus of the amygdala (Bmg), and auditory cortex (AC), send direct descending projection to the IC by using a retrograde tract tracing method. All three structures projected to bilateral IC although the ipsilateral projection was dominant. Comparisons of pattern of retrogradely labeled cells across animals suggested that ipsilateral AC projection to the IC is tonotopically organized. Projections from other forebrain structures did not show clear tonotopicity. Together with evidence of previous studies, these results demonstrated the triadic descending projections to the IC which make loops between forebrain and IC. As IL, Bmg, and AC relate to navigation, emotional value, and spatial coding, respectively, the loops may quickly optimize active sensation during navigation.

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Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 573.02/X8

Topic: D.06. Auditory & Vestibular Systems

Title: Elevated cochlear adenosine-mediated metabolic disruption causes hearing loss

Authors: *J. M. MANALO[1], H. LIU[2], M. ADEBIYI[2], D. DING[3], T. NEMKOV[4], A. D'ALESSANDRO[4], R. SALVI[3], F. PEREIRA[5], Y. XIA[2]
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Abstract: Over 360 million people in the world have been diagnosed with disabling hearing loss (HL). Current treatments for HL are limited to hearing aids and cochlear implants, with no FDA-drugs available. Patients who lack adenosine deaminase (*Ada*), the enzyme that degrades adenosine, have high levels of adenosine that yield severe health problems, including HL.

Previous studies have shown noise-exposed mice have elevated cochlear adenosine, but the pathogenic mechanisms behind these phenomena remain elusive. Our lab has found a HL phenotype in *Ada*-deficient mice (*Ada*$^{-/-}$) that parallels *Ada*-deficient humans. We also identified an accumulation of metabolites paired with elevated cochlear adenosine, acyl-carnitines, succinate, and glutamate, using unbiased high-throughput metabolomics profiling in *Ada*$^{-/-}$ mice. Elevated levels of succinate, acyl-carnitines, and glutamate imply a perturbation of the TCA cycle, beta-oxidation, and glutamate uptake, respectively. Additionally, out of all the four adenosine receptors, adenosine a_2b (ADORA2B) was found to have the highest genetic and protein expression in *Ada*$^{-/-}$ mice. On a cellular level, there is an increase in hair and neuronal cell loss that is commonly found in other models of sensorineural HL in mice, and in humans with age-related HL. In addition, lowering adenosine levels in the *Ada*$^{-/-}$ mice attenuated hearing deficiencies, decreased the aforementioned levels of metabolites, and reduced hair and neuronal cell losses. With these findings, we hypothesize that elevated adenosine-mediated hearing loss is dependent on ADORA2B signaling that leads to mitochondrial damage and excitotoxicity. Identifying the pathological signaling pathway induced by elevated cochlear adenosine will expand treatment options for millions of individuals suffering from HL.

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Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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Program #/Poster #: 573.03/X9

Topic: D.06. Auditory & Vestibular Systems

Support: Wellcome Trust DBT India Alliance to SB
IIT Kharagpur SRIC Challenge Grant to SB
IIT KGP Institute Fellowship to HKS

Title: Both lemniscal and non-lemniscal pathways define auditory responses in the mouse orbitofrontal cortex (OFC)

Authors: *H. K. SRIVASTAVA[1], S. BANDYOPADHYAY[2]
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Abstract: OFC, a part of the prefrontal cortex (PFC), is involved in assigning value to a stimulus depending upon the behavioral or contextual demands in a dynamic environment. Our studies show presence of auditory responses in the OFC of an anesthetized and awake mouse to pure tones as well as to low probability oddball sounds in a sequence of repeating sounds showing

pure deviance detection. The sources of the auditory inputs to and the mechanism of deviance detection in the OFC are unclear. We show that both of the two parallel auditory pathways, lemniscal and non-lemniscal, operating in tandem with different response characteristics to auditory stimulation participate in creating responses and deviance detection in the OFC. The differential contribution of both lemniscal and non-lemniscal pathways to the auditory response profile of OFC neurons are presented. Irreversible blocking (with muscimol and baclofen) of the lemniscal ventral division of the medial geniculate body, (MGBv) completely abolishes responses in the OFC. On the contrary blocking the non-lemniscal medial division (MGBm) and also subsequently blocking only the basolateral amygdala (BLA; receiving projections from MGBm and projecting to OFC) cause auditory responses in the OFC to become persistent, a usual response pattern known to be involved in working memory in the PFC. These results suggest that an MGBm driven inhibitory input from the BLA controls the temporal response profile of OFC auditory responses. Further, blocking primary (lemniscal) auditory cortex (A1, ACX) has no significant effect on OFC auditory responses, whereas blocking secondary ACX (thought to be non-lemniscal) areas abolishes auditory responses in the OFC. We show that non-primary ACX regions receiving MGBv projections (and hence lemniscal) and also projecting to the OFC contribute to the main auditory responses in the OFC, which are temporally sharpened to provide deviance detection through the non-lemniscal pathway. Together these findings suggest a critical interplay of lemniscal and non-lemniscal auditory pathways underlying the mechanism of creation and shaping of auditory sensory responses in the OFC and hence subsequently should be involved in value assignment to auditory stimuli in the OFC.

Disclosures: H.K. Srivastava: None. **S. Bandyopadhyay:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 573.04/X10

Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01 DC016641
David M. Rubenstein Fund for Hearing Research

Title: Plasticity in the ventral cochlear nucleus in response to age-related hearing loss

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Abstract: The central auditory system shows a remarkable ability to compensate in response to insults to the inner ear, such as exposure to noise or ototoxic drugs. However, it is not known whether the auditory system reacts similarly to age-related hearing loss. Previously, we have

shown that young adult mice exposed to damaging noise exhibit sound-evoked hyperactivity in bushy-cell driven pathways, indicative of an increase in central gain. Furthermore, immunolabeling indicated that this hyperactivity was not associated with much change in excitatory terminals, but rather with a widespread loss of inhibitory terminals in the ventral cochlear nucleus (VCN). In the present study, we investigate whether similar changes occur in the aging auditory system. We allowed adult CBA/CaJ mice to age to at least 20 months. We measured auditory brainstem responses (ABRs) to assess auditory sensitivity and processing. To investigate synaptic plasticity in the brainstem, we labeled excitatory and inhibitory terminals in the VCN. We found that ABR thresholds were mildly increased at about 20 months compared to young animals (~10 week old), but that thresholds increased quickly at older ages. Similarly, the amplitude of the ABR was slightly decreased at 20 months, but decreased rapidly as a function of age thereafter. While inhibitory labeling in the VCN was approximately halved in animals over 20 months compared to young animals, the ABR did not provide strong evidence of hyperactivity. This dissociation may be explained by a parallel loss of excitatory labeling in animals over 20 months of age. Loss of synapses was confirmed with transmission electron microscopy. We also generally find that many of these changes in the auditory system are accelerated in males compared to females. The data in older animals suggest that aging may not initiate the same compensatory mechanisms as acute trauma to the auditory system.

Disclosures: K.M. Schrode: None. **H. Javaid:** None. **J. Engel:** None. **A.M. Lauer:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 573.05/X11

Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01 DC004391

Title: Descending projections from the auditory cortex and inferior colliculus contact GABAergic cells in the ventral nucleus of the trapezoid body

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Abstract: The ventral nucleus of the trapezoid body (VNTB), an auditory nucleus in the superior olivary complex (SOC), is a major target of descending projections from the auditory cortex (AC) and inferior colliculus (IC). Previous investigators reported that these descending projections contact cholinergic olivocochlear cells in the VNTB (Mulders and Robertson, 2000; Suthakar and Ryugo, 2017). The VNTB also contains GABAergic cells, which can project to the cochlear nuclei or IC. Here, we asked whether GABAergic cells of the VNTB are contacted by

descending projections from the AC and the IC.
We injected traditional tracers (e.g. FluoroRuby) or adeno-associated viruses carrying fluorescent protein genes (e.g., AAV2-hSyn1-EYFP) into the AC and/or IC of pigmented guinea pigs and Long-Evans rats. After 5-28 days, we perfused the animals and stained brain sections for glutamic acid decarboxylase (GAD) to label GABAergic cells.
Injections of anterograde tracer into the IC labeled axons and boutons in the thalamus, nuclei of the lateral lemniscus, SOC, and cochlear nucleus. In the VNTB, we saw many putative contacts onto GAD+ cells in both guinea pigs and rats. Injections of anterograde tracer into the AC labeled axons and terminals in the auditory thalamus, IC, and SOC. In the VNTB, putative contacts were present on GABAergic VNTB cells in both guinea pigs and rats, although these contacts were fewer than those observed after IC tracer injections. In animals with injections of different tracers into AC and IC, we observed convergence of AC and IC inputs onto single GABAergic cells of the ipsilateral VNTB.
These results show that descending projections from the AC and the IC contact VNTB GABAergic cells. Both AC and IC projections arise from excitatory cells, so their targeting of VNTB GABAergic cells could provide for top-down inhibition of nuclei innervated by the VNTB. Moreover, the convergence of AC and IC projections onto individual VNTB cells suggests integration of these descending inputs. Thus, the VNTB is well-situated to act as an inhibitory hub of the descending auditory system. It is possible that activation of these descending pathways contributes to habituation or attentional mechanisms to suppress or facilitate processing of auditory stimuli based on salience.

Disclosures: N.L. Beebe: None. **W.A. Noftz:** None. **B.R. Schofield:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

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Program #/Poster #: 573.06/X12

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Title: Cortico-subcortical monosynaptic excitatory loops that originate and terminate in the auditory cortex

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Abstract: Various types of information are associated with auditory information in auditory perception. However, the neuroanatomical mechanisms for such association are unclear. We challenged to reveal neuroanatomical mechanisms for associating emotional meaning with auditory information. We visualized mesoscopic- and synaptic-scale connective patterns between the amygdala and auditory cortex in mice by neural tracer injection. The mouse auditory cortex, which can be functionally identified using flavoprotein fluorescence imaging, has at least four tonotopic regions including the secondary auditory field (A2). Injection of cholera toxin subunit b (CTB) revealed that A2 had strong reciprocal connections with the lateral amygdala (La). The primary auditory cortex (A1) had only weak connections with La, and other tonotopic fields had no connections with the amygdala. Further investigation using a confocal microscope revealed that the feedback loops running through A2-La-A2 were directly connected by axosomatic excitatory synapses at La neurons: numerous glutamatergic axon terminals derived from A2 make synapses directly with somas of excitatory La neurons that project back to A2. These data suggest that the feedback loops relayed in La must be inevitably activated by A2 activities, and signals come back to A2 rapidly. Moreover, similar rapid feedback loops were projected from A2 to various other subcortical structures and relayed back to A2. Together, these findings suggest that the precise feedback loops which are relayed in subcortical structures including the amygdala operate as functional units, and the current study revealed the presence of potential anatomical platform, which associates non-auditory activities with auditory information, with a hub in A2.

Disclosures: H. Tsukano: None. **X. Hou:** None. **M. Horie:** None. **H. Takebayashi:** None. **S. Sugiyama:** None. **K. Shibuki:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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Program #/Poster #: 573.07/X13

Topic: D.06. Auditory & Vestibular Systems

Support: FAPESP 2016/01607-4

Title: Control of firing of dorsal cochlear nucleus cartwheel neurons by ATP-sensitive K^+ channels

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Abstract: Glycinergic cartwheel neurons from the dorsal cochlear nucleus (DCN) provide a strong inhibitory force on the glutamatergic DCN fusiform neuron. Most cartwheel neurons present spontaneous action potential firing contributing to most of inhibitory post-synaptic currents on fusiform neurons. Several evidences suggest that a decrease in the inhibitory drive in the DCN could be related to the increased in the firing of fusiform neurons observed in animal models of tinnitus. We performed whole-cell patch-clamp recordings of DCN cartwheel neurons of young rats (p18-22) in order to investigate the ion channels influencing spontaneous firing of cartwheel neurons, and consequently the tonic inhibition on fusiform neurons. Most of cartwheel neurons (>80%) fired action potentials spontaneously at rest (active) while the other present a stable resting membrane potential (quiet). The spontaneous firing was not abolished by perfusion of glutamatergic or glycinergic synaptic blockers. Active neurons had bigger membrane input resistances and longer membrane time constants. Application of barium chloride (0.1 mM) a blocker of inwardly rectifying potassium channels (K_{ir}), which controls spontaneous firing of fusiform neurons, depolarized the membrane of both active and quiet cartwheel neurons, and induced firing of quiet neurons. Interestingly, the current blocked by barium had a similar conductance in the non-rectifying part, but in quiet neurons we observed a smaller rectification. ATP-sensitive K^+ channels (K_{ATP}) are formed by the K_{ir6} subunits and have small rectification. Application of the K_{ATP} blocker tolbutamide (0.2 mM) depolarized quiet neurons, leading to spontaneous firing, and inhibited a non-rectifying potassium conductance. In active neurons tolbutamide did not have any effect on both firing and membrane currents. On the other hand, the K_{ATP} activator diazoxide hyperpolarized the membrane potential of both quiet and active neurons, and silenced active neurons. Thus, K_{ATP} channels have an active role in controlling the spontaneous firing of DCN cartwheel neurons. Because K_{ATP} channels couple membrane potential with the energetic status of the cell and continuous action potential firing is energetically demanding, these channels can be an important mechanism to decrease tonic firing in cartwheel neurons in response to decreased ATP levels during situations of high intensity firing.

Disclosures: R.M. Leao: None. **P.S. Strazza:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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U.S. Army Research Laboratory Grant W911NF-10-2-0022

Title: Inhibition differentially affects auditory evoked P1-N1 response generation in a morphologically realistic model of auditory cortex

Authors: *D. BEEMAN[1], P. KUDELA[2], D. BOATMAN-REICH[3], W. S. ANDERSON[2]
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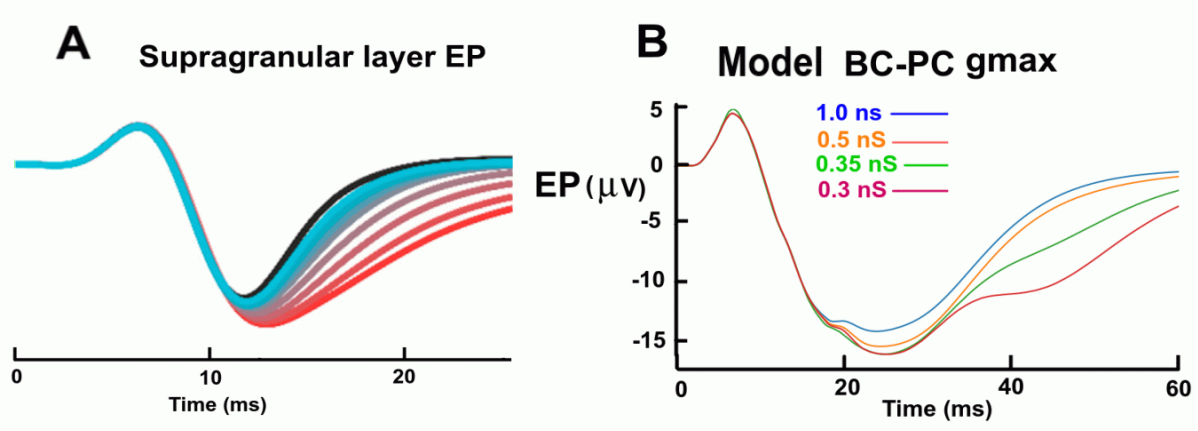
Abstract: The ACnet2 GENESIS model of primary auditory cortex (AI) has been used to reproduce and understand adaptation, as measured by cortical surface electrodes in an 'oddball paradigm' experiment. Both the vertex-positive P1 peak in the evoked potential (EP) and the vertex-negative N1 peak were found to arise from excitatory currents in the pyramidal cells (PCs). This study uses the model to understand how basket cell (BC) inhibition of PCs affects the shape of the P1 and N1 peaks in the EP. Bruyns-Halett et al. (Neuroimage 2017) recorded EPs from rat barrel cortex and applied a GABA antagonist to vary the amount of inhibition. Panel (A) of the figure shows that decreasing inhibition (dark red) widens N1, but has no effect on P1 or the initial portion of N1. For the AI layer 2/3 model, tone pulses were applied to PC basal dendrites, as if they were coming from layer 4, as in the two-layer version of the model. Inhibition from the BCs was applied to a proximal apical dendrite compartment of the PCs. EPs were calculated from a trial-averaged sequence of short 1000 Hz tones. Panel (B) shows that decreasing the maximal inhibitory conductance widens the latter part of N1, with minimal effect on P1. Features of the EP are explained by the orientation of electric dipoles that are formed when synaptic currents enter or leave the cell at one point and compensating leakage and capacitive return currents flow through other dendrite sections. A multi-compartmental PC model that accurately reproduces these spatially separated currents is essential for computing and understanding effects of inhibition on evoked responses. Here, BC inhibition produces delayed outward inhibitory currents and return currents in the lower dendrites, resulting in a dipole that is oriented oppositely to that produced by PC-PC excitation, reducing the late contribution to N1.



Disclosures: D. Beeman: None. **P. Kudela:** None. **D. Boatman-Reich:** None. **W.S. Anderson:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 573.09/Y1

Topic: D.06. Auditory & Vestibular Systems

Support: NSF Grant IOS 1147117

Title: Voltage-sensitive potassium currents contribute to sound processing during prepulse inhibition in the goldfish startle circuit

Authors: *D. R. BRONSON[1], T. PREUSS[2]
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Abstract: In fish, a pair of reticulospinal decision neurons, the Mauthner cells (M-cells), initiate startle in response to auditory stimuli. The experimental accessibility of the M-cells for *in vivo* intracellular recordings makes them ideally suited to study mechanism/s of prepulse inhibition (PPI). M-cell PPI is partly mediated by a voltage-dependent conductance that has been indirectly linked to a potassium current. However, the latter notion has yet to be tested. Potential candidates include voltage-gated Kv1.1 and inward rectifying potassium (GIRK) channels. Thus, we used here brainstem applications of a specific antagonist for Kv.1.1 (DTX-K 10 nM, n=10) or a general GIRK channels blocker (Tertiapin-Q, 2 µM, n=5) and assessed their effect on baseline M-cell membrane properties, auditory evoked post synaptic potentials (PSPs) and PPI. DTX-K and Tertiapin-Q decreased M-cell firing threshold in current ramp experiments by 64.2 ± 12.3 nA ($p < .01$, paired t-test, n=9) and 59.3 ± 7.9 nA ($p < .01$, paired t-test, n=5), respectively, with no effect on RMP. The antidromic action potential (AP) exhibited differential effects to the antagonists. Specifically, blocking GIRK increased AP magnitude (4.5 mV ± 1.7, paired t-test, $p < .05$, n=5), whereas blocking Kv1.1 increased AP width (0.13 ± .03 ms, paired t-test, $p < .05$, n=9). Both antagonists also modulated the waveform of sound-evoked PSPs, as indicated by a lingering membrane depolarization that persisted up to 150 ms. This was quantified by an increase in the membrane decay constant (τ) (increase in τ DTX-K = +17.7 ± 4.7 ms, $p < 0.01$, n=9, Tertiapin-Q = +31.6 ± 5.7 ms, $p < 0.01$, n=5, paired t-tests). The lingering depolarization added to the depolarization of secondary auditory PSPs in a prepulse/pulse stimulus paradigm, particularly at shorter interstimulus intervals (membrane depolarization increase at 50 ms, DTX-K = +0.49 ± 0.2 mV, $p<0.05$, n=9, Tertiapin-Q = +0.42 ± 0.1 mV, $p<0.05$, n=5, paired t-tests). Consequently, functional PPI, as quantified by the reduction in PSP height induced by the preceding prepulse, was reduced (DTX-K, $F(1,105) = 4.7$, $p<0.05$, n=8, Tertiapin-Q, $F(1,60) = 9.251$, $p < .05$, n=5, two-way repeated measures ANOVAs). Our results suggest that both Kv1.1

and GIRK conductances play a partially overlapping role in sensory processing, which indirectly affect functional aspects of PPI.

Disclosures: D.R. Bronson: None. **T. Preuss:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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Program #/Poster #: 573.10/Y2

Topic: D.06. Auditory & Vestibular Systems

Support: NSF Grant DGE1342536

Title: Auditory representation in cortex and striatum during audiomotor learning

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Abstract: Animals respond to sensory stimuli with appropriate motor actions. Some of these actions are innate, while others are learned. Models of sensorimotor learning argue that to learn the association between a sensory input and a motor output, the sensory stimulus must be reliably represented in the brain. Previous work in the auditory system has focused on how learning influences the representation of behaviorally-relevant auditory stimuli in auditory cortex (Polley, et al., 2006; Reed, et al., 2011; Takahashi et al., 2010). However, it is unclear how this information is represented in downstream areas and influences behavioral performance. The auditory striatum, a posterior area of striatum, receives input from the auditory system (via auditory cortex) and is required for audiomotor association tasks (Znamenskiy and Zador, 2013; Guo, et al., 2018). Are sensory representations affected by learning similarly or separately in the auditory striatum and cortex?
To address these questions, we developed a two-alternative forced-choice head-fixed audiomotor association task for mice. Animals report tones as either target or foil tones by licking right or left water ports, respectively, after a delay. If the animal is correct, it receives a small water reward from the same port. Animals reached criterion (80% correct) in this task after 9-21 days. In trained animals, we injected muscimol in either auditory cortex or auditory striatum bilaterally. Muscimol infusions in either area substantially reduced behavioral performance. Using *in vivo* whole-cell recordings, we measured the representation of auditory stimuli before and after learning in auditory striatum. The target tone was overrepresented in trained animals, but not in naive animals. This could arise from increased excitatory input from cortex, increased intrinsic excitability, or decreased inhibition within striatum. Based on previous literature,

dopamine can impact corticostriatal plasticity (Reynolds, et al., 2001), intrinsic excitability, and/pr inhibition within striatum (Dobbs, et al., 2016). Additionally, auditory striatum receives strong innervation from dopaminergic areas (the ventral tegmental area and substantia nigra pars compacta). However, dopamine antagonists in both either auditory cortex or auditory striatum in trained animals did not affect performance. This might suggest that if dopamine is playing a role, it may be during learning. Taken together, these results indicate that auditory representation in auditory striatum is important for audiomotor learning and imply that dopamine receptor signaling is not required to maintain these learned representations.

Disclosures: K.A. Martin: None. **R.C. Froemke:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 573.11/Y3

Topic: D.06. Auditory & Vestibular Systems

Title: Contributions of feedforward inhibition to feature selectivity and critical period plasticity in primary auditory cortex

Authors: *S. MASRI[1], S. BAO[2]
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Abstract: Thalamocortical projections conveying sensory stimulus information activate pyramidal neurons in Layer 4 of cortex while also recruiting strong bursts of disynaptic feedforward inhibition from Parvalbumin-expressing interneurons (PV cells) which attenuate the magnitude of the sensory response. This canonical microcircuit provides the first cortical transformation of sensory information. Studies using optogenetic stimulation to activate fast spiking PV cells have failed to achieve a consensus with regards to their influence on tuning properties and feature selectivity in sensory cortex. Conversely, similar studies performed on slow spiking Somatostatin-expressing interneurons have returned generally consistent results. Hypothesizing that these inconsistencies may arise from time dependent dynamics of fast spiking interneurons and inconsistent experimental stimulation protocols, we investigated the impact of optogenetic activation of PV cells on acoustic response propterties in Primary Auditory Cortex in a time dependent fashion. We show that activating PV cells causes significant suppression of stimulus evoked activity in putative pyramidal neurons and an increase in feature selectivity, but that these effects are time dependent.
Critical period plasticity is functionally distinct from adult cortical plasticity, but models of its neural underpinnings are still being developed. While PV cells are playing an increasingly important role in that story, their contributions to the process have not been demonstrated. We used these experiments to explore the neural basis of critical period plasticity by exposing mouse

pups to pure tone pips of a single frequency, producing distortions in the normal development of tonotopy in A1. We show that changes in feature slectivity occur in an idiosyncratic fashion relative to naive mouse pups.
Overall these experiments have new implications for the effects of feedforward inhibition on sensory processing and provide support for a new methodology when using optogenetics to study fast spiking neurons.

Disclosures: S. Masri: None. **S. Bao:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

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Topic: D.06. Auditory & Vestibular Systems

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Title: Layer 6b modulation of sensory processing in the adult mouse auditory cortex

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Abstract: At the base of the cortical sheet lies a band of cells that is well-known for its crucial role in development as a transient relay for thalamic input. While many of these cells die off early in development, a fraction persists into adulthood, becoming the layer 6b (L6b) sublamina. In the adult animal, layer 6b cells receive input from thalamic and cortical sources, send processes all the way to layer 1, and are highly responsive to neurotransmitters of arousal. As such, these cells are well-situated to sculpt activity in the cortical column. To investigate the role of L6b in sensory processing, we made use of the Ctgf-T2A-dgCre mouse line, in which Cre recombinase is expressed in the subset of Ctgf+ L6b cells. Ctgf is thought to define a large class of morphologically heterogeneous excitatory L6b cells. To achieve optogenetic control over L6b, we crossed this line with reporter mice expressing Cre-dependent opsins. We then performed acute extracellular recordings in awake mouse auditory cortex using multichannel probes to span many cortical layers. From these recordings, we identify L6b based on responsiveness to light stimulation, and show that this sublamina exhibits atypical and often reduced responses to tones

when compared with more superficial responses. We further show that light activation of Ctgf+ cells elicits both increases and decreases in firing rate at varying cortical depths, suggesting that these cells make multiple types of functional connections within the cortex. L6b light activation also affects responses to sounds, both enhancing and suppressing tone responsiveness relative to the light-off condition. We interpret these results in the context of a circuit model in which L6b cells sculpt sensory responsiveness through both excitation and inhibition, and speculate about their influence on sensory processing and their behavioral relevance.

Disclosures: R.J. Morrill: None. **A.R. Hasenstaub:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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Program #/Poster #: 573.13/Y5

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant T32DC000023-33
NIH Grant R21NS095232-02
Rubenstein Internal Grant (80038868)

Title: The cerebellar vermis robustly modulates neural activity in the inferior colliculus

Authors: *R. J. SIMA[1], T. KODAMA[2], H. FUJITA[2], S. DU LAC[1,2,3]
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Abstract: Accumulating lines of evidence from anatomical, functional, and imaging studies indicate that the cerebellum is involved in sensory processing, including in the auditory domain. However, how the cerebellum affects auditory processing is understudied. To investigate how the cerebellum influences the auditory system, we performed extracellular recordings from neurons in the inferior colliculus (IC) of awake, moving, head-fixed mice while optogenetically stimulating the contralateral cerebellar vermis. We discovered that 20 ms photoinhibition of Purkinje cells robustly excited approximately half of all recorded IC units with response latencies of ~45ms (Type 1) and ~92ms (Type 2) following stimulation onset. A smaller subset of IC neurons (~8%) was inhibited. Type 1 units were found preferentially in the dorsal cortex of IC, though were also present in the IC external cortex and central nucleus. Type 2 units were more abundant in IC external cortex and central nucleus. These results indicate that the cerebellar vermis is functionally connected to the IC, potentially through polysynaptic circuits. The functional significance of cerebellar influence on IC remains to be determined, but this circuit could serve as a channel for the auditory system to integrate sensorimotor and internal state information processed in the cerebellar vermis.

Disclosures: R.J. Sima: None. **T. Kodama:** None. **H. Fujita:** None. **S. du Lac:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

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Program #/Poster #: 573.14/Y6

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R21DC015124 (DEV)

Title: Central auditory pathway corticotropin releasing factor signaling elements and their correlation to known regional and cellular markers in central auditory target regions

Authors: *K. T. YEE[1], S. G. COLLINS[3], J. S. GILBERT[2], D. E. VETTER[2]
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Abstract: We have previously identified corticotropin releasing factor (CRF) and CRF receptor (CRFR)1 and CRFR2 in the cochlear epithelium (Graham et al 2010), and CRF in spiral ganglion neurons which innervate the cochlear nucleus (CN). Using two reporter mice, tdTomato CRF-Cre and BAC Tg CRFR1-GFP (Justice et al, 2008), we visualized CRF and CRFR1 expression patterns, respectively, over the dynamic postnatal maturational process in the central auditory pathway. There is broad CRFR1 expression in the developing CN that is down-regulated to the mature expression pattern, suggesting a transitory role for CRFR1 signaling during early dynamic synapse formation within various CN cell types. In the inferior colliculus (IC), CRFR1 is detected throughout the main subdivisions. In the medial geniculate nucleus (MGN), CRFR1 fibers exist as a meshwork and CRFR1 cell bodies are located ventral and medial to the ventral MGN (vMGN).
CRF is also expressed across the central auditory pathway. In the CN, CRF is expressed in neurons with varied morphologies and in distinct regions within the ventral and dorsal subdomains. In the IC, CRF expression consists of a small number of neurons in each of the major subdivisions, with a heavy axonal terminal field in a sub-region of the central nucleus of the inferior colliculus (CIC). In the MGN, CRF terminals exist, but are sparse centrally, and many positive neurons are positioned ventral and medial to the vMGN.
To correlate CRF expression with known regional demarcations and cellular populations, we double-labeled with cytochrome oxidase (CO) in IC and MGN and with the calcium-binding proteins, calbindin (CB), calretinin (CR) and parvalbumin (PV) from the cochlear nucleus through auditory cortex. The heavy field of CRF-positive terminals in CIC lies within an area of strong CO staining. CRF-positive terminals are also evident in the CO-positive domain in the vMGN while CRF-positive cells are positioned outside of the heavy CO domain of vMGN.

Further, few CRF-positive neurons are double labeled with CB, CR, or PV in the CN and IC. Relative to other auditory regions, the MGN shows the most CRF- calcium-binding protein double labeled cells. Within auditory cortex, a subset of CRF-positive neurons show double labeling with CR. CRF-positive terminals, however, appose soma that are positive or negative for CB, CR or PV.
In summary, CRF signaling across the central auditory pathway does not share common rules for correlation to CO, CB, CR, or PV, suggesting that CRF elements may 1) represent a unique population of neurons and 2) perform unique functions along the central auditory pathway.

Disclosures: K.T. Yee: None. **S.G. Collins:** None. **J.S. Gilbert:** None. **D.E. Vetter:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 573.15/Y7

Topic: D.06. Auditory & Vestibular Systems

Support: Wellcome Trust DBT India Alliance to SB
IIT Kharagpur SRIC Challenge grant to SB
MHRD Signals and Systems for Life Sciences Scheme grant to SB
IIT KGP Institute fellowship to SS and HKS

Title: Modulation of auditory responses by visual inputs in the mouse auditory cortex (ACX)

Authors: *S. SHARMA[1], H. K. SRIVASTAVA[1], S. BANDYOPADHYAY[2]
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Abstract: Multisensory (MS) integration allows seamless assimilation of information from different sensory modalities in order to better perceive the environment. Cross-modal interaction of incoming sensory information is important since each sensory system is capable of encoding the same stimulus in uniquely advantageous way. For example, the auditory system has better temporal resolution whereas the visual system has better spatial resolution and each may influence the other depending on context or requirement. Circuit and synapse based mechanism of visual input based modulation of responses in the ACX is poorly understood. We first elucidate the local broad cortical circuitry that could be involved in the above through neuroanatomical experiments. Retrograde tracer injections in the ACX show labelled cell bodies in both primary and secondary visual cortices indicating a direct interaction between the two sensory systems at the earliest stages of the sensory cortical hierarchy. Previous studies show the presence of single neuron responses to visual stimulus in infragranular layers but rare in supragranular layers of ACX. Using two-photon calcium imaging with GCaMP6s in awake

mouse we probed layer 2/3 (supragranular) of ACX with auditory, visual and audio-visual stimuli. In addition to responses to auditory stimulation (tone based tuning and broadband noise), we found robust Ca^{2+} based responses to visual as well as MS stimulus in layer 2/3 of auditory cortex in subpopulations of both excitatory (EXN) as well as inhibitory (IN) neurons. Most of the neurons show differential responses to multimodal stimulus as compared to their unimodal counterparts with linear as well as non-linear additive and suppressive effects. Noise correlations between pairs of neurons, a measure of functional connectivity, are significantly lower for the multimodal stimulus compared to that of unimodal stimuli suggesting better fidelity in population coding of multimodal stimuli as compared to that of unimodal stimuli. This decrease in noise correlations is strongly evident in similarly tuned EXN-EXN connections where as EXN-IN and IN-IN connections are less affected. The results have strong implications in coding of sounds in the mouse early auditory cortical regions.

Disclosures: S. Sharma: None. **H.K. Srivastava:** None. **S. Bandyopadhyay:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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Program #/Poster #: 573.16/Y8

Topic: D.06. Auditory & Vestibular Systems

Support: F31 DC015695-02
NIH-NIBIB R01 EB020740

Title: An atlas of the subcortical auditory system from post mortem human MRI

Authors: *K. R. SITEK[1], E. CALABRESE[2], G. A. JOHNSON[3], S. S. GHOSH[1]
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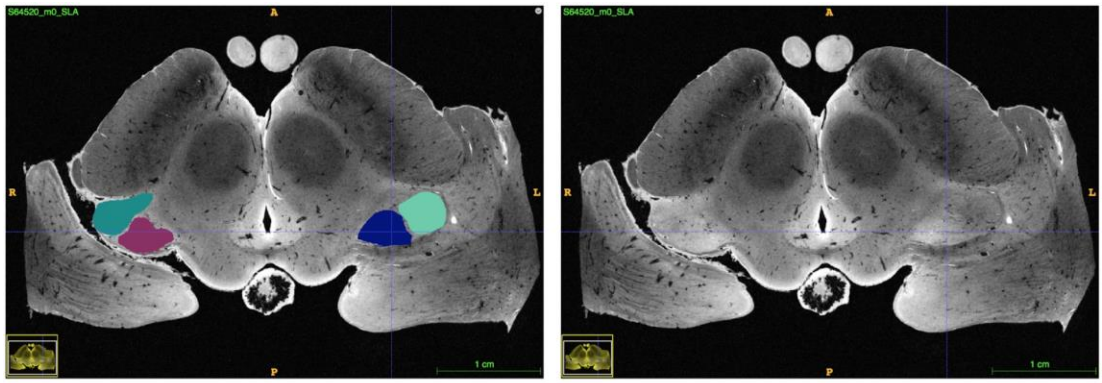
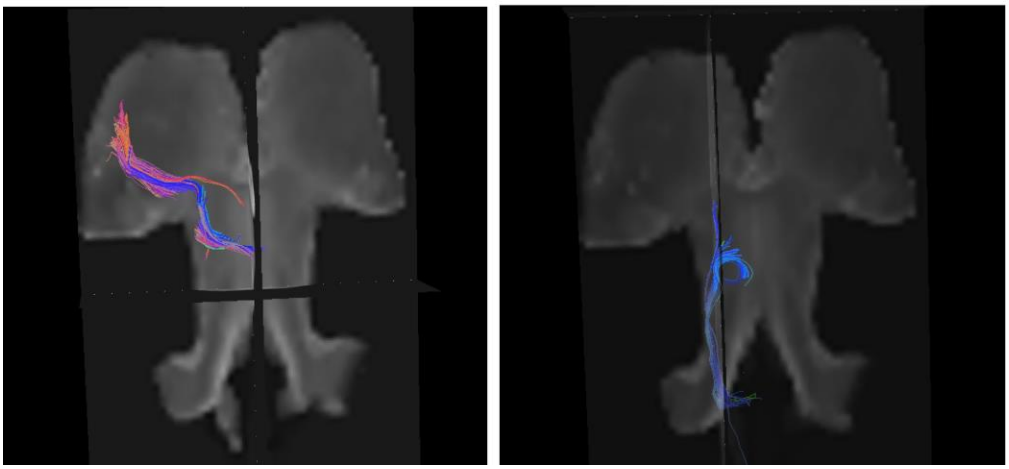
Abstract: Investigating the subcortical auditory system is challenging, particularly in humans, due to the technical difficulty of in vivo MR imaging: the small brainstem auditory nuclei require high spatial resolution, but decreasing voxel sizes reduces the signal-to-noise ratio, making the nuclei difficult to identify. A priori information about the location of auditory structures can focus in vivo investigations in the appropriate anatomical locations and reduce the number of voxels being analyzed, improving the statistics of detecting meaningful signal from auditory nuclei. In this study, we created an atlas of subcortical auditory structures based on high resolution, high quality anatomical MRI of a post mortem human brainstem and thalamus. The specimen was imaged at the Duke Center for In Vivo Microscopy in a 7-Tesla small-bore MRI scanner at 50 μm isotropic voxel sizes. This resolution allows for identification and segmentation of substructures along the entire subcortical auditory pathway, from the root of the cochlear nerve and the dorsal and ventral cochlear nuclei, through the superior olivary complex and

inferior colliculus, to subdivisions of the medial geniculate of the thalamus. By registering the atlas to a common reference space, we can apply the atlas to standard in vivo human MRI. In addition, registering the atlas to diffusion-weighted images (200 μm) from the same post mortem specimen allows us to constrain tractography to streamlines between specific auditory subnuclei, yielding the highest quality, highest resolution connectivity map of the subcortical auditory pathway. Auditory pathway streamlines were still visible after downsampling the diffusion images to resolutions feasible in vivo (1 mm), suggesting in vivo identification of subcortical auditory streamlines should be possible with high quality data. In total, this work contributes novel information about the auditory pathway using high resolution, high quality ex vivo MRI and facilitates further in vivo research on the structure and function of the human auditory system.





Disclosures: K.R. Sitek: None. **E. Calabrese:** None. **G.A. Johnson:** None. **S.S. Ghosh:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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Program #/Poster #: 573.17/Y9

Topic: D.06. Auditory & Vestibular Systems

Support: Operational Programme Research, Development and Education in the framework of the project "Centre of Reconstructive Neuroscience", registration number CZ.02.1.01/0.0./0.0/15_003/0000419

Title: The effects of early postnatal noise exposure on the development of perineuronal nets inthe rat auditory cortex

Authors: *J. SVOBODOVA BURIANOVA, J. SYKA
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Abstract: In the rat auditory system, a period of increased vulnerability to external stimuli (known as the critical period, CP) starts with the onset of hearing (PD 12) and ends around three weeks later. It is expected that the closing of the critical period is paralleled with the maturation of perineuronal nets (PNNs); lattice-like extracellular matrix structures that appear around the soma and proximal dendrites of mainly parvalbumin expressing inhibitory neurons. Exposure to loud sound during the CP, can significantly affect the neuronal morphology and electrophysiology of neurons in the rat auditory cortex. Whether the exposure can also change the pattern of PNN maturation remains unknown. Long-Evans rats were exposed at PD14 to a 125 dB SPL broad-band noise for 8 min. The content of PNNs was evaluated in brain sections, stained for Wisteria floribunda agglutinin in exposed rats aging from PD14 to PD106, and compared with non-exposed controls. We observed no visible nets at PD14, either in the exposed animals or in the controls. The first signs of PNN appeared at PD21 in both groups however, they were more expressed in the exposed animals. In principle, the development of PNNs appeared to be more accelerated in the noise-exposed animals than the non-exposed controls. These results suggest that noise exposure may lead to the premature closing of the CP window, thus limiting the plasticity of early postnatal development in the auditory cortex.

Disclosures: J. Svobodova Burianova: None. **J. Syka:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 573.18/Y10

Topic: D.06. Auditory & Vestibular Systems

Title: Ultra-small, transparent and genetically accessible vertebrate brain with rich behavior

Authors: *L. SCHULZE[1], J. HENNINGER[1], T. CHAIGNE[1], M. KADOBIANSKYI[1], A. FAUSTINO[1], S. ALBADRI[2], N. HAKIY[1], M. SCHUELKE[3], L. MALER[4], F. DEL BENE[2], B. JUDKEWITZ[5]
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Abstract: Information processing in the brain is based on the interactions of distributed neuronal populations. However, studying neuronal networks at single-cell resolution across the entire adult brain has so far been impossible in vertebrates due to their size and opacity. Here, we address this challenge by introducing a new model organism to neuroscience. The freshwater fish Danionella translucida (DT) combines small size and near complete transparency even in the adult when neural circuits and behaviour have matured. We found this close relative of the zebrafish to have the smallest known adult vertebrate brain (0.6 mm^3), containing over one order of magnitude fewer neurons than zebrafish. DT adults display a rich set of complex behaviors, including courtship, shoaling, schooling and, remarkably, acoustic communication. To enable optical network activity measurements and perturbations, we established CRISPR/Cas9 genome editing and Tol2 transgenesis techniques that allowed us to image neural activity in response to acoustic stimuli, including mimics of DT's own vocalizations. Small size, transparency, genetic access and rich behavior make Danionella translucida a highly promising model organism for the study of adult vertebrate brain function at single-cell resolution.

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Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

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Program #/Poster #: 573.19/Y11

Topic: D.06. Auditory & Vestibular Systems

Support: NIH grant DC10000

Title: Presynaptic modulation and inhibitory feedback in the avian cochlear nucleus angularis

Authors: *K. M. MACLEOD, S. L. EISENBACH, S. E. SOUEIDAN
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Abstract: Inhibition plays a critical and varied role in auditory processing, ranging from sound level gain control, sculpting frequency tuning, and enhancing temporal processing. The cochlear nuclei in the avian brain are unusual in that they lack local inhibitory circuitry, and instead receive feedback from the superior olivary nucleus, a third order brainstem area. Cochlear nucleus angularis (NA) is a key encoder of overall sound level for gain control, but also subserves spectrotemporal processing. Since NA encodes multiple aspects of sound intensity, the nature and dynamics of the inhibitory feedback are of keen interest, as well as how feedback mechanisms might differ from the timing pathways of the brainstem. Using whole cell patch clamp recordings from NA neurons in brainstem slices, we investigated the short-term synaptic plasticity of fast, mixed $GABA_A$- and glycine-receptor mediated currents, presynaptic inhibition of excitatory and inhibitory inputs, and the effects of presynaptic inhibition on synaptic dynamics. Trains of IPSCs elicited by electrical stimulation showed substantial transient and sustained facilitation across a wide range of frequencies (5-200 Hz). In addition, trains of stimulation elicited numerous asynchronous events that contributed substantially to the overall current. Summation of facilitated synchronous events and increased frequency of asynchronous events resulted in total inhibitory current that showed >50% enhancement over that expected from trains of IPSCs without facilitation. Presynaptic inhibition via GABA-B receptors ($GABA_BR$) suppressed inhibitory synaptic transmission, but also shifted the dynamics further toward facilitation. Presynaptic modulation of excitatory transmission via $GABA_BRs$ also suppressed glutamatergic responses, but had little effect on the synaptic plasticity of these inputs. At inhibitory synapses, basal spontaneous release of GABA/glycine may be at sufficient levels to tonically suppress release. These results suggest that both excitatory and inhibitory synapses in the avian cochlear nucleus angularis can be strongly modulated via presynaptic metabotropic $GABA_BR$. The modulation of excitatory and inhibitory inputs of NA neurons via $GABA_BR$ activation appears to parallel that in the timing pathway.

Disclosures: K.M. MacLeod: None. **S.L. Eisenbach:** None. **S.E. Soueidan:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

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Program #/Poster #: 573.20/Y12

Topic: D.06. Auditory & Vestibular Systems

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The Sandler Foundation
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The Klingenstein Foundation
The Coleman Memorial Foundation

Title: Modulation of auditory cortical information processing by movement and VIP interneuron activation

Authors: *J. BIGELOW, J. DEKLOE, R. MORRILL, A. HASENSTAUB
UCSF, San Francisco, CA

Abstract: Information processing in sensory cortex is highly sensitive to contextual variables such as anesthetic state, arousal, and task engagement. Recent work in visual cortex (VCtx) has established that local circuitry responsible for extracting information from visual environment is highly sensitive to inputs originating from motor circuits activated during locomotion, finding that evoked firing rates and stimulus information increase when animals are engaged in movement. A specific inhibitory interneuron circuit appears to be critical for this change. Inhibitory interneurons expressing vasoactive intestinal peptide (VIP) are differentially activated during movement, which typically suppress other inhibitory interneurons, ultimately disinhibiting excitatory pyramidal cells. Although VIP activation has been observed during movement in somatosensory and auditory cortices, it remains unclear whether these activations similarly elevate evoked responses and stimulus information. The present study examined auditory cortical (ACtx) responses evoked by tone cloud stimuli in awake, headfixed mice during spontaneous movement and still conditions. To test the role of a VIP-mediated circuit in motor-related activity modulation, we crossed VIP-Cre mice with Ai32 mice to express channelrhodopsin in Cre-expressing VIP interneurons. VIP+ cells were optogenetically activated for half of the stimulus presentations, permitting independent analysis of the consequences of movement and VIP activation, as well as their intersection. Preliminary analyses suggest heterogeneous influences of both movement and VIP activation on ACtx responses. In contrast to VCtx, stimulus-evoked spike counts tend to decrease during movement in our neuron sample. Loss of spikes during movement appears to be associated with a reduction in total information (bits) as well as information efficiency (bits/spike). Consistent with the disinhibitory circuit observed in VCtx, VIP interneuron activation tends to elevate evoked firing rates in ACtx

neurons. Importantly, however, the additional spikes produced by VIP activation seem to be unrelated to the stimuli, usually contributing zero additional information (bits), thus undermining information efficiency (bits/spike). The effects of simultaneous movement and VIP activation appear to sum linearly: although evoked spike counts during locomotion generally return to baseline levels with concurrent VIP activation, information remains abnormally low. Our findings raise intriguing possibilities about asymmetric consequences of motor circuit activation on information propagation in VCtx and ACtx.

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Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 573.21/Y13

Topic: D.06. Auditory & Vestibular Systems

Support: UT Austin School of Biological Sciences

Title: Short-term synaptic plasticity evoked by electrical and optical stimulation in neurons of the gerbil medial geniculate body

Authors: A. MILLER, K. V. NGUYEN, C. MARTINEZ, J. BRODEUR, A. J. CARRERA, D. MANDALAPU, S. GOOCH, M. HOOPER, L. A. MORENO-ELLIS, S. D. STOLLE, L. E. WAGNER, B. WILKINS, L. J. KREEGER, D. B. HAIMES, *N. L. GOLDING
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Abstract: The dynamics of synaptic transmission plays a critical role in shaping the encoding of auditory information to the cortex from the medial geniculate body (MGB). However, due to the extensive convergence of multiple ascending and descending auditory inputs to the MGB, it is not clear whether the release properties of excitatory and inhibitory terminals on MGB neurons are dictated by the target or source neurons. To understand whether the properties of short-term plasticity depend on input pathway, we made whole-cell current clamp recordings from MGB neurons in thalamocortical brain slices of Mongolian gerbils (30-38 days old, ~35°C). In initial experiments, we activated pharmacologically isolated excitatory or inhibitory synaptic inputs to thalamic neurons through local electrical stimulation. In a second set of experiments, we injected the inferior colliculus of P23-25 gerbils with an adeno-associated virus (synapsin promoter driving GFP and channelrhodopsin-2 (ChR2)), 13 days prior to electrophysiological slice experiments in which ChR2 was activated with 470 nm field illumination. Recorded MGB neurons were labeled with biocytin and slices were processed for streptavidin conjugated to Alexa-568 for subsequent morphological analysis and localization within the MGB. Labeling with an antibody against calretinin was used to distinguish the dorsal from the ventral

subdivisions of the MGB. For electrical stimulation experiments, most recorded neurons were from the ventral MGB, and exhibited electrophysiological properties consistent with prior studies in mouse and rat. Resting potentials were maintained between -60 and -65mV with direct current injection. In response to paired electrical stimuli, excitatory inputs exhibited strong short-term potentiation (2.99±0.33 fold change, n=6), whereas inhibitory inputs exhibited short-term depression (0.58 to 0.72 fold change, n=3). By contrast, in preliminary recordings, cells subjected to optical activation of both excitatory and inhibitory IC inputs to thalamic neurons all exhibited short-term depression (n=4). Taken together, our results in gerbils are in agreement with data from studies in rats and mice demonstrating there is input specificity in the release properties of synapses. The strong short-term potentiation observed during intra-thalamic stimulation is consistent with predominant activation of descending corticothalamic inputs, which may be preferentially preserved in these slices.
slices.

Disclosures: A. Miller: None. **K.V. Nguyen:** None. **C. Martinez:** None. **J. Brodeur:** None. **A.J. Carrera:** None. **D. Mandalapu:** None. **S. Gooch:** None. **M. Hooper:** None. **L.A. Moreno-Ellis:** None. **S.D. Stolle:** None. **L.E. Wagner:** None. **B. Wilkins:** None. **L.J. Kreeger:** None. **D.B. Haimes:** None. **N.L. Golding:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 573.22/Y14

Topic: D.06. Auditory & Vestibular Systems

Support: DARPA BAA-16-24

Title: Vagal nerve stimulation strongly activates cortical networks, centered around sensorimotor cortex

Authors: L. N. COLLINS[1], *L. J. BODDINGTON[1], P. J. STEFFAN[1], D. NESTVOGEL[1], S. JO[1], R. C. FROEMKE[2], M. J. MCGINLEY[3], D. A. MCCORMICK[1]
[1]Inst. of Neurosci., Univ. of Oregon, Eugene, OR; [2]Neurosci. Inst., New York Univ. Sch. of Med., New York, NY; [3]Duncan Neurolog. Res. Inst., Baylor Col. of Med., Houston, TX

Abstract: Stimulation of the vagus nerve is used for treatment of some forms of epilepsy and depression, although the mechanisms of these effects are not well known. Many forms of epilepsy are state-dependent, occurring more prominently at some states (e.g. drowsiness, transition between sleep and waking, etc.) than others. We hypothesized that vagal nerve stimulation may alter the state of the brain, perhaps through the activation of ascending sensory or modulatory pathways. Indeed, activation of ascending neuromodulatory pathways is known to

strongly influence arousal state, which in turn substantially alters sensory processing, task performance, and propensity to generate epileptic seizures. For example, our lab has previously demonstrated that when in an intermediate state of arousal (as determined by pupil size), mice perform significantly better on an auditory detection task than mice in either low or hyper-aroused states (McGinley et al., Neuron, 2015). We hypothesize that vagal nerve stimulation may strongly control the state of activity in the forebrain through activation of either ascending neuromodulatory pathways involved in arousal, or through activation of sensorimotor cortical regions, or both.
To test this hypothesis, we performed wide-field imaging of the entire dorsal surface of the mouse brain in awake, behaving mice, monitored pupil diameter (arousal) and walking while periodically delivering varying intensities of vagal nerve stimulation. Our data demonstrate that vagal nerve stimulation reliably induces pupil dilation (arousal), with the magnitude of the pupillary response being directly related to stimulus intensity. Increasing the intensity of vagal nerve stimulation also leads to a dose-dependent increase in neuronal activity (calcium signals) in widespread cortical regions, centered around motor and sensory areas, even in the absence of walking (measured using widefield imaging of Thy1-GCaMP and CamKII-GCaMP mice). Ongoing work is underway to examine these effects in detail at the neuronal and neuromodulatory level and how they affect the ability of the mouse to learn, retain, and perform detection and discrimination tasks.

Disclosures: L.N. Collins: None. **L.J. Boddington:** None. **P.J. Steffan:** None. **D. Nestvogel:** None. **S. Jo:** None. **R.C. Froemke:** None. **M.J. McGinley:** None. **D.A. McCormick:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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Support: Universidad Nacional de Colombia. DIB,
Convocatoria Nacional de Proyectos para el
Fortalecimiento de la Investigación, Creación e Innovación
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Title: Inactivation of primary auditory cortex decrease the overall cortical burst activity in isoflurane-induced burst suppression state

Authors: *M. J. ROJAS, M. D. SUAREZ
Salud Animal, Univ. Nacional De Colombia, Bogota, Colombia

Abstract: Cortical Burst suppression (BS) is an electroencephalographic pattern present under a number of conditions such as deep isoflurane-induced anesthesia. The BS pattern shows very low amplitude EEG (silent EEG periods) followed by bursts of very high amplitude, and the ratio of these periods is the burst suppression ratio BSR. Auditory stimulation during isoflurane-induced BS evoke burst activity modifying the BSR. It might be an intracortical transmission of this signals from the auditory primary cortex (APC), but there is no data about the role of the APC in the generation of cortical BS. The aim of this exploratory study was to unveil the role of APC on BS, thus, we performed a bilateral reversible inactivation of the APC in 4 male Wistar rats under isoflurane anesthesia while recording temporal and frontal electroencephalogram (EEG). The animals were instrumented with frontal and temporal EEG electrodes, and a reference electrode was inserted into the neck muscles. A cooling technique was used to inactivate the APC by positioning a 3.5mm diameter cryoloop on the surface of each APC (AP: -4.5mm to Bregma, DV: 5mm); also, body temperature, and the surface cortical temperature was monitored while EEG recordings were carried out prior to cooling down the APC, during, and after the cooling procedure. The results from this study showed that inactivation of PAC significantly increased the BSR from 56.3% (±14.2) to 94.3% (±6.6) ($p=0.002$), and this effect was reversible after cooling when BSR went back to 70% (±9.8) ($p=0.01$). These results indicate that the PAC plays a key role in the generation of burst activity during isoflurane-induced BS. However, there is still the need of research the mechanisms involved in this phenomenon.

Disclosures: M.J. Rojas: None. **M.D. Suarez:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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Program #/Poster #: 573.24/Y16

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC016169

Title: Cholecystokinin neurons of the inferior colliculus provide direct and powerful excitation and inhibition to the medial geniculate body of the gerbil

Authors: *L. KREEGER, P. MEHTA, B. V. ZEMELMAN, N. L. GOLDING
Inst. for Neurosci., The Univ. of Texas At Austin, Austin, TX

Abstract: Neurons in the central nucleus of the inferior colliculus (ICC) exhibit diverse morphologies, electrophysiological properties, and projection targets. To understand whether molecular-genetic classes of ICC neurons form distinct cell classes, we targeted neurons with interdependent adeno-associated viruses and evaluated their anatomy, physiology, and neurochemical markers. This technique enabled us to label and optogenetically activate

cholecystokinin (CCK) expressing neurons in the ICC of the Mongolian gerbil. Using patch-clamp recordings in slices, we found that CCK neurons comprise two classes, one excitatory and one inhibitory, which can be distinguished by endogenous neurochemical markers and electrophysiological properties. Excitatory CCK neurons comprise 30% of ICC excitatory neurons, whereas inhibitory CCK neurons comprise 20% of ICC inhibitory neurons.
To characterize the relationship between neurochemical markers and electrophysiology, we targeted CCK neurons in the ICC for *in vitro* whole-cell current clamp recordings (P35-50 gerbils, 35°C). Excitatory and inhibitory CCK neurons have an adapting firing pattern. However, the inhibitory group exhibited more moderate levels of adaptation and an additional slow phase of spike after-hyperpolarization. To determine the connectivity of CCK neurons, we made recordings from non-CCK (non-fluorescently labeled) ICC neurons, and activated channelrhodopsin-expressing CCK neurons and axons with blue light. Evoked excitatory and inhibitory post-synaptic potentials (PSPs) were small (<2 mV) and widespread, making connections with >50% of recorded ICC neurons of all firing phenotypes.
To assess the role of CCK neurons in the ascending pathway, we activated channelrhodopsin-expressing CCK axons to putative medial geniculate body (MGB) targets. CCK neurons in the ICC exclusively target the ventral division of the MGB, suggesting a role in the thalamocortical pathway. Interestingly, terminal fields of virally labeled axons were highly branched and polymorphic, consisting of both small boutons with *en passant* swellings, as well as medium to large axons with large complex endings. Optogenetic activation of CCK inputs evoked large suprathreshold EPSPs and IPSPs (10-15 mV). Large EPSPs and terminal sizes are consistent with proposed models of thalamic drivers.
Taken together, our results indicate that excitatory and inhibitory CCK neurons are functionally distinct cell types. CCK neurons comprise a large proportion of ICC neurons and are well positioned to drive and shape thalamocortical activity.

Disclosures: L. Kreeger: None. **P. Mehta:** None. **B.V. Zemelman:** None. **N.L. Golding:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

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Burroughs Wellcome Fund (BWF) Career Award at the Scientific Interface

Pennsylvania Lions Club Hearing Research Fellowship

Title: Amygdala-TRN projections amplify tone-evoked activity in auditory thalamus and cortex

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Abstract: Many forms of behavior require selective amplification of neuronal representations to relevant sensory signals. Associating emotional responses with sensory cues can lead the nervous system to alter behavior to future representations of these cues. Here, we identify a novel pathway between the baso-lateral amygdala (BLA), an emotional learning center in the mouse brain, and the inhibitory nucleus of the thalamus (TRN), and demonstrate that activation of this pathway amplifies sound-evoked activity in the central auditory pathway. We stimulated BLA using channelrhodopsin (ChR2) with a laser via implanted optic cannulas, while recording neuronal activity in the auditory cortex (AC) in response to a presentation of random tone sequences in awake, head-fixed male or female mice. Optogenetic activation of the BLA suppressed spontaneous activity (*paired t-test, p=0.0007*), while amplifying tone-evoked response magnitude in AC (*paired t-test, p=8.5e-5, n= 8 mice*). Inspection of fluorescence following RetroBead injections in TRN revealed direct projections from BLA to TRN. These projections were further confirmed by retrograde labeling of neurons in the BLA using a CAV-2 virus in TRN. We next directly activated projections from the BLA to TRN by repeating the initial experiment, but positioning the optic cannula over TRN. We found that there was a significant suppression of spontaneous activity (*paired t-test, p=0.003, n= 7 mice*), and a significant increase in tone-evoked responses in AC (*paired t-test, p=3.9e-8*). We found that activation of the BLA projections to TRN also led to inhibition of spontaneous activity (*paired t-test, p=4.3e-9*) and an increase in tone-evoked responses in auditory thalamus (Medial Geniculate Body, MGB) (*paired t-test, p=3.4e-7, n = 5mice*), consistent with the hypothesis that the changes in AC responses with BLA activation are a result of projections from BLA to TRN via MGB. These results demonstrate a novel circuit mechanism for amplification of sensory representation of behaviorally relevant signals and provide a potential target for treatment of neuropsychological disorders, in which emotional control of sensory processing is disrupted.

Disclosures: S. Rolón-Martínez: None. **M. Aizenberg:** None. **M.N. Geffen:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 573.26/Y18

Topic: D.06. Auditory & Vestibular Systems

Support: NIH NIDCD R01DC013102

Title: Pharmacologic and optogenetic manipulation of the dopamine system alters auditory processing in the inferior colliculus

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Abstract: Background: The ability to understand speech relies on accurate auditory processing of complex sounds. Individuals with Parkinson's disease suffer from speech perception deficits, suggesting that dopamine is involved in the encoding of complex sounds. Recent studies from our lab demonstrated that dopamine has heterogeneous effects on the responses of many neurons in the inferior colliculus (IC) of mice, although the strongest effect is to suppress neural activity. It is currently unknown, however, which dopamine receptors are involved in modulating neuronal responses, and whether the observed preponderance of depressive effects reflects the endogenous dopamine system in the IC. In this study, we tested whether dopamine acts via D1- and/or D2-like receptors to alter responses of IC neurons, as well as tested the effect of optogenetically induced dopamine release on auditory responses in the IC. **Methods:** *Iontophoretic experiments*: We recorded extracellular responses of single neurons in the IC of awake, restrained mice. We compared neuronal responses to tones and mouse vocalizations before and after iontophoretic application of dopamine and D1- or D2-like agonists or antagonists. *Freely behaving experiments*: We recorded multi-unit activity in the IC of freely behaving mice. We compared global response to tones and mouse vocalizations before and after pressure injection of dopamine or a D2-like agonist or antagonist into the IC. *Optogenetic experiments*: We created a DAT/ChR2 mouse line and recorded extracellular responses of single neurons in the IC of awake, restrained mice. We compared neuronal responses to tones and mouse vocalizations before and after stimulation with blue light pulses through an optrode to evoke local dopamine release. **Results:** In iontophoretic and freely behaving experiments, both the single-unit and global effects of dopamine and a D2-like agonist were heterogeneous as both either increased or decreased responses of IC neurons to tones and vocalizations, while a D2-like antagonist had opposite effects. Similar to the effects of exogenous dopamine application, optogenetic induction of endogenous dopamine release via blue light decreased responses to tones and vocalizations in the majority of cells in mice expressing ChR2. **Conclusions:** We found that dopamine alters auditory responses in the IC, and that such modulation occurs via D2-like receptors. We also found that activation of the endogenous dopamine system in the IC suppresses responses of auditory neurons. Understanding how dopamine modulates auditory processing will ultimately inform therapies targeting mechanisms of auditory and communication disorders.

Disclosures: J.M. Hoyt: None. **D.J. Perkel:** None. **C.V. Portfors:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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Program #/Poster #: 573.27/Z1

Topic: D.06. Auditory & Vestibular Systems

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Title: Connectional modularity within the lateral cortex of the mouse inferior colliculus gives rise to partially segregated processing streams for auditory and multisensory information

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Abstract: The lateral cortex of the inferior colliculus contains a network of modules characterized by dense staining 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, it is unknown whether the intrinsic connections in the lateral cortex also exhibit connectional modularity. In the present study, we sought to characterize the intrinsic inputs to GABAergic and non-GABAergic neurons in both modular and extramodular regions of the lateral cortex. 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. GABAergic and non-GABAergic cells in both regions were filled and 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. Morphological reconstructions of biocytin-filled cells revealed that the dendrites of neurons in the lateral cortex are largely confined to the domain (modular or extramodular) in which their cell body resides, with the exception of GABAergic modular cells. Photostimulation maps generated under synaptic blockade further support these results; direct stimulation of extramodular cells is elicited from extramodular sites, direct stimulation of non-GABAergic modular cells arises from modular sites, but direct stimulation of GABAergic modular cells can be driven from both domains. Pre-synaptic photostimulation and spatial analysis revealed that extramodular cells receive input almost exclusively from the extramodular domain, non-GABAergic modular cells receive mixed input from both domains, and GABAergic modular cells receive the majority of their input from the extramodular domain. Overall, these results indicate that there is a unidirectional flow of information within the lateral cortex, such that

modular cells receive inputs from auditory-recipient (extramodular) and somatosensory-recipient (modular) areas of the lateral cortex, while extramodular cells only receive input from the extramodular domain. This modularity in the intrinsic connectivity of the lateral cortex may give rise to partially segregated processing streams for auditory and multisensory processing.

Disclosures: A.M. Lesicko: None. **D.A. Llano:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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Program #/Poster #: 573.28/Z2

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant 5R01DC00427418
NIH Grant 5R01DC01293805

Title: Astrocytes and potassium buffering at a fast auditory synapse

Authors: *B. J. LUJAN, H. VON GERSDORFF
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Abstract: The nerve terminals of auditory neurons are responsible for the efficient release and recycling of synaptic vesicles that enable the secure chemical transmission that underlies auditory perception. The fidelity and modulation of these synaptic events is important in the superior olivary complex (SOC) of the mammalian brainstem, which is involved in sound source localization. The calyx of Held nerve terminal is an integral component of this afferent projection pathway and a hallmark of this synapse is the ability to maintain synaptic fidelity during high frequency transmission (i.e. at frequencies ≥ 800 Hz *in vivo*), a range at which conventional synaptic boutons cannot reliably fire. The regulation of local ionic environments in the extracellular spaces during synaptic transmission is not well understood. Because of the high-fidelity necessary for the functional output of this synapse, extracellular K^+ accumulation is likely higher at auditory synapses than traditional synaptic boutons during persistent spiking activity. Astrocytes probably play a major role in the homeostatic maintenance of extracellular K^+ concentration; implicating the tripartite synapse as an important regulator of synaptic strength. Using the mature mouse brainstem slice preparation containing the calyx of Held-MNTB synapse as a model of tripartite synaptic function, we investigated the functional role of compromised K^+ buffering on synaptic vesicle release. We inhibited extracellular K^+ uptake produced as a byproduct of synaptic firing by antagonizing inward rectifying K^+ channels with Ba^{2+}. Bath application of Ba^{2+} increased evoked excitatory postsynaptic current (EPSC) amplitudes and led to a concomitant increase in the frequency of spontaneous EPSCs. Additionally, paired-pulse ratio was decreased after Ba^{2+} application, suggesting an increase in

presynaptic release probability (Pr). Finally, we made whole-cell patch clamp recordings from astrocytes juxtaposed to synapses, and observed an inward current in the astrocyte immediately following high-frequency synaptic firing. The amplitude of the inward current was dependent on the synaptic firing frequency and was completely blocked by Ba^{2+}, implicating astrocytes as a major regulator of extracellular K^+ concentration. Our data suggest that astrocyte K^+ buffering plays a putative role in the maintenance of extracellular K^+ concentration and loss of K^+ homeostasis increases presynaptic Pr in a mouse auditory synapse.

Disclosures: B.J. Lujan: None. **H. von Gersdorff:** None.

Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

Location: SDCC Halls B-H

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Program #/Poster #: 573.29/Z3

Topic: D.06. Auditory & Vestibular Systems

Support: DFG PP1608-Fr7/1

Title: Ca^{2+}-dependent vesicle replenishment in inhibitory synapses in the auditory brainstem allows for faithful and robust inhibition during high frequency activity

Authors: *D. J. WEINGARTEN[1,2,3], N. MÜLLER[3], E. FRIAUF[3], H. P. VON GERSDORFF[2]
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Abstract: A key function of the auditory brainstem is the precise localization of sound sources in the horizontal plane. For the ascending auditory pathway to perform this feat, a system which is able to distinguish minute intensity and timing differences at high frequencies is needed. Indeed, reliable synaptic transmission is a hallmark of the highly specialized excitatory synapses of this system, which allow the processing of auditory information with exquisite temporal precision. However, less is known about how inhibitory synapses shape the processing of sound signals and if they further develop after hearing onset. Here, we investigated synapses of the lateral superior olive (LSO) in the mouse auditory brainstem. A speeding of evoked excitatory and inhibitory postsynaptic currents has been observed in the LSO during postnatal development. Yet, if changes occur in the presynaptic parameters of vesicle release remains unknown. Using whole-cell patch clamp recordings in acute brainstem slices we characterized inputs from the medial nucleus of the trapezoid body (MNTB) and the cochlear nucleus (CN) to principle neurons of the LSO (MNTB-LSO and CN-LSO synapses) via electrical afferent fiber stimulation. Recordings were done at 36±1°C from pre-hearing mice at postnatal day P10-12 and young adults at P28-34. Using high-frequency stimulation (50 stimuli of 50, 100, and 200 Hz), synaptic parameters could be determined. After hearing onset CN-LSO synapses showed an

increase in their number of readily releasable vesicles (RRP), which has also been previously described for other excitatory synapses in this system. Surprisingly, the RRP of MNTB-LSO synapses dropped from 600 vesicles at P10-12 to below 300 vesicles at P28-34. To counteract this reduced number of RRP vesicles, after hearing onset these synapses develop a frequency-dependent vesicle replenishment. While the slow Ca^{2+}buffer EGTA and the K^{+}-channel blocker TEA had little effect on vesicle replenishment in young mice, mature animals showed a twofold higher vesicle replenishment with increased Ca^{2+} and a drop to ~50% compared to P10-12 under EGTA. To investigate how this activity-dependent replenishment affects recovery after depression, gaps of 10 ms to 5 seconds were introduced in between stimulation trains. In the absence of activity the time constant of recovery of MNTB-LSO synapses was significantly slower (2.7 ± 0.4s) than that of CN-LSO synapses (1.2 ± 0.4s). In summary, mature MNTB-LSO synapses develop a remarkably fast vesicle replenishment specialized for faithful and sustained inhibition during high-frequency activity.

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Poster

573. Auditory Processing: Circuits, Synapses, and Neurotransmitters

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Program #/Poster #: 573.30/Z4

Topic: D.06. Auditory & Vestibular Systems

Support: NIH RO1DC009607
NIDCD DC-00046

Title: Development of functional responses to sound in primary auditory cortex

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Abstract: Early sensory experience is critical for normal structural and functional neural development. Altricial animals are born with closed ears and so ear opening is thought to be the earliest time point at which auditory stimuli can shape sensory circuits. However, recent reports from ferret (Wess et al. 2017) indicate that auditory cortex can respond to sound over one week before ear opening. These responses emerged in a deep cortical layer, the subplate, which is known to receive thalamic projections before thalamorecipient layer 4 circuits. We therefore investigated if mouse auditory cortex(ACX) responds to sounds before ear opening (~P11) using

neonatal transgenic Thy1-GCaMP6s mice to track the evoked calcium fluorescence of neurons during sound presentation.
To image activity in these young mice we head-fixed the animal and exposed their skull. GCaMP fluorescence was elicited through a 473nm LED located above the skull surface. Emitted fluorescence was collected by a CMOS camera (ThorCam), allowing for recording of calcium activity across ACX fields. We presented broadband white noise (4-48kHz) or single tones (4-48kHz). Since the ear canals are closed at young ages we presented sounds at 90dB. Sounds were presented for 2 seconds in separate blocks of 30 trials each. Fluorescence data were then extracted and the time-courses of the 25th percentile most responding regions were plotted for each animal. Our analysis revealed an increase in GCaMP fluorescence during the period of sound presentation. Acute widefield imaging in anesthetized animals also showed evoked fluorescence signals before ear opening. To confirm that these responses were coming from single neurons, we used 2-photon in-vivo calcium imaging of ACX in mice before the ages of ear opening. We found that neurons in both layer 2/3 and layer 4 show sound-evoked activity. These results indicate that the ascending auditory pathway is functional in mice before ear opening. Therefore, studies of experience-dependent development of the auditory system have to take into account early auditory experience through closed ears.

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Poster

574. Auditory Processing: Adaptation, Learning, and Memory

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 574.01/Z5

Topic: D.06. Auditory & Vestibular Systems

Support: NSERC

Title: Neural indices of auditory reflective attention during word-in-noise identification

Authors: *T. M. V. CHAN[1,2], C. ALAIN[1,2]
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Abstract: Speech-in-noise (SIN) comprehension is greatly enhanced when semantic context is provided prior to degraded speech. Evidence suggests that semantic context can also help identify words-in-noise even when the context is provided after. The latter is attributed to attention being retrospectively allocated to representations in short-term memory. However, it is unclear whether the use of context following degraded speech employs the same neural mechanisms as contextual cues prior to degraded speech. Here we present EEG findings from 15

healthy young adults using a word-in-noise identification paradigm. Participants listened to a target word embedded in speech-shaped noise that was either preceded (pre-cued) or followed (retro-cued) by one of three auditory cue conditions: a word related to the target, a word unrelated to the target, or a burst of white noise. Event-related potentials time-locked to retro-cue onset showed an effect of relatedness at about 400-700 ms over bilateral parietal scalp areas. These data are consistent with a proposed model of the employment of auditory reflective attention in the ongoing comprehension of SIN, where context provided after a degraded speech target engages attention to short-term memory representations of speech.

Disclosures: T.M.V. Chan: None. **C. Alain:** None.

Poster

574. Auditory Processing: Adaptation, Learning, and Memory

Location: SDCC Halls B-H

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Program #/Poster #: 574.02/Z6

Topic: D.06. Auditory & Vestibular Systems

Support: NCN Grant UMO 2013/14/D/NZ5/03337

Title: Functional polymorphism of MMP9 and BDNF as a potential biomarker of neuroplasticity in prelingual deafness treatment with cochlear implantation - A retrospective cohort analysis

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Abstract: Aims: Genetic biomarkers of neuroplasticity in prelingually deaf children treated with cochlear implantation could facilitate their clinical management, giving higher chances for development of robust proficiency of spoken language. We investigated whether carrying of a certain variants of genes encoding matrix metaloproteinase *MMP-9* and neurotrophin *BDNF* is a prognostic marker of auditory skills acquisition outcome. Method: We performed a retrospective analysis of functional *MMP9* variant (rs3918242, c.1562 C>T) known to affect *MMP-9* gene expression level and *BDNF* variant (rs6265, c.196G>A, p.Val66Met) known to affect the protein function in a group of 106 deaf children, aged below 2, treated with unilateral cochlear implantation. We studied associations between the presence of relevant *MMP9* and *BDNF* genotypes and auditory development of the implanted children. Language acquisition was assessed with Little Ears Questionnaire (LEAQ) over 14 months post intervention. Results: Prevalence of *MMP-9* variants in the studied group was C/C - 66%, C/T- 34%, *BDNF* - G/G - 75,5%, G/A- 24,5% and this data are consistent with Caucasian population dispersion. In the subgroup of subjects implanted below 1 year, showing no response in pre-implant Auditory

Brainstem Responses median rate of auditory development for carriers of rs3918242 C/T genotype (median 5.0, IQR 4.0-.9.0) 1 month after CI activation is statistically significantly higher than for carriers of rs3918242 C/C (median 2.0, IQR 1.5-5.0) (p=0.0102). This predominance remains in 5th month of auditory development (p=0.0424), but not in further follow up. (U Mann Whitney test). Applied regression model predicating LEAQ score after 1st month of CI use in this subgroup includes genetic status of both *MMP9* and *BDNF* (p<0.0001). It reveals that, assuming the same pre-implant LEAQ score, 1 month post CI a subject carrying both rs6265 G/G genotype and rs3918242 C/T genotype will score 7.6 points in LEAQ higher than a carrier of both rs6265 G/A genotype and rs3918242 C/C genotype, 4.1 points in LEAQ higher than a carrier of rs6265 G/G genotype and rs3918242 C/C genotype, and 3.5 points higher than a carrier of rs6265 G/A genotype and rs3918242 C/T genotype.
Conclusions: rs6265 G/G genotype and rs3918242 C/T genotype predisposes their deaf carriers to better response to a sensory stimulation delivery to cochlea in first months after CI activation than carriers of the rs6265 G/A and rs3918242 C/C genotypes. Further studies should address potential biomarker value of those genetic variants as well as possible functional role of *MMP9* and *BDNF* in neuroplasticity evoked by cochlear implantation in the prelingually deaf children.

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Poster

574. Auditory Processing: Adaptation, Learning, and Memory

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 574.03/Z7

Topic: D.06. Auditory & Vestibular Systems

Support: Boramae Medical Center 03-2018-5

Title: PET and MR evidence of functional connectivity between hearing and working memory in rat model

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Abstract: Purpose: The effect of hearing loss on memory area of brain and is still not completely understood. Recently, several studies suggested that hearing loss aggravated decline of cognitive and memory function. In this present study, changes of cerebral volume and glucose metabolism were evaluated using PET, MR and RT-qPCR study in deafened animal model.

Materials & Methods: Twenty rat of F-18 FDG PET and T2W MR were scanned using Siemens Inveon PET scanner and agilent 9.4T MR scanner (baseline study). Single sided deafness (right sided deafness) model (n=10) and bilateral deafness model (n=10) were constructed. One month and 3 month after deafness induction, FDG PET and T2W MR were acquired. To perform SPM and VBM analysis, brain was extracted using rectangular masking. Individual PET and MR data was spatially normalized onto template and smoothed with 2 mm Gaussian kernel. To conserve gray matter concentration, modulated VBM was performed. Two sample t-test was performed ($p < 0.005$). Another sets of animals (n=42) were sacrificed for RT-qPCR at 1 and 3 month after deafening procedure. Age-matched normal hearing animals were also included for control. After sacrifice, entorhinal cortex was quickly harvested. Quantitative RT-PCR was performed for measuring mRNA expression of aldehyde dehydrogenase 1 family member L1 (ALDH1L1), glial fibrillary acid protein (GFAP), oligodendrocyte transcription factor 2 (OLIG2), and neurofilament heavy chain (NEFH).

R**esults:**

SPM analysis shows that cerebral glucose metabolism was decreased in the region of bilateral primary auditory cortex compared to that of baseline after 1 month of deafness for both single sided / bilateral deafness group. After 3 months of deafness, additionally, cerebral glucose metabolism was decreased in the region of medial entorhinal cortex compared to that of baseline for both single sided / bilateral deafness group. VBM analysis shows that regional gray matter concentration was decreased in the region of left medial entorhinal cortex for single sided deafness group and bilateral medial entorhinal cortex for bilateral deafness group. ALDH1L1 mRNA expression was significantly decreased at both entorhinal cortices after 1 month later in both single sided and bilateral deafness group.

Conclusions: SPM and VBM analyses showed decreased metabolic activity and volume of gray matter and an astrocyte marker mRNA expression was also decreased in medial entorhinal cortex. Entorhinal cortex is located between auditory cortex and hippocampus and known to relate to working memory. So these findings suggest a functional connectivity between hearing and working memory.

Disclosures: M. Park: None. **H. Lee:** None. **J. Kim:** None.

Poster

574. Auditory Processing: Adaptation, Learning, and Memory

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Program #/Poster #: 574.04/Z8

Topic: D.06. Auditory & Vestibular Systems

Title: Dopaminergic modulation of noise vocoded speech learning in patients with Parkinson's disease

Authors: *C. M. THIEL[1,2], M. CONTY[1], L. WURST[1], A. PFEIFFER[1], A. ENGELHARDT[1], S. PUSCHMANN[1,3]
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Abstract: Human and animal studies provide evidence for a central role of the dopaminergic neurotransmitter system in auditory learning and brain plasticity (Bao, Chan, & Merzenich, 2001; Knecht et al., 2004; Weis, Puschmann, Brechmann, & Thiel, 2012). We here aimed to test the role of dopamine in learning noise vocoded speech, which consists of spectrally degraded signals and is commonly used to simulate the sensation after cochlear implantation. To investigate the role of the dopaminergic system, we used a between subject design and patients with Parkinson's disease (male/female; age range:42-83 yrs) on (n=10) or off (n=11) their regular l-dopa medication and an age-matched healthy control group (n=10). Subjects were presented with 60 noise vocoded sentences taken from a matrix test (Uslar et al., 2013) and asked to repeat back what they heard. In addition, they underwent neuropsychological and motor assessment. We found significantly reduced learning rates in patients off l-dopa as compared to on l-dopa. Our findings in this sample of patients with Parkinson's disease provide first evidence that dopamine promotes adaptation to degraded speech which may be of relevance for auditory rehabilitation after cochlear implantation.
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Poster

574. Auditory Processing: Adaptation, Learning, and Memory

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Burroughs Wellcome Fund (BWF) Career Award at the Scientific Interface Maria Neimark Geffen
Pennsylvania Lions Club Hearing Research Fellowship Maria Neimark Geffen

Title: Reorganization of cortical population neuronal activity following auditory fear conditioning

Authors: *K. WOOD[1], R. BETZEL[2], D. S. BASSETT[2], M. N. GEFFEN[1]
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Abstract: Auditory perception relies on learning-driven neuronal plasticity within the auditory pathway. Here, we investigated how associative learning, differential auditory fear conditioning (DAFC), affects neuronal population responses to sounds in auditory cortex (AC). In DAFC, the subject is presented with two different frequency tones, one of which is paired with a foot-shock. Previously, we found that AC is required for expression of DAFC-driven changes in sound-frequency discrimination acuity (Aizenberg and Geffen, 2013) and that modulating inhibitory neuronal activity in AC leads to similar bi-directional changes in discrimination acuity (Aizenberg, 2015). However, how DAFC affects tone-evoked population neuronal activity remained unknown. We hypothesized that DAFC would drive changes in population tone-evoked neuronal activity corresponding to either and increase or a decrease in neurometric frequency discrimination acuity, as a function of fear learning specificity.
To understand the transformation of sound representation in AC before and after DAFC we imaged calcium activity in hundreds of neurons simultaneously in AC of awake, head-fixed mice, tracking the same neurons over days under a two-photon microscope before and after two DAFC sessions. We quantified changes in tone frequency-dependent responses of individual neurons, as well as in population functional connectivity. DAFC drove heterogeneous changes in individual neuronal responses for either shock-paired or unpaired tone frequencies. At the same time, mean population neuronal response strength to tones across frequencies was preserved. However, neuronal responses to tones following DAFC became more consistent after DAFC. Neuronal populations formed clusters driven by correlated activity, neurons within clusters exhibit heterogeneous response patterns. The neuronal cluster structure changed between days in the absence of DAFC, but the network structure became more consistent over days following DAFC. These findings suggest that DAFC drives cortical population activity toward a more stable state.

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Poster

574. Auditory Processing: Adaptation, Learning, and Memory

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 574.06/Z10

Topic: D.06. Auditory & Vestibular Systems

Support: TCU SERC Grant 170306

Title: Effects of auricular vagus nerve stimulation on novel orthography acquisition

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Abstract: For typically developing adults as well as for children with dyslexia, the acquisition of a novel orthography is a difficult task and fluency is often unattainable. While the brain is hardwired for language, the reading network must be allocated and optimized from scratch in every individual brain. In dyslexia, the visual word form area is often hypoactivated and recent studies suggest this lack of involvement may lead to poor fluency. Invasive cervical vagus nerve stimulation (cVNS) can drive plasticity in the adult brain and has shown recent promise in the treatments of tinnitus and post- stroke motor impairment. This invasive approach is not practical for reading-based interventions. However, the auricular nerve, a branch of the cervical vagus nerve, innervates the cymba conchae region of the outer ear and projects to similar brain regions as cVNS. This study evaluated whether this non-invasive form of vagus nerve stimulation could be useful in driving plasticity for newly learned letter-to-sound correspondences. Adults between the ages of 18-24 years old completed ten 30-minute training sessions in which they learned letter-to-sound correspondences in Hebrew. Participants were randomly assigned to one of two control conditions (training with an in-person tutor vs. a customized computer program) or the active stimulation group. In the active group, participants completed the computer-based training program while receiving low levels of electrical stimulation to the left cymba conchae. Participants were tested at 3 time points to track progress on letter identification, rapid letter reading, and pseudoword reading: once at the halfway point, (on Day 6) once on the final day of lessons (on Day 10), and once more at least a week following their final lesson (retention). Participants receiving stimulation were monitored daily to ensure no adverse reactions to the intervention and to ensure the level of current was well within tolerable levels. There were no differences in performance between the two control groups, so these groups were combined for comparison with the active stimulation group. The active stimulation group exhibited faster reading times and higher accuracy on pseudoword reading compared to the control group as early as day 6. We present these findings as well as the longer-term performance of the stimulation group and possible behavioral predictors of individual success in the training

program. This study demonstrates for the first time that non-invasive auricular vagus nerve stimulation may be a valuable tool in improving reading acquisition and fluency. Ongoing research is evaluating the neural correlates of this approach.

Disclosures: T.M. Centanni: None. **V. Thakkar:** None. **A. Jefferson:** None. **C. Stacey:** None. **N. Khodaparast:** None.

Poster

574. Auditory Processing: Adaptation, Learning, and Memory

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 574.07/Z11

Topic: D.06. Auditory & Vestibular Systems

Support: Natural Science Foundation of China 31600798

Title: Brain activities under culturally familiar and unfamiliar music: fMRI evidence of musical culture effect

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Abstract: Musical culture is an important aspect of musical cognition that affects our understanding of music, as well as our daily lives. Although previous studies have provided culture-related evidence on neural activities, the influence of musical culture on neuroplasticity has been scarcely considered. In this work, 20 pianists and 20 Chinese traditional instrument players were recruited as the musician group for the experiment, while 20 non-musicians were recruited as the baseline group. Using a paradigm of listening to culturally familiar and unfamiliar music under functional magnetic resonance imaging (fMRI) technology, we found that greater activation of the superior temporal gyrus was elicited by culturally unfamiliar music. The result showed that culturally unfamiliar music might strengthen the abilities to judge melodic familiarity. These findings, which provide evidence for functional neuroplasticity based on the familiarity of musical culture, could enrich our insights into the musical brain.

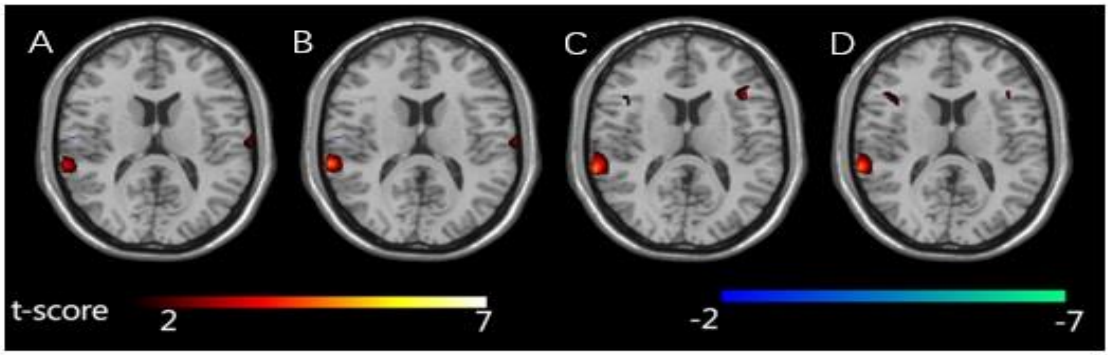

Figure1. The difference of brain activities when musicians listen to music compared with non-musicians. Superior temporal gyrus was significantly different in all conditions. A: the result of Chinese traditional musicians listening to Chinese music vs. non-musicians listening to Chinese music; B: the result of Chinese traditional musicians listening to western music vs. non-musicians listening to western music; C: the result of western musicians listening to Chinese music vs. non-musicians listening to Chinese music; D: the result of western musicians listening to western music vs. non-musicians listening to western music. Moreover, we also found that the greater activation of the superior temporal gyrus was elicited when subjects listening to the culturally unfamiliar music.

Disclosures: S. Guo: None. **Y. He:** None. **J. Lu:** None.

Poster

574. Auditory Processing: Adaptation, Learning, and Memory

Location: SDCC Halls B-H

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Program #/Poster #: 574.08/Z12

Topic: D.06. Auditory & Vestibular Systems

Support: JSPS KAKENHI JP17H06034
AMED Brain/MINDS

Title: Predictive coding on auditory processing: Spatio-temporal structure of signal flow in whole-cortical electrocorticograms

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Abstract: To acquire a model of external world into our brain, the brain encodes sensory inputs by constracting generative model of the external world via synaptic modulations. Recently, this process is referred as "predictive coding (PC)", and some kinds of PC models inspired by the brain have become basis of the deep learning models in terms of the machine learning. However,

it remains unclear how the brain updates its generative model incessantly. In this study, we investigated the spatiotemporal structure of neuronal signal flow in a whole cortical level, which related to PC on auditory processing. We recorded the electrocorticograms (ECoGs) from three common marmosets with epidurally implanted electrodes covering entire hemispheres. The 85-96 channel ECoG array was epidurally implanted on the left or right hemisphere of each marmoset. ECoG recordings were conducted in passive listening condition with " roving oddball sequences" of 20 types of pure tones (250-6727 Hz with an interval of 1/4 octaves). Repetitive sequences of each 20 tone were randomly presented. We considered the last tones of sequences as standard, and the first tones of sequences as deviants. First, we investigated spatiotemporal propagation of auditory evoked potentials. We firstly observed significant activity in primary auditory area. And then, the activity moved to higher auditory areas, parietal, and frontal corteces. Second, we investigated brain regions correlated with prediction errors. The significant corelations firstly appeared in auditory belt regions, and then the corelations observed in frontal cortex. Third, we inferred functional connectivity by calcurating corretions of neuronal signals aligned on onset of standard and deviant stimuli, and compared the connectivity between standard and deviant stimuli at 50 msec time window with 10 msec step. The significant diffenrences of the connections were observed between temporal and frontal corteces. As results, a cortical processing model of auditory predictive coding have arrised. Information of auditory stimuli travels in wide range of cortical areas including temporal, frontal, and parietal areas. Onece stimulus comes into the brain, prediction errors firstly occure auditory belt regions, then propagate to other higher areas including frontal and parietal regions. Then, those signals update the generative model via changes of functional connectivities within auditory areas, and between auditory area and higher brain areas.

Disclosures: N. Ichinohe: None.

Poster

574. Auditory Processing: Adaptation, Learning, and Memory

Location: SDCC Halls B-H

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Program #/Poster #: 574.09/Z13

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R01
DARPA Grant

Title: Effects of peristimulus vagal nerve stimulation on responses in ferret auditory cortex

Authors: *J. B. FRITZ[1,2], A. MOHAMMED[2], J. VISWANATHAN[2], P. YIN[2], D. ELGUEDA[2], E. CAUSEY[2], J. LAI[3], S. V. DAVID[3], S. A. SHAMMA[2]
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Abstract: Vagal nerve stimulation (VNS) has been shown to be an effective therapy for treatment of anxiety, epilepsy, inflammation and improves rehabilitation following stroke or brain injury. VNS is known to activate the Nucleus Tractus Solitarius which elicits CNS release of the neuromodulators acetylcholine (Nucleus Basalis) and norepinephrine (Locus Coeruleus), each of which are known from previous studies to affect responses in auditory cortex and enhance neuroplasticity. The goal of the present research was to explore the cortical effects of VNS in the awake animal, which could provide the neurobiological basis for VNS targeted neuroplasticity training – the use of VNS to enhance auditory learning. In the present study, we investigated the effects of VNS on auditory cortical responses in the awake, quiescent ferret to tonal, noisy and Mandarin Chinese phonemic stimuli with or without pairing with peristimulus VNS. We varied VNS duration, interstimulus interval, current amplitude and stimulation rate to explore parameter space for optimal stimulation effects. We also varied the site of VNS stimulation, using either cuff stimulation of the cervical vagus (c-VNS) or transdermal stimulation of the concha of the external ear, known to be innervated by the auricular branch of the vagus (a-VNS). We recorded from 120 neurons in primary auditory cortex (A1) and found that 32/120 cells showed response enhancement of 30% or more and 12/120 showed suppression effects of 30% or more. In some neurons, VNS lead to striking enhancement of cortical responses by 100-200%. Effects lasted for minutes and sometimes for up to one hour post-VNS, but gradually decreased and cell responses returned to baseline. Effects could be replicated with a second application of VNS. A parallel set of effects was observed with pupillary responses which were also modulated by VNS. In addition to measuring effects of VNS in a quiescent, listening animal, we also measured responses to VNS during active auditory task engagement in auditory go-nogo tasks, pairing target stimuli with or without peristimulus VNS. We shall describe the differences between the effects of a-VNS and c-VNS on cortical responses to acoustic stimuli during active behavior and passive listening.

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Poster

574. Auditory Processing: Adaptation, Learning, and Memory

Location: SDCC Halls B-H

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Program #/Poster #: 574.10/Z14

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant FP00017074
Vilcek MSTP Scholarship

Title: Neuromodulation and plasticity for a rodent model of cochlear implant use

Authors: *E. G. GLENNON[1,2], J. MULTANI[2], I. CARCEA[3], M. SVIRSKY[4,2,5,6], R. C. FROEMKE[4,3,2,5]
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Abstract: Cochlear implants are neuroprosthetic devices that can provide hearing to deaf patients. However, learning rates and peak performances of speech perception with cochlear implants are highly variable (Blamey et al. 2013). Adaptation to cochlear implants is believed to require neuroplasticity within the central auditory system (Fallon et al. 2009). However, mechanisms by which behavioral training enables plasticity and improves outcomes are poorly understood. Here we investigate the hypothesis that neural mechanisms that promote plasticity in the rodent auditory system are key to optimizing cochlear implant usage, and might be especially helpful in cases of poor performance. We focus on noradrenergic modulation of rat auditory cortex by the locus coeruleus, which can enable robust and long-lasting neural and behavioral changes (Manunta and Edeline 2004; Martins and Froemke 2015; Sara 2015).
We developed a new surgical approach for cochlear implantation in adult rats (King et al. 2016). Our approach optimizes insertion depth of an 8-channel electrode array and allows rats to freely behave while using the implant to perform auditory tasks. Normal hearing rats are trained on a go/no-go task, and self-initiate trials to respond to a target tone. Previously, we showed that this task requires auditory cortex, and that this task is sensitive to cortical modulation and plasticity (Carcea et al. 2017).
We tested if changes in locus coeruleus activity affect or improve auditory learning in normal hearing and cochlear-implanted rats. Prior to each daily behavioral training session, rats underwent a 5-10 min locus coeruleus pairing session. We examined how locus coeruleus stimulation might affect reversal learning when a new sound became the target. The new target was paired with locus coeruleus stimulation as for cochlear-implanted animals. Locus coeruleus stimulation accelerated learning in each case. We used fiber photometry to monitor neural activity of noradrenergic locus coeruleus neurons, showing strong responses to novel auditory stimuli and noxious stimuli. These studies indicate that neuromodulation can play a powerful role in shaping outcomes with cochlear implant use and training.

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Poster

574. Auditory Processing: Adaptation, Learning, and Memory

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Program #/Poster #: 574.11/Z15

Topic: D.06. Auditory & Vestibular Systems

Support: Havas & Dib Lawyers to JN
Western Sydney University Scholarship to JN

Title: Binaural interactions in the inferior colliculus following unilateral noise induced hearing loss

Authors: J. NGUYEN, J. W. MORLEY, *C. H. PARSONS
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Abstract: The auditory system is a bilateral system, integrating information from both ears to perform auditory functions such as sound localisation and detection of signals within noisy environments. The binaural functioning of the auditory pathway implies a balance of excitation and inhibition between inputs from either ear. Disruption of this balance, as may occur following unilateral noise-induced hearing loss (NIHL), may profoundly affect the normal functioning of the auditory system. There is growing consensus that the imbalance of excitation and inhibition due to hearing loss may contribute to the perception of tinnitus. Investigating changes in binaural processing following NIHL may aid us in understanding the neural basis of tinnitus. We investigated the consequences of unilateral NIHL on the response properties of cells in the inferior colliculus (IC). Four normal hearing Wistar rats were used as controls and four were unilaterally exposed to 115 dB SPL of 16 kHz for 1-hour. ABRs confirmed permanent threshold shifts. Using 32-channel, single shank electrodes, we simultaneously recorded from left and right ICs. We characterized the monaural response properties of IC neurons from normal hearing and NIHL animals, and investigated responses due to contralateral (dominant) and ipsilateral (non-dominant) stimulation. In these same neurons, we characterized binaural response profiles. Overall, in both normal (61%) and NIHL (71%) animals, the dominant binaural response was EI. Interestingly, the underlying monaural response properties of these EI neurons differed between the controls and NIHL groups. In the controls, the majority of EI neurons were derived from a population of V-shaped neurons, whilst in the NIHL group the EI responses were distributed amongst O and V-shaped neurons. We also examined the change in output of ipsilaterally driven IC neurons in response to introduction of contralateral stimulation. Under these conditions, introduction of contralateral stimulation produced an increase of excitatory responses. This was the dominant output in control (91%) and NIHL (61%) animals. In the control group, there was little inhibitory effect from contralateral stimulation with 4% of neurons being inhibited by contralateral input. However, in NIHL group, a significantly higher proportion of neurons were inhibited by contralateral stimulation. This result was consistent in the lesion (20%) and the intact ear (27%). These results show specific changes occur in the monaural and binaural response properties of IC neurons following unilateral NIHL. Moreover, the majority of binaural changes occur in a defined population of IC neurons.

Disclosures: J. Nguyen: None. **J.W. Morley:** None. **C.H. Parsons:** None.

Poster

574. Auditory Processing: Adaptation, Learning, and Memory

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Program #/Poster #: 574.12/Z16

Topic: D.06. Auditory & Vestibular Systems

Support: APA RD-2015-5A
APA RD-2015-5B
PSL Data-Science SDDS

Title: Circuits for opposite valences learning in auditory cortex studied by inference of plastic connectivity

Authors: *J.-F. LEGER[1], X. LIU[1], A. LOURDIANE[1], C. VENTALON[1], L. BOURDIEU[1], Y. BOUBENEC[2], S. SHAMMA[2], S. WOLF[3], S. COCCO[4], R. MONASSON[3]
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Abstract: Listening and understanding our sound environment is a behavior that requires training and relies on our past experience. Auditory-cued behavioral training can alter neural circuits in primary auditory cortex (A1), but the mechanisms and consequences of experience-dependent cortical plasticity are far from being fully understood. This work addresses the following open questions: is there a general pattern of changes in the neuronal properties of A1 local networks when a sound becomes behaviorally relevant? Does the representation of a sound depend on whether it is associated with reward or punishment? Are there different circuits recruited during these opposite motivation auditory learning, and can we identify them? We explore these issues with mice that learn to perform two tasks with the same acoustic discrimination but with differential reward valence—one with water reward and the other with shock punishment. By taking advantage of the imaging capability of two-photon microscopy, we follow the same GCaMP6f expressing neurons in A1 throughout successive learning. Awake head-fixed recordings provide a rich observation of A1 activity in its layer 2/3. We find that in A1 superficial layers, both learning tasks induce strong patterning of neuronal sound selectivity, with an increase of marked spatial contrasts between zones representing the target sound and the surrounding areas. Neuronal assemblies representing the target sounds after learning the two tasks are distinct but overlapping. Finally, we apply approaches inspired by statistical physics to analyze our optical recordings and infer the underlying functional connectivity. They suggest that internal connectivity within A1 and external connection onto A1 are both reshaped by learning, and contribute to the observed modifications of sound selectivity. This work will

improve our understanding of the various circuits involved in learning associated with opposite values.

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Poster

574. Auditory Processing: Adaptation, Learning, and Memory

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Program #/Poster #: 574.13/Z17

Topic: D.06. Auditory & Vestibular Systems

Support: NRF-2017R1A2B4006604

Title: Influence of various auditory stimuli conditions on P3-like auditory event-related potentials in rodent model

Authors: *J. LEE[1], Y. LEE[1], Y. A. CHO[2], S. KIM[2], K. KIM[4], J. SUNG[5], S. JUN[3]
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Abstract: P300 (P3) wave is an event-related potential (ERP) recorded approximately 300 ms after stimulus in electroencephalography (EEG). P3 waves have been considered as a potential marker for cognitive brain function. Also, it is known that the P3 component may reflect information processing and consecutive decision making. However, there have been few studies for P3 waves using a rodent animal model due to the difficulty in dealing with animals' cognitive behaviors. In this study, we recorded P3-like ERPs from rats after behavior training during oddball paradigm. After the animal is trained to respond to a specific auditory stimulus, distinct P3 ERPs were successfully obtained. Further investigation is performed to propose the underlying mechanisms of P300 ERP component. We observe the aspects of P3-like ERP components under several different auditory target stimuli to accumulate the results from different cases and verify further underlying mechanisms of auditory cognitive perception in the rodent model.

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Poster

574. Auditory Processing: Adaptation, Learning, and Memory

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Topic: D.06. Auditory & Vestibular Systems

Support: Wellcome Trust DBT India Alliance Fellowship to SB
IIT Kharagpur SRIC Challenge Grant to SB
CSIR fellowship to M.Mehra

Title: Role of inhibitory interneurons in long time scale adaptation based changes in coding of sound sequences in the mouse auditory cortex (ACX)

Authors: *M. MEHRA[1], M. PARASHAR[2], H. K. SRIVASTAVA[3], A. MUKESH[4], S. BANDYOPADHYAY[5]
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Abstract: Cortical encoding of sound sequences with embedded rare stimuli, used in animal behavior of detection/discrimination/working memory tasks, can play a role in perception and behavior associated with streams of sounds. We investigate auditory cortical representation of deviant/rare stimuli in different long-term contexts using single unit extracellular recordings and 2-photon Ca^{2+} imaging in the mouse ACX. We use stimuli that are either periodic with a fixed relative location of the oddball sound (FF) or randomized location of the deviant (RF) with both tonal (T) and broadband noise (N) stimuli. Single unit recordings in Layer 2/3 show distinct patterns of response adaptation in the ACX over repetitions of stimuli in the two contexts. In the FF stream case (FF) ACX responses adapt to the entire sequence while in the RF case with an unpredictable deviant location there is a general increase followed by the adaptation of responses. Further, the adaptation observed in the FF case with T as deviant is absent with N as deviant amidst T sound stream. In order to understand the mechanisms underlying the differential adaptive coding of sound sequences, we hypothesize a role of inhibitory interneurons (INNs). Using 2-photon Ca^{2+} imaging, we probe coding of such sound sequences in excitatory and inhibitory neurons. We find differential selectivity of parvalbumin+ (PV) and somatostatin+ (SOM) INNs to N and T stimuli and thus further hypothesized that they play a differential role in the coding of Tone as deviant and Noise as deviant stimuli. We find the differential nature of adaptation of the PV and SOM neurons to the different contexts and types of sound sequence stimuli compared to excitatory neurons (EXNs) underlie the differential coding of sound sequences by EXNs in the supra-granular layers of the mouse ACX.

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Poster

574. Auditory Processing: Adaptation, Learning, and Memory

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH ZIA MH001101-25
NIH ZIA MH002928-01

Title: Auditory oddball response in dorsolateral prefrontal cortex and basolateral amygdala is distinct from that in auditory cortex of macaque

Authors: *C. R. CAMALIER[1], K. SCARIM[2], M. MISHKIN[3], B. B. AVERBECK[4]
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Abstract: The mismatch negativity (MMN) is an event-related potential component seen in response to unexpected "novel" stimuli, such as in an auditory oddball task. The MMN is of wide interest and application, but a key limitation of current understanding is that neural responses that generate it are poorly understood. This is in part due to differences in design and focus between animal and human oddball paradigms. For example, one of the main explanatory models, the "predictive error hypothesis", posits differences in timing and selectivity between signals carried in auditory and prefrontal cortex. However, these predictions have not been fully tested because 1) noninvasive techniques used in humans lack the combined spatial and temporal precision necessary for these comparisons, and 2) single neuron animal models of oddball, which combine spatial and temporal precision, have not focused on higher order contributions to novelty signals. In addition, accounts of the MMN traditionally do not address contributions from subcortical areas known to be involved in novelty, such as the amygdala. To better constrain hypotheses and to address methodological gaps between human and animal studies, we recorded single neuron activity from the (AC, n=690), dorsolateral prefrontal cortex (PFC, n=598) and the basolateral amygdala (AMY, n=627) of two macaque monkeys during an auditory oddball paradigm modeled after that used in humans. Consistent with predictions of the predictive error account, novelty signals in prefrontal cortex were generally later than in auditory cortex, as well as abstracted from stimulus specific effects seen in auditory cortex. However, we found signals in amygdala that were comparable in magnitude and timing to those in prefrontal cortex, and both prefrontal and amygdala signals were generally much weaker than those in auditory cortex. These observations place useful quantitative constraints on putative generators of the auditory oddball-based MMN, and indicate that subcortical areas, such as the amygdala, may need to be included in future explanatory accounts.

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Poster

574. Auditory Processing: Adaptation, Learning, and Memory

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Topic: D.06. Auditory & Vestibular Systems

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NIMH F32-MH106265
NIMH K99MH115082-01
NIMH R01MH101218
NIMH R01MH100561
NARSAD 19944

Title: Ensemble encoding of redundant and novel stimuli in auditory cortex

Authors: *Y. SHYMKIV[1], J. P. HAMM[2], R. YUSTE[2]
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Abstract: Processing of sensory information in the brain strongly depends on prior context, whereby repetitive (redundant) stimuli are ignored, while novel (deviant) stimuli are amplified. The peak response to deviant stimuli also typically shows a delay relative to the redundant response, suggesting that deviance detection involves additional local or top-down feedback. To explore this we imaged the activity of neuronal populations in both primary and higher order auditory cortices and examined the dynamics of population activity involved in context processing and deviance detection. First, we mapped the auditory cortex with wide-field calcium imaging to locate primary auditory cortex (AI), anterior auditory field (AAF), and secondary auditory cortex (AII). Then, we focused on layer 2/3 of each area and record neuronal activity with volumetric two-photon calcium imaging. Awake mice were presented with an acoustic "oddball" paradigm, where a given stimulus was displayed in three contexts, repetitive, deviant, and neutral. The types of stimuli were either simple amplitude modulated tones of different frequencies (2-80 kHz), or more complex frequency grating of different orientations and mouse vocalizations. The population average of neuronal responses in each cortical area showed context dependency, where they were attenuated to redundant stimuli, i.e. stimulus specific adaptation, and amplified to deviant ones, i.e. deviance detection. We used cluster analysis and identify ensembles of neurons encoding context across levels of auditory cortex and as a function of stimulus complexity. We find elements of hierarchical organization, where context encoding is more complex in higher order cortex. We conclude that neuronal ensembles can specifically code

redundant or deviant stimuli, consistent with the hypothesis that they are involved in primary and higher order processing of sensory information.

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Poster

574. Auditory Processing: Adaptation, Learning, and Memory

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Program #/Poster #: 574.17/AA3

Topic: D.06. Auditory & Vestibular Systems

Support: DARPA Targeted Neuroplasticity Training Program N66001-17-2-4008

Title: Vagus nerve stimulation modulates cortical activity in the common marmoset

Authors: *S. D. KOEHLER[1], L. SANTOS[2], X. WANG[2]
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Abstract: Vagus nerve stimulation (VNS) has been shown to modulate resting state cortical activity and induce cortical plasticity in motor and sensory cortex. These observations motivate VNS as a treatment modality for epilepsy and a neuromodulation modality for enhancing rehabilitation and learning. While a number of studies have investigated VNS stimulation effects in rodents, there is currently little data from non-human primates. The common marmoset, a highly vocal and social monkey species, has emerged in recent years as a promising model for neuroscience research. We have established the first marmoset model for studying the effects of VNS on cortical physiology and plasticity. Chronic cuff electrodes were implanted around the left cervical vagus nerve, and electrode status was monitored chronically with electrode impedance spectroscopy. We recorded electroencephalography (EEG) and local field potential (LFP) and single-unit activity from auditory cortex of awake marmosets in response to parametric variations of VNS pulse train parameters including current amplitude (0 - 2 mA), pulse duration (50 - 600 us), and pulse train frequency (10 - 100Hz). We observed that VNS modulated EEG power, predominantly in the alpha (8-12Hz), beta (13-30Hz), and gamma (30-75Hz) bands with modulation strength increasing with pulse train frequency. High frequency pulse trains also suppressed spontaneous activity in some cortical neurons. These data provide the first evidence for VNS modulation of physiological and cortical responses in the marmoset.

Disclosures: S.D. Koehler: None. **L. Santos:** None. **X. Wang:** None.

Poster

574. Auditory Processing: Adaptation, Learning, and Memory

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 574.18/AA4

Topic: D.06. Auditory & Vestibular Systems

Support: R01 DC009836 grant
Hearing Health Fundation grant

Title: Recalibration of excitatory and inhibitory local cortical networks supports neural and perceptual recovery of simple - but not complex - sound processing

Authors: *J. RESNIK, D. B. POLLEY
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Abstract: The auditory system employs a variety of rapid gain control mechanisms to adjust neural coding sensitivity to match transient shifts in acoustic signal energies. In addition to these "fast acting" gain control systems, central auditory neurons also exhibit slower gain control that adjusts neural excitability following long lasting reductions in auditory input strength, for example, deprivation of afferent inputs from the ear. While there is a general notion that increased neural amplification following a partial blockade of input from the ear is enabled by changes in inhibitory strength, the time course and cell type specific circuitry modifications that underlie slow changes in auditory gain remain unknown.
We performed chronic, cell type specific 2-photon calcium imaging to simultaneously visualize sound evoked GCaMP signals in genetically identified inhibitory PV (parvalbumin expressing) neurons alongside neighboring PPy (putative pyramidal) cells in the auditory cortex of awake adult mice, before and after a controlled loss of afferent input from the cochlea. This approach allowed us to track the daily dynamics in identified cell types, at different spatial scales - single cell to network activity, and temporal scales - hours to weeks following peripheral insult. We found an increase in spontaneous activity in PPy cells on the day of the insult followed by an increase in PV spontaneous activity 24 hrs later. Both excitatory and inhibitory cells exhibited a major decrease in toned evoked responses, which recovered almost back to baseline levels two weeks post injury. For more temporally complex stimuli, such as tones embedded in background noise, both PPy and PV cells showed an increase in response thresholds that didn't recover. Network activity showed a permanent increase in noise correlation between PV cells during complex, but not simple, sound presentations.
Our imaging data demonstrated complete cellular and network recovery for simple stimuli, but persistent coding deficits for more complex stimuli such as tones in noise. To explore the perceptual implications of these observations, auditory operant behavioral measurements were performed in head-fixed mice before and following damage to cochlear nerve afferents. As

predicted from our imaging data, mice showed complete perceptual recovery for detecting tones in silence despite a massive loss of auditory nerve input. However, tone detection in noise remained impaired.
Collectively, our work provides new insight into slow compensatory plasticity in PV and PPy neurons in the auditory cortex that restores neural encoding of rudimentary, but not complex, sounds after peripheral deafferentation.

Disclosures: J. Resnik: None. **D.B. Polley:** None.

Poster

574. Auditory Processing: Adaptation, Learning, and Memory

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 574.19/AA5

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R01DC014279

Title: Adaptive noise reduction in human auditory cortex

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Abstract: Speech communication in real-world conditions requires a listener's auditory system to continuously adapt to sudden changes in the acoustic environment and selectively suppress the noise features relative to speech. How adaptation occurs in the human auditory cortex and how it affects the representation and perception of phonetic features as a new noise source appears in the acoustic scene remains unclear. We directly measured neural activity in perisylvian cortical regions of six human subjects as they listened to speech in abruptly changing background noise. We found rapid and selective suppression of acoustic features of noise in the neural responses, which resulted in enhanced representation and perception of spectrotemporal and phonetic features of speech. We further show that the degree of adaptation to different background noises varied across electrodes and was predictable from the tuning properties and speech-specificity of electrodes. Finally, electrical brain stimulation of highly adaptive electrodes significantly improved the perceived quality and the intelligibility of speech in noise. The convergence of these neural, perceptual, and stimulation effects reveal novel representational properties for speech processing in human perisylvian areas and shed light on intrinsic dynamic mechanisms that enable a listener to filter out irrelevant sound sources in a changing acoustic scene.

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Poster

574. Auditory Processing: Adaptation, Learning, and Memory

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 574.20/AA6

Topic: D.06. Auditory & Vestibular Systems

Support: R01NS089679

Title: Dissociation in behavioral effects of perineuronal net degradation in premotor nuclei of adult songbirds

Authors: *L. DARKWA[1,2], V. NERURKAR[2], D. SEMU[2], T. M. OTCHY[2,3]
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Abstract: During sensitive periods, enhanced neural plasticity enables environmental factors to shape developing circuits and behavior. In primary visual cortex, where developmental regulation of plasticity has been studied most extensively, the absence of perineuronal nets (PNNs), extracellular matrix containing chondroitin sulfate proteoglycans, is thought to be a key permissive factor for experience dependent plasticity. The assembly of PNNs around parvalbumin (PV)-expressing inhibitory interneurons is thought to contribute to critical-period closure, and consistent with this notion the degradation of PNNs in adults returns primary visual cortex plasticity and dynamics to a less mature state. In contrast to these sensory circuits, relatively little is understood regarding the regulation of plasticity in sensorimotor circuits and the behaviors they underlie. Song learning in the zebra finch (*Taeniopygia guttata*) occurs during a sensitive period, and it has been previously shown that PNN formation around PV-expressing interneurons in key song system nuclei is correlated with song stereotypy. Drawing an analogy with primary visual cortex, we hypothesized that the degradation of PNNs in premotor nuclei would similarly revert the adult song system to a state more characteristic of the juvenile songbird. To test this hypothesis, we compared the song structure before and after degrading PNNs in either HVC (used as a proper name) or the robust nucleus of the arcopallium (RA) in adult zebra finches by bilateral injections of chondroitinase ABC (ChABC), an enzyme shown to digest PNN proteoglycans *in vivo*. For birds receiving injections targeting RA, we found no significant difference in song structure following PNN degradation, nor between ChABC-injected birds and sham controls. In contrast, birds receiving ChABC injections targeting HVC showed significant and persistent increases in spectral and temporal variability in comparison to both pre-injection songs and sham controls. A subset of these birds also showed elevated syntactic variability. The elevated behavioral variability we observed in birds receiving HVC-targeted injections was reminiscent of the song structure of uncrystallized juvenile birds and is

consistent with prior mechanistic studies showing a correlation between song maturation and the increase of inhibitory tone within HVC. Whether degradation of PNNs in HVC additionally recapitulates other aspects of juvenile song system structure and function remains to be determined. Future experiments will focus on understanding the role of PNNs in shaping singing-related neural dynamics and behavioral flexibility.

Disclosures: L. Darkwa: None. **V. Nerurkar:** None. **D. Semu:** None. **T.M. Otchy:** None.

Poster

574. Auditory Processing: Adaptation, Learning, and Memory

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 574.21/AA7

Topic: D.06. Auditory & Vestibular Systems

Support: DARPA Grant 16-A0-00-006862
NIH Grant DC012557

Title: Chronic vagus nerve stimulation enables long-term plasticity in mouse auditory cortex

Authors: *E. PAPADOYANNIS[1,2], K. A. MARTIN[1,2], J. K. SCHIAVO[1,2], N. Z. TEMIZ[1,2,3], D. A. MCCORMICK[4], M. J. MCGINLEY[5,6], R. C. FROEMKE[1,2,7]
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Abstract: Vagus nerve stimulation is a medical treatment for severe epilepsy and depression, but the mechanisms underlying the neural effects are poorly understood. The vagus connects essentially all peripheral organs to the brain via afferents through the nucleus tractus solitarius to several neuromodulatory centers. Vagus nerve stimulation has been shown to produce long-lasting plasticity in the cerebral cortex to improve sensory processing after stroke (Boreland et al. Brain Stimul 2016). Additionally, recent work has shown that neuromodulatory signaling via the cholinergic and noradrenergic systems lead to enhanced learning (McGinley et al. Neuron 2015). Understanding the circuit mechanisms by which vagus nerve stimulation modulates neural plasticity is important for developing non-invasive neuromodulatory therapies and expanding their application to learning.
Mice provide an opportunity to monitor and manipulate neural circuits during stimulation but vagus nerve cuff electrodes are not available for mice due to their small size. We first designed a novel cuff electrode for mice and demonstrated reliable low-impedance measurements and stimulation for months during behavior in chronically implanted animals. Vagus nerve stimulation was calibrated to transiently reduce respiration without affecting heart rate or blood

oxygen saturation levels. We next wanted to see if vagus nerve stimulation could affect neural representation and behavior. During two-photon calcium imaging in auditory cortex, we found that pairing a tone with stimulation led to a short-term enhancement of auditory representation. After several days of pairing sessions, the representation of the paired tone increased across the population. The observed changes in neural activity following pairing are reminiscent of the effects of basal forebrain stimulation (Froemke et al. Nature 2007). To test if neural changes could influence behavioral performance, animals were trained on either a paired go/no-go or two-alternative forced choice auditory discrimination task (Martins & Froemke Nat Neurosci 2015; Kuchibhotla et al. Nat Neurosci 2017). We are now investigating how vagus nerve stimulation might lead to direct or indirect activation of central modulatory systems to enable plasticity and improve learning.

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Poster

574. Auditory Processing: Adaptation, Learning, and Memory

Location: SDCC Halls B-H

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Program #/Poster #: 574.22/AA8

Topic: D.06. Auditory & Vestibular Systems

Support: NIH DC004682
NIH DC015239

Title: Interhemispheric projections regulate sensory processing in primary auditory cortex

Authors: *B. J. SLATER, J. S. ISAACSON
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Abstract: Interhemispheric (callosal) connections between the right and left auditory cortex are proposed to participate in sound localization and speech processing. Intriguingly, pathophysiology of auditory callosal projections has also been proposed to underlie language deficits and auditory hallucinations in disorders such as autism and schizophrenia. Despite the potential importance of cortical callosal projections in auditory processing, the functional properties of interhemispheric connections are not well understood. Here we combine reversible optogenetic silencing of the left auditory cortex with linear silicon probe recordings in the right primary auditory cortex (A1) of awake, head fixed mice to determine how one cortex influences tone-evoked responses in the other. Pure tones (4-60 kHz) were applied free field to the left ear and the right ear was plugged. Under these conditions, cortical silencing caused a rapid and sustained reduction in spontaneous firing of fast spiking units preferentially located in deep layers. Cortical silencing caused a slow increase in spontaneous firing of regular spiking cells

across all layers and suppressed tone-evoked responses in the majority of cells (39/52) with classical v-shaped tuning curves. The suppressive action on evoked responses scaled with firing rate and there was no consistent change in best frequency. These findings indicate that interhemispheric connections provide both subtractive and multiplicative operations on sensory processing. Thus, cortical callosal projections regulate both the signal to noise ratio and gain control of sound representations in A1.

Disclosures: B.J. Slater: None. **J.S. Isaacson:** None.

Poster

574. Auditory Processing: Adaptation, Learning, and Memory

Location: SDCC Halls B-H

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Program #/Poster #: 574.23/AA9

Topic: D.06. Auditory & Vestibular Systems

Support: NSF GRFP 2015215385
NIH DC004682
NIH DC015239

Title: Brain state-dependent modulation of sensory representations in layer 2/3 of primary auditory cortex

Authors: *P.-A. LIN[1], S. K. ASINOF[2], J. S. ISAACSON[3]
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Abstract: Sensory processing in the neocortex is continuously modulated by changes in behavioral and cognitive state. Thus, deconstructing the complex and multi-faceted relationship between brain state and sensory responses is key to understanding how our brains represent the world around us. In deep layers of the primary auditory cortex (A1) of mice, the magnitude and reliability of sound-evoked responses have been shown to be maximal at intermediate states of arousal (McGinley et al., 2015). However, the manner in which brain state modulates sensory responses in superficial layers of A1—where the bulk of intracortical auditory processing is thought to occur—is less well understood. To address this question, we monitored pure tone-evoked responses using two-photon calcium imaging of GCaMP6s-expressing layer 2/3 (L2/3) pyramidal cells in awake, head-fixed mice situated on a linear treadmill. We simultaneously measured fluctuations in arousal and locomotion via pupillometry and treadmill activity tracking, respectively. In contrast to previous observations in deep layers of A1, we found that the magnitude and reliability of L2/3 tone-evoked responses increased linearly with arousal, peaking at high states of arousal in the absence of locomotion. Thus, the relative sparseness of sensory representations rapidly changed depending on the level of arousal. Furthermore, although

changes in arousal did not alter the best frequency of individual cells, increases in arousal broadened frequency tuning. Taken together, our results suggest that arousal alters the density of population responses and tuning broadness in L2/3 on a moment-by-moment basis. We are currently examining responses of local interneurons to explore potential mechanisms underlying arousal-dependent changes in L2/3 activity.
McGinley, M.J., David, S.V., and McCormick, D.A. Cortical membrane potential signature of optimal states for sensory signal detection. Neuron. 2015; 87: 179-192.

Disclosures: P. Lin: None. **S.K. Asinof:** None. **J.S. Isaacson:** None.

Poster

574. Auditory Processing: Adaptation, Learning, and Memory

Location: SDCC Halls B-H

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Program #/Poster #: 574.24/AA10

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC005779

Title: Adaptive efficient coding of correlated acoustic features in primary auditory cortex of the awake ferret

Authors: *K. LU, W. LIU, J. B. FRITZ, S. A. SHAMMA
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Abstract: Natural sounds such as vocalizations often have co-varying acoustic attributes where one acoustic feature can be predicted from the other. In such cases, neural encoding of one acoustic feature would overlap with that of the other, resulting in coding *redundancy*. It has been proposed that sensory systems are able to detect such covariation and adapt so as to reduce redundancy leading to more efficient neural coding (Barlow and Földiák, 1989). Recent psychoacoustic studies provide evidence supporting this Efficient Coding Hypothesis (Stilp et al., 2010, Stilp and Kluender, 2011, 2012, 2016). Following passive exposure to a set of complex sounds in which the waveform amplitudes and the spectral envelopes covaried in a correlated fashion,,subjects' discriminability for sounds along the correlated dimensions remained intact. However, their discriminability of sounds on the orthogonal dimension was significantly impaired. These results suggest that passive exposure induced the auditory system to efficiently encode the two co-varying dimensions as a single dimension, at the cost of lost sensitivity to the orthogonal dimension. Here we explore the neural underpinnings of this phenomenon by recording single-unit responses from neurons (n=80) in the primary auditory cortex (A1) in awake ferrets (n=4) following a similar passive exposure procedure The stimuli in our study were harmonic tones with two correlated stimulus attributes - amplitude modulation (AM) rate and peak frequency of the spectral envelope (SP). We found that: (1) cortical responses driven by

sounds with correlated attributes rapidly became adapted to these stimuli, (2) while their neuronal spike rate coding signal-to-noise ratio remained unchanged along the covaried dimension, the SNR along the orthogonal dimension decreased, (3) correlation between neurons tuned to the two covarying attributes decreased after exposure, (4) these exposure effects still occurred if sounds were correlated along two acoustic dimensions (AM and SP), but varied randomly along a third dimension (pitch). These neurophysiological results support the Efficient Learning Hypothesis and deepen our understanding of how the auditory system represents acoustic regularities and covariance.

Disclosures: K. Lu: None. **W. Liu:** None. **J.B. Fritz:** None. **S.A. Shamma:** None.

Poster

574. Auditory Processing: Adaptation, Learning, and Memory

Location: SDCC Halls B-H

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Program #/Poster #: 574.25/AA11

Topic: D.06. Auditory & Vestibular Systems

Support: NSF GRFP
NIH DC004682
NIH DC015239

Title: Brain state regulates network-level control of the strength and tuning of tone-evoked responses in primary auditory cortex

Authors: *S. K. ASINOF[1], P.-A. LIN[2], J. S. ISAACSON[3]
[1]Neurosciences Grad. Program, Univ. of California San Diego, La Jolla, CA; [2]Neurosciences, Univ. of California San Diego, San Diego, CA; [3]UCSD, La Jolla, CA

Abstract: Recent rodent studies demonstrated that brain state, as measured by changes in pupil diameter or behaviors like whisking or running, can have profound effects on the cortical encoding of sensory stimuli. However, the synaptic mechanisms underlying brain-state dependent changes in cortical sensory processing are unclear. Here we combine pupillometry and whole-cell recording in awake, head-fixed mice to determine how arousal modulates sensory-evoked activity in layer 2/3 cells of primary auditory cortex (A1). We studied responses to pure tones (100-200 ms duration) over a range of frequencies. In current-clamp, we found that increases in arousal enhanced the amplitude and duration of tone-evoked excitatory postsynaptic potentials (EPSPs). Furthermore, subthreshold responses were tuned to a broader range of frequencies as arousal increased. We next used voltage-clamp recordings to study the excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) underlying tone-evoked responses. Surprisingly, increases in arousal were associated with modest decreases in short-latency, evoked EPSCs and IPSCs locked to tone onset. However, we observed that lateral inhibition generated

by a slow, tone-evoked withdrawal of ongoing, recurrent excitation ("network suppression", Kato et al., 2017) was strongly suppressed as arousal increased. These results reinforce the notion that A1 operates as an inhibition stabilized network and show that the modulation of recurrent activity underlies stronger and more broadly tuned tone-evoked membrane voltage responses in aroused brain states.
Kato HK, Asinof SK, Isaacson JS. Network-Level Control of Frequency Tuning in Auditory Cortex. Neuron. 2017 Jul 19; 95(2):412-423.e4. doi:10.1016/j.neuron.2017.06.019.

Disclosures: S.K. Asinof: None. **P. Lin:** None. **J.S. Isaacson:** None.

Poster

574. Auditory Processing: Adaptation, Learning, and Memory

Location: SDCC Halls B-H

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Program #/Poster #: 574.26/BB1

Topic: D.06. Auditory & Vestibular Systems

Support: NIH-SC1 grant SC1GM118242

Title: Functional investigation of a brainstem excitatory connection relevant to sensorimotor gating

Authors: *L. E. MARTINETTI, E. PERU, A. TENA, C. D. LOYOLA, K. FÉNELON
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Abstract: In order to focus attention, the brain has to "gate" or block irrelevant sensory information that could lead to cognitive overload. This is done by way of a neuronal pre-attentive mechanism termed sensorimotor gating (SG). Therefore, deficits in the SG mechanism prevent patients from focusing attention. SG deficits have been observed in patients suffering from various neurological disorders, and it is a hallmark of schizophrenia. Previous work has identified key brain areas, such as the pedunculopontine tegmental nucleus (PPTg), that send inputs to the brainstem caudal pontine reticular nucleus (PnC). The PNC is the area at the center of the SG circuitry. However, there is still a knowledge gap concerning what cell types are involved and what other brain areas could potentially contribute to SG. It has been long known that the PPTg contains cholinergic, glutamatergic and GABAergic neurons and sends direct inputs to the PnC which contains large glutamatergic neurons as well as glycinergic neurons. These projections are known to modulate SG. Recently, the contribution of PPTg cholinergic neurons to SG was debated. Therefore, it is not known whether other PPTg neurons project to the PnC and whether they contribute to SG. We investigated the role of the PPTg glutamatergic inputs onto the PnC as well as the possible role of glycinergic neurons present in PNC in the context of SG in mice, which had not been demonstrated before. To test our hypothesis, we used neuronal dyes to label cellular pathways, immunohistochemistry to reveal cellular

neurochemistry and *in vivo* optogenetics to functionally study the contribution of the PPTg-PnC glutamatergic connection as well as the possible role of glycinergic interneurons present in the PNC in SG. Additionally, whole cell recordings were obtained from glycinergic PNC neurons in order to characterize their intrinsic and synaptic properties. Our data show for the first time that there is a direct bilateral glutamatergic connection between the PPTg and the PnC. In addition, silencing these PPTg excitatory fibers in the PnC lead to altered prepulse inhibition (PPI), showing a contribution to SG *in vivo,* possibly via glycinergic PnC neurons.

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Poster

574. Auditory Processing: Adaptation, Learning, and Memory

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Program #/Poster #: 574.27/BB2

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant 1SC1GM118242-01

Title: Shining light on a key amygdala-brainstem connection important for attention processing

Authors: J. CANO, *K. FENELON
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Abstract: Sensorimotor gating is a pre-attentive neural filtering mechanism that prevents sensory or cognitive overload, and contributes to attention processing. Patients suffering from neuropsychiatric disorders, such as schizophrenia and anxiety, present sensorimotor gating deficits that greatly impact their daily lives. Clinically, sensorimotor gating can be assessed 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). Numerous *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). However, recent *in vivo* rat and fish studies suggest that different neurotransmitters released from other brain regions might be more critical for PPI. The amygdala is another region directly connected to the PnC. Interestingly, the amygdala modulates PnC neuronal activity, and lesions to the amygdaloid complex can disrupt PPI. Furthermore, anatomical and functional abnormalities of the amygdaloid complex are a phenotypic marker of schizophrenia. However, the potential role of the amygdala-PnC connection in sensorimotor gating remains to be further investigated. We previously showed that the amygdala sends monosynaptic and glutamatergic

inputs to the PnC, using mice. Therefore, here, we investigated the functional contribution of this excitatory connection to sensorimotor gating *in vivo* using an optogenetic approach. We show that silencing this connection significantly affects PPI. Furthermore, we performed tract-tracing, immunohistochemical and *in vitro* electrophysiological experiments to further identify the PnC neurons targeted by this amygdala glutamatergic input. These 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. Cano: None. **K. Fenelon:** None.

Poster

574. Auditory Processing: Adaptation, Learning, and Memory

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Program #/Poster #: 574.28/BB3

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC005779

Title: Habituation of neural responses to complex sounds in secondary auditory cortex of ferrets reflects long-term auditory memory

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Abstract: Auditory neurons encode sound features and also stimulus history (Ulanovsky et al., 2003, 2004). The most commonly reported time course for context or history effects on auditory responses in mammalian auditory cortex ranges from a few hundreds ms up to minutes (Yaron et al., 2012). In the secondary auditory areas in the forebrain of songbirds (Chew et al., 1995, 1996), however, stimulus-specific habituation to acoustic stimuli has been shown to last for hours, even days. This long-term habituation effect in the songbird requires RNA synthesis and is also correlated with behavioral responsiveness to familiar sounds. It is correlated with immediate gene expression and is believed to be a form of long-term auditory memory. However, such long-term habituation effects in the mammalian auditory system have not been previously described. In the current study, we investigated the long-term effect of stimulus history on single-unit responses in secondary auditory cortex (areas PPF and PSF in the dorsal PEG (posterior ectosylvian gyrus)) of awake ferrets (n = 3). We used standard neurophysiological technique and recorded from neurons (n=81) while the animal was repetitively presented with short clips (duration of 2-5 seconds) of novel complex sounds in blocks of 50 same-sound repetitions. Sounds were diverse (n = 73) and included music samples,

animal vocalizations and human speech, For all stimuli we consistently observed marked habituation which decreased the response to asymptote after ~20-30 repetitions. Responses to the same stimuli were then measured again after a delay. We found habituation to stimuli persisted, demonstrating that stimulus habituation could last for at least 20 minutes, thus reflecting a form of long-term memory. With a parallel set of pupillometric studies in the ferret (n=3) we compared pupillary size in response to familiar and novel stimuli and plotted habituation curves for pupillary responses that were similar to the neural habituation curves. The correlated neural and pupillary indices showed that long-term habituation to passively presented repeated stimuli was correlated with recognition of familiar sounds.

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Poster

574. Auditory Processing: Adaptation, Learning, and Memory

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant 1SC1GM118242-01

Title: The protective effects of ferrostatin-1 (fer-1) in response to excitotoxicity in mouse hippocampal slices

Authors: *V. I. NAVARRO[1], M. N. RAMIREZ[2], C. D. LOYOLA BALTAZAR[2], R. SKOUTA[3], K. FENELON[2]
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Abstract: The development of different neurological disorders has been associated with the accumulation of reactive oxygen species (ROS). This phenomenon has been seen during epilepsy and neurodegenerative diseases like Parkinson's disease. Furthermore, an excess of ROS and the development of such disorders have been linked to neuronal cell death partly due to excessive, non-physiological glutamate release. Ferroptosis is a defined iron-dependent cell death mechanism. Interestingly, both ferroptosis and glutamate excitotoxicity are associated with an increase in ROS levels. Ferroptosis and glutamate-induced cell death can be inhibited by another small molecule named Ferrostatin-1 (Fer-1). This suggest that both cell death mechanisms share a similar lethal pathway that can be rescued by Fer-1. The goal of the present study is to better understand the neuroprotective properties of Fer-1. To test our hypothesis, we bath applied glutamate in order to induce epileptiform activity in an *in-vitro* brain slice model. Extracellular field electrophysiological recordings were then performed on hippocampal slices in the presence

and absence of Fer-1. In addition to these studies immunohistochemical experiments were used on 150 µm thick mouse hippocampal slices in order to assess soma size of CA3 region neurons exposed to glutamate-induced excitotoxicity, in the presence and absence of Fer-1. Preliminary data show that Fer-1 decreases the frequency of the glutamate-induced epileptic-like events and prevents the morphological changes subsequent to the exposure to an excess of glutamate.

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Poster

575. Auditory Processing: Perception, Cognition, and Action II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 575.01/BB5

Topic: D.06. Auditory & Vestibular Systems

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Title: Cortical network topology across awareness states during sleep and anesthesia: An intracranial electrophysiology study

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Abstract: Introduction: The sharing of information between nodes in the cortical network plays a central role in leading theories of consciousness, and disruption in connectivity has been proposed to occur upon loss of consciousness (LOC) during anesthesia and sleep. However, whether LOC during these two conditions shares a common mechanism is unclear. To investigate this issue, resting state network topology was compared across brain states during natural sleep and propofol anesthesia.
Methods: Subjects were neurosurgical patients implanted with intracranial electrodes placed to identify epileptic foci. A combination of subdural grids and depth electrodes provided dense coverage of temporal, parietal and frontal cortex. We focused on nodes in the cortical hierarchy activated during both pre-attentive and conscious auditory novelty detection: core and non-core auditory cortex on the superior temporal gyrus including the superior temporal plane, auditory-related cortex on the middle temporal and supramarginal gyrus, and prefrontal cortex. Resting state data were recorded in the same subjects during overnight natural sleep and during induction

of general anesthesia with incrementally titrated propofol infusion. Six brain states were compared: wake (WS) and NREM stages 1 and 2 (N1, N2) during natural sleep, and pre-drug wake (WA), sedated/responsive (S) and unresponsive (U) during propofol anesthesia. Adjacency matrices (A), computed as thresholded, weighted, alpha (8-13 Hz) phase lag index, were compared pairwise for brain states using the operator norm of the difference between adjacency matrices (i.e. $d_{i,k} = ||A_i - A_k||_{op}$).
Results: Changes in network topology were more dramatic for transitions into the unconscious states (N2, U) than for transitions into states of diminished but maintained awareness (S, N1) (i.e., $d_{WA,S} < d_{S,U}$ and $d_{WS,N1} < d_{N1,N2}$). Network topology was most similar between brain states hypothesized to be equivalent under sleep and anesthesia (i.e. WA vs. WS, S vs. N1, U vs. N2); d values comparing hypothesized equivalent states (i.e. $d_{WA,WS}$, $d_{S,N1}$, $d_{U,N2}$) were smaller than d values for corresponding non-equivalent states (e.g. $d_{S,N2}$).
Conclusions: Pronounced changes in network topology for the transitions S -> U and N1 -> N2 likely reflect changes in cortical connectivity mediating transition between conscious and unconscious states. The similarity in network topology between equivalent brain states during anesthesia and sleep suggests common mechanisms in transitions to and from unconsciousness.

Disclosures: M.I. Banks: None. **K.V. Nourski:** None. **H. Kawasaki:** None. **M.A. Howard:** None.

Poster

575. Auditory Processing: Perception, Cognition, and Action II

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Topic: D.06. Auditory & Vestibular Systems

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Title: Auditory and premotor cortex connectivity in the rat brain

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Abstract: Sensorimotor synchronization (SMS) is the coordination of rhythmic movements with an external rhythm. This SMS ability is essential for a number of human behaviors such as

language comprehension, dance and music performance, activities that depend on a dynamic interaction between the auditory and motor system. Neuroimaging and electrophysiological studies have shown that the motor cortico-basal ganglia-thalamocortical circuit (mCBGT), which includes SMA, pre-SMA and putamen, is involved in rhythm, perception and motor execution or rhythmic behaviors. Although it has been demonstrated that neurons in premotor regions (SMA and preSMA) respond to the presentation of auditory stimuli it is not clear how the auditory cortex reach this premotor area. Before the characterization of the audio-premotor pathway in the monkey, we standardized the neuronal tracing technique in the rat brain to elucidate the auditory-premotor cortex circuit in this model. Using the retrograde fluorescent tracer Fluoro-Gold (FG) and the anterograde tracer Dextran Tetramethylrhodamine (TMR), we found that neurons in superficial and deep layers of the auditory cortex (A1) project directly to the supplementary tor cortex (M2), and axons of other areas such as motor, visual and somatosensory cortex also target M2 with different magnitudes. Preliminary data also propose that this pathway M2-A1 is reciprocal. These findings were corroborated using high field 7T magnetic resonance tract-graphs in the same animals. Hence, these results suggest a strong and direct audio-premotor loop in the rat.

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Poster

575. Auditory Processing: Perception, Cognition, and Action II

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Topic: D.06. Auditory & Vestibular Systems

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Title: Oscillatory correlates of auditory working memory as revealed by electrocorticography

Authors: *T. D. GRIFFITHS[1], P. GANDER[2], K. V. NOURSKI[2], C. KOVACH[2], H. OYA[2], H. KAWASAKI[2], M. HOWARD, III[2], S. KUMAR[1]
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Abstract: Working memory is the capacity to hold and manipulate behaviourally relevant information in mind in the absence of ongoing sensory input. Here we explored the hypothesis that working memory for tones requires a network of oscillatory activity in auditory cortex, frontal cortex, and hippocampus, and examined the form of such activity in neuronal ensembles. We recorded local field potentials from six human subjects undergoing invasive monitoring for pre-surgical localization of epileptic foci. The subjects were implanted with depth electrodes

along the axis of Heschl's gyrus (HG) containing primary cortex in the medial part, subdural electrodes over temporal and frontal cortex, and hippocampal depth electrodes. Following a visual cue, subjects were presented with a pair of tones (0.5 s duration, 750 ms inter-stimulus interval) belonging to one of the two different categories ('Low': 300-570 Hz; 'High': 2000-2800 Hz). A visual cue (750 ms) then informed the subjects which tone (first or second) to remember. A 3 s retention period was followed by a tone which could be the same or different (frequency difference ±20%) from the tone held in mind. The subjects made a same/different judgement over a total of 160 trials (80 each of 'Low' and 'High' tone retention). We measured averaged evoked potentials and carried out single-trial time-frequency analysis using a wavelet transform. During retention, a sustained increase (compared to rest period) in power in the beta band (15-20 Hz) was observed in the lateral part of HG. Increase in power in the gamma band (60-100 Hz) was observed in recording sites on the posterior superior temporal gyrus (pSTG) and inferior frontal gyrus (IFG). In the hippocampus, power increase in low frequencies (less than 10 Hz) in the retention period was observed. The data demonstrate a network of brain regions during auditory working memory that includes auditory, frontal, and hippocampal cortex and is consistent with the network shown in our previous functional neuroimaging study (Kumar et al., J Neurosci 2016 36:4492-505). The results provide a foundation for analysis of effective connectivity to test the hypothesis that the auditory cortex activity during retention is driven by the activity in inferior frontal gyrus or hippocampus.

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Poster

575. Auditory Processing: Perception, Cognition, and Action II

Location: SDCC Halls B-H

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Program #/Poster #: 575.04/BB8

Topic: D.06. Auditory & Vestibular Systems

Title: Modulation of low frequency oscillations by human speech control

Authors: *A. RAMÍREZ-CÁRDENAS[1], D. R. PETERS[2], R. BEHROOZMAND[3], R. M. KELLEY[2], C. KOVACH[1], H. KAWASAKI[1], M. A. HOWARD, III[1], J. D. W. GREENLEE[1]
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Abstract: Speech motor control requires the timely integration of vocal-motor and sensory information. Particularly, auditory feedback is crucial for online monitoring of speech production. To study the neural networks involved in speech control, we introduced a sudden and short perturbation in the auditory feedback that human subjects received when speaking. Particularly, feedback was shifted in time (delayed) by 200-300 ms for a variable short period in

pseudorandomized trials. Voice was recorded and subsequently played back to the subjects for comparisons between auditory and motor activity. Fifteen surgical epilepsy patients performed the task while we recorded local field potentials from multicontact intracranial electrodes implanted for clinical purposes. All subjects exhibited some degree of speech disruption in trials with delayed auditory feedback. Moreover, significantly more utterances were rated as abnormal in trials in which feedback was disrupted. The abnormalities more frequently identified in these trials were utterance prolongation and a change in speech rate. Consistently across patients, the perturbation in auditory feedback induced modulation of high gamma power (HGP, 70-150 Hz) and ERPs (filter <30 Hz) in the posterior superior temporal gyrus (STG) during vocal production. Other high-order auditory (MTG, SCG) and prefrontal also showed modulation by the disrupted feedback. During vocal production, ERPs were more rapidly and specifically modulated by disrupted feedback than HGP. Both HGP and ERPs were also modulated in these areas when subjects listened to their delayed vocalizations. However, during listening, HGP exhibited a larger and faster modulation by the disruption than ERPs. Indeed, ERPs were minimally modulated in non-disrupted trials and while listening disrupted utterances, but exhibit a strong response in the speaking phase of disrupted trials. A suppression of beta power (12-20 Hz) seems to drive this effect, which is specific of vocal motor control. These preliminary results show how a sudden and short delay in auditory feedback affects speech production and reveal the neural mechanisms of speech control in the human brain.

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Poster

575. Auditory Processing: Perception, Cognition, and Action II

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Topic: D.06. Auditory & Vestibular Systems

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Title: Alpha oscillations index the temporal dynamics of exerted cognitive effort during listening

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Abstract: The ability to exert cognitive effort during listening is crucial for speech perception in the presence of background sound, and may be an important factor that determines listening success in older people with hearing difficulties. Neural alpha oscillations in parietal cortex may provide a promising index for the assessment of effort as its power increases when individuals listen for subtle acoustic changes in sounds. It is, however, less clear how alpha oscillations are modulated by prior knowledge about when in time cognitive effort must be exerted during listening. In electroencephalography (EEG) and magnetoencephalography (MEG) experiments (N>100), we investigated how alpha power in a gap-detection task is affected by knowledge about when a near-threshold gap occurs within 10-s white noise sounds. Within one recording block, the gap occurred either within the first or the second half of the sound, and participants (21-33 years) were informed prior to each block. The precise position of the gap within the specified half was unknown to participants. Reaction times indicate that participants shifted their attention to either the first or second half of the sound, depending on the anticipated gap occurrence. EEG data showed a peak in alpha power at parietal electrodes either within the first or the last 5 seconds after sound onset, depending on whether participants were instructed to focus on the first versus second half of the sound. When detecting supra-threshold gaps, reaction times again indicated that participants shifted their attention to either the first or second half of the sound, but alpha power was less sensitive to this manipulation. These results suggest that investment of effort is needed to modulate alpha power. MEG data show that alpha power in parietal cortex was sensitive to the manipulation of gap occurrence (first vs. second half), but that alpha-power in auditory cortex remained enhanced throughout sound presentation (relative to baseline), independent of gap occurrence. MEG data from older people (54-72 years) show similar patterns of brain activity, but also subtle differences. The data show that alpha oscillations in parietal cortex are sensitive to when in time cognitive effort is exerted during listening.

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Poster

575. Auditory Processing: Perception, Cognition, and Action II

Location: SDCC Halls B-H

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Program #/Poster #: 575.06/BB10

Topic: D.06. Auditory & Vestibular Systems

Support: U.S. Army Research Office Grants W911NF-17-1-0532 and W911NF-14-1-0491
David M. Rubenstein Fund for Hearing Research

Title: Cross-frequency coupling in human auditory cortex measured by complex modulation

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[1]Neurol., [2]Johns Hopkins Sch. of Med., Baltimore, MD; [3]Physics, Univ. of Warsaw, Warsaw, Poland; [4]Human Res. and Engin. Directorate, US Army Res. Lab., Aberdeen Proving Ground, MD

Abstract: Human electrophysiology studies have demonstrated that interactions between low-frequency and high-frequency cortical oscillations are important for sensory processing, including auditory perception. Prior studies in humans have focused mainly on phase-amplitude coupling (PAC) between cortical frequencies, leaving other potential cross-frequency interactions, such as power coupling, largely unexplored. Here we implemented a novel method, complex modulation, to measure stimulus-related changes in power coupling between theta (4-7 Hz) and high-gamma (70-150 Hz) frequency bands. We analyzed electrocorticographic (ECoG) recordings to tone and speech stimuli from three right-handed epilepsy patients (ages 24-56 years; 3 female) who had subdural electrode arrays implanted over lateral left temporal cortex for clinical purposes of seizure localization. The ECoG time-series was frequency-shifted by complex demodulation transform. Cross-frequency power coupling was then quantified using linear coherence measures. To compare results with established phase-amplitude coupling measures, we computed the phase-locking values of theta phase and high gamma amplitude from the same ECoG recordings. Results from the complex modulation method showed significant increases in cross-frequency power coupling (Wilcoxon sign test) at electrode sites in auditory responsive cortex for tones (N=5) and speech (N=11), comprising a subset of sites that showed significant increases in PAC (tones: N=10; speech: N=12). These results suggest that cross-frequency interactions during auditory perception are not limited solely to changes in phase-amplitude coupling, but also involve changes in power coupling. These findings underscore the complexity of cortical frequency interactions as well as the potential utility of the complex modulation method.

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Poster

575. Auditory Processing: Perception, Cognition, and Action II

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Program #/Poster #: 575.07/BB11

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC016353

Title: Neural oscillations predict stuttering disfluency on a single trial basis

Authors: *J. MYERS, J. MOCK, E. GOLOB
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Abstract: Fluent speech requires precise coordination between sensory processing and motor planning areas in the brain. Impaired communication between sensory and motor areas may underlie stuttering disfluency. Most stuttering events occur at the beginning of an utterance, so, in principle, the state of the speech network before speaking should differ between fluent and stuttered speech. Here we provide evidence that neural oscillations during speech preparation predict stuttering on a single trial basis. Brain activity was recorded with EEG in people who stutter (n = 3) in two sessions on separate days. Subjects read aloud pseudo-word pairs during 4 different 'Cue-Go' behavioral tasks (n=100 trials/task/session) and each trial was classified as either a fluent or stutter trial by a speech-language pathologist. Independent component analysis (ICA) identified neural sources underlying speech preparation. For each neural source, a custom algorithm extracted event-related spectral perturbation and neural coherence (i.e., phase synchrony between sources) data from the time window of maximum activation. During the speech preparation phase of stuttering trials, we observed abnormal coherence between independent components localized to speech/motor planning (e.g., inferior frontal gyrus) and sensory regions (e.g., auditory cortex) ($p < 0.001$). In all three subjects, a discriminant function fitted to the spectral and coherence data predicted fluent vs. stuttered speech on > 80% of the trials. These results support the feasibility of developing a brain-computer interface (BCI) system to detect stuttering before it occurs, with potential for therapeutic application.

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Poster

575. Auditory Processing: Perception, Cognition, and Action II

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Topic: D.06. Auditory & Vestibular Systems

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Title: Neural substrates of auditory rhythm processing and language skill in early-to-mid adolescence

Authors: *M. GRUBE[1,2], F. SMITH[2], S. KUMAR[2], H. SLATER[2], T. D. GRIFFITHS[2]
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Abstract: This study seeks the neural substrates of auditory-sequence processing skills in the adolescent brain by seeking correlation between grey matter density and a systematic battery of tests to measure timing and rhythm skill. The study is part of a large initiative at a local high school where we carry out extensive behavioral testing to assess auditory skills and literacy skills on whole-year group cohorts (see PMID 22951739 for initial report). This study was carried out

on subgroups form the larger study. Previous work in 42 twelve-to-fourteen year-olds suggested a correlation between grey matter density in the left intra-parietal sulcus (IPS) and the first principal components of both auditory and language skills (Grube et al., Society for Neuroscience 2011: XX27 509.10). We also found a correlation between rhythm processing and grey matter density in the right cerebellum. The current work assesses rhythm and language skill and their corresponding structural correlates of in two separate, new cohorts of mean age 12 (n = 20) and 14 (n = 24), respectively. Structural MRI was carried out at 3T on a Phillips xxx scanner, and voxel-based morphometry (VBM) implemented in SPM 8 sought correlation between grey matter density and auditory and language skill, taking non-verbal intelligence into account. We test the hypotheses: i) there is a critical correlation between left IPS grey matter density and both sound sequence and literacy skill; ii) there is a correlation between cerebellar grey matter density and skill in rhythmic perception; iii) the alteration in the behavioral link between auditory sequencing and language skill from early to mid-adolescence that we have observed (Grube et al., Society for Neuroscience 2016: HHH3 85.07) is reflected in altered correlations between grey matter density and the sequence and rhythm measures.

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Poster

575. Auditory Processing: Perception, Cognition, and Action II

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Title: Dissociating task acquisition from expression during learning reveals latent knowledge

Authors: *T. HINDMARSH STEN[1], K. KUCHIBHOTLA[2], E. PAPADOYANNIS[3], R. KUMAR[4], Y. BOUBENEC[4], P. C. HOLLAND[2], S. OSTOJIC[4], R. C. FROEMKE[5]
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Baltimore, MD; [3]Skirball Inst. of Biomolecular Med., New York Univ. Sch. of Med., New York, NY; [4]Ecole Normale Superieure, Paris, France; [5]Otolaryngology, NYU Med., New York, NY

Abstract: Performance on cognitive tasks during learning is often used to measure progress and intelligence, yet remains controversial since such testing is susceptible to contextual factors. To what extent does performance depend on the testing context, rather than underlying knowledge? Here, we report that acquisition and expression of task knowledge can be dissociated during learning by manipulating the testing context. We trained head-fixed mice to discriminate between a "target" tone to which the animal was trained to lick for a water reward provided through a licktube, and an unrewarded "foil" tone to which the animal was trained to withhold licking (Kuchibhotla et al., Nat Neurosci 2017). To examine how testing context impacts acquisition versus expression, we interleaved the reinforced context with a smaller number of trials without reinforcement by removing the licktube ("probe context"). Surprisingly, in probe trials, all mice (n=14) discriminated between the tones much earlier in learning than in reinforced trials (trials to expert: reinforced=4728±647; probe=1765±108; n=7, t(6)=4.359, p=0.0055). Moreover, the inter-animal variability in learning curves was strikingly reduced in the probe context showing that the underlying acquisition of sensorimotor associations is highly stereotyped across mice. These results generalized to other species (rats and ferrets), motor action (licking and lever press), animal restraint (head-fixed and freely moving), sensory modality, and task structure. A computational model that explicitly dissociates between reward-driven plasticity of sensorimotor projections (representing task knowledge) and expression modulated by context in a decision circuit parsimoniously captured all aspects of these observations. These results suggest that reinforcement is critical for learning but paradoxically masks underlying task acquisition. Probing behavior in the absence of reinforcement, therefore, uncovers latent knowledge and identifies testing context, rather than sensorimotor abilities, as the critical driver of individual variability.

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Poster

575. Auditory Processing: Perception, Cognition, and Action II

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Title: Gender differences in DPOAE in mice

Authors: *T. MAKISHIMA, T. SUZUKI, J. MARUYAMA, S. PAESSLER
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Abstract: Mice have become the model animal of choice for studying the inner ear auditory and vestibular system due to its ease of genetic manipulations. Auditory testing in mice have been extensively done using methods such as auditory brainstem response (ABR), otoacoustic emission (OAE), or acoustic startle reflex (ASR). Although there is a large amount of reports using these methods in association with different auditory function altering conditions, not much has been reported on gender differences. Most studies have traditionally not taken into account gender differences, which in recent years have been identified as a major factor affecting outcome in most studies. Our goal was to determine whether there were gender differences in DPOAE in several different mice strains used as infectious disease model mice. We tested wild type C57BL6J mice (n=3 each males and females), Stat1 mice (n=5 each males and females), Stat1 wt mice (n=5 each males and females) and IFNa/bg mice (n=3 each males and females). The mice were age 6 weeks to 14 weeks. We performed ABR with 8, 16, 24 and 32kHz tone pip and click stimulus. We tested DPOAE with F2 value of 8kHz - 16kHz. Weekly recordings were done and results of males and females were compared. We observed significant difference in ABR and DPOAE results between males and females in the Stat1 mice ($p<0.05$), but not in the other mice tested. We conclude that there is a gender difference in auditory function in mice frequently used in infectious disease models. Therefore, the results of any auditory test must be interpreted with caution, and needs to account for gender differences at the planning stage of experiments to study this effect.

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Poster

575. Auditory Processing: Perception, Cognition, and Action II

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH K99DC015014

Title: Sensory-evoked cholinergic dynamics in auditory cortex during sensorimotor learning

Authors: *K. KUCHIBHOTLA[1], T. DESBORDES[2], S. OSTOJIC[2]
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Abstract: The basal forebrain cholinergic projection system modulates behavioral state and signals reinforcement feedback in fully trained animals. To date, however, the sensory-evoked

dynamics of cholinergic projections are unknown and whether and how these representations change during learning has never been recorded. Here we use two-photon calcium imaging in head-fixed behaving mice to monitor the activity of cholinergic axons in auditory cortex in response to conditioned auditory stimuli during learning. We find that cholinergic axons in naïve mice exhibit robust, broadly tuned, and spatially homogeneous sound-evoked responses. We next trained mice to lick for a water reward in response to a "target" tone and withhold licking to an unrewarded "foil" tone. We interleaved the reinforced context with a smaller number of trials without reinforcement by removing the licktube ("probe context"). Surprisingly, in the probe context, mice discriminated correctly between the tones far earlier than in reinforced context pointing to two distinct timescales of learning: rapid acquisition and slower expression. We then monitored cholinergic dynamics daily during learning. Remarkably, the population-level representation by cholinergic axons rapidly discriminated between target and foil tones on the fast learning timescale of sensorimotor acquisition with individual axons exhibiting selectivity for the conditioned stimuli. This simple behavioral dissociation points to a novel role for phasic, sensory-evoked cholinergic signaling during sensorimotor acquisition. Moreover, these results suggest that cholinergic projections may play a critical role in opening up a plasticity window in sensory circuits during real-time learning.

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Poster

575. Auditory Processing: Perception, Cognition, and Action II

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Program #/Poster #: 575.12/BB16

Topic: D.06. Auditory & Vestibular Systems

Title: Understanding word representations in the brain using ECoG

Authors: S. RAHIMPOUR[1], M. M. HAGLUND[1], S. R. SINHA[2], C. R. MUH[1], *G. B. COGAN[1]
[1]Dept. of Neurosurg., [2]Dept. of Neurol., Duke Univ., Durham, NC

Abstract: Understanding speech forms the basis for complex language functioning in the human brain. In milliseconds, the brain effortlessly transforms sound into words. The first contact that sound has with cognition is through words: words, but not sound, interface with representations of meaning. We still do not however, understand how sounds are transformed into words nor how words are processed in the brain. Here, therefore, we studied word representations in the brain using 5 patients undergoing phase II monitoring for pharmacologically intractable epilepsy (4 sEEG, 1 Grid - 515 electrodes total). We had subjects perform a task in which there were 2 conditions: a lexical decision condition in which after a short delay, subjects were asked to state whether the auditory stimulus was a word or not (yes or no) and a repetition condition in which

subjects were to repeat the word or nonword after a delay. In both conditions, we presented subjects with 41 words and 41 nonwords that also varied in their sublexical properties (phonotactic probability/neighborhood size - high vs. low). We first assessed significance of the high-gamma neural responses using a permutation test with the baseline period for each electrode (189/515 electrodes - 37%). We then used the significant electrodes in a linear model with lexicality (words vs. nonwords), phonotactic probability/neighborhood size (high vs. low), and task (decision vs. repeat) as predictor variables. We find that 59 electrodes demonstrate a significant effect of lexicality, with the majority (45 - 76%) showing greater high-gamma power for words as compared to nonwords. We find that 52 electrodes demonstrate a main effect of sublexicality, with the majority demonstrating greater high-gamma power for low phonotactic probability/low neighborhood size (41 - 79%). Finally, wefind89 electrodes demonstrating a significant effect of task with a majority demonstrating higher power for decision (67 - 75%) as compared to repetition. An analysis of the time course of the beta weights for each factor reveals that the processing of sublexical properties arises first (100 ms) while lexical processing and task processing occur later (~400ms). Taken together, these results suggest that word representation is a sequential process involving sublexical, lexical, and task representations.

Disclosures: S. Rahimpour: None. **M.M. Haglund:** None. **S.R. Sinha:** None. **C.R. Muh:** None. **G.B. Cogan:** None.

Poster

575. Auditory Processing: Perception, Cognition, and Action II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 575.13/BB17

Topic: D.06. Auditory & Vestibular Systems

Title: The neural processing of phonemes is shaped by linguistic analysis

Authors: *J. C. LEE, T. OVERATH
Duke Univ., Durham, NC

Abstract: Speech perception entails the mapping of the acoustic waveform to stored linguistic representations, such as phonemes, syllables, or words. Recent evidence suggests that different phoneme classes (e.g. plosives, fricatives, etc.) have characteristic neural signatures, or phoneme-related potentials (PRPs; Khalighinejad et al., 2017). What remains to be understood is the extent to which the temporal scale of linguistic analysis, and linguistic knowledge, influence the processing of this fundamental linguistic unit.
To control the scale of linguistic analysis, we used a modification of our speech quilting algorithm (Overath et al., 2015) to generate stimuli that maintain linguistic structure at one of 4 linguistic units: phoneme, syllable, word or sentence; to control for linguistic knowledge, we constructed speech quilts from both familiar (English) and foreign (Korean) languages.

We recorded EEG from 28 native English speakers (with no knowledge of Korean); data were epoched to the phoneme onset boundary. Grand average PRPs across all phonemes showed a similar sequence of P50, N100, and P200 components at fronto-central regions. Comparisons between linguistic-unit levels (phoneme, syllable, word, sentence), showed significant differences ($p < 0.05$) at time points corresponding to the P50 and N100 components for English, but not for Korean. In addition, the N100 component showed a main effect of language and an interaction between language and linguistic unit. The classification of phonemes based on articulatory manner revealed unique PRPs for each of these classes, and forms were similar between the two languages. The similarity in articulatory-class responses across languages suggests that acoustic features play an important role in the processing of phonemes. However, the main effect of language and the interaction with linguistic unit suggest that the processing of a fundamental linguistic unit, the phoneme, is already shaped by linguistic analysis as early as 100 ms after phoneme onset.

References:
Khalighinejad et al. (2017), J Neurosci 37: 2176-2185.
Overath et al. (2015), Nat Neurosci 18: 903-911.

Disclosures: J.C. Lee: None. **T. Overath:** None.

Poster

575. Auditory Processing: Perception, Cognition, and Action II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 575.14/CC1

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC04290
UL1RR024979
Hoover Fund

Title: Electrocorticographic (ECoG) analysis of dialog-based paradigms for assessing speech, language and cognitive functions: A case report

Authors: *M. STEINSCHNEIDER[1], K. V. NOURSKI[2]
[1]Neurol., Albert Einstein Col. of Med., Bronx, NY; [2]The Univ. of Iowa, Iowa City, IA

Abstract: Acquisition of ECoG data while subject/patients engage in a dialog-based study permits high resolution analysis of multiple speech, language and cognitive functions in a concise question/answer format. Analysis focuses on neural processing associated with listening, performing mental calculations, and verbal responses. Here, we demonstrate the utility of this paradigm by presenting data from a single neurosurgical subject with normal hearing and cognitive functions, and who had extensive right hemisphere electrode coverage of temporal,

temporo-parietal, and frontal cortices. The paradigm was an expanded version of the Mini-mental State Examination, which included additional spelling, naming, and memory-based tasks. Recording of the verbal exchange was parsed using Praat software based upon natural articulatory breaks in the conversation. Cortical recording sites were categorized based upon their location, within anatomically defined regions of interest (ROIs), including e.g., Heschl's gyrus, posterior, middle and anterior portions of the lateral superior, middle and inferior temporal gyri (STG, MTG). A key analysis was the degree of high gamma (70-150 Hz) activation during listening to the interviewer vs. during one's own speech (Nourski, Steinschneider, Rhone, Front Hum Neurosci 2016 10:202). ROIs where listening was associated with stronger high gamma activity than speaking were restricted to the posterior and middle portions of the STG. ROIs where speaking was associated with stronger high gamma activity than listening included Heschl's gyrus, planum polare, anterior STG, middle/anterior MTG, ITG, supramarginal gyrus and the temporal pole. A second analysis examined the relationship between high gamma and activity in lower ECoG frequency bands. In all ROIs, there was a positive correlation between high and low gamma (30-70 Hz). Relationships between high gamma and alpha (8-14 Hz) and theta (4-8 Hz) bands were inconsistent across ROIs. Largest high gamma responses were generally associated with relatively difficult tasks, naming favorite items and task completion. We conclude that monitoring one's own speech can extend beyond the classically defined dorsal auditory-motor speech pathway into the ventral pathway involved in listening and decoding speech at progressively higher processing levels. A consistent relationship between high and low gamma activity supports the utility of low gamma acquired in non-invasive studies as a proxy for high gamma activity. Comparing neural activity across subjects may assist in defining the natural variability in language and cognitive processing strategies utilized by individuals.

Disclosures: K.V. Nourski: None.

Poster

575. Auditory Processing: Perception, Cognition, and Action II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 575.15/CC2

Topic: D.06. Auditory & Vestibular Systems

Support: US ARMY MRAA W81XWH-13-1-0494

Title: Resting-state functional imaging of chronic tinnitus

Authors: *L. B. HINKLEY[1], A. FINDLAY[2], D. MIZUIRI[2], S. W. CHEUNG[4], S. S. NAGARAJAN[3]
[1]Radiology, UC San Francisco, San Francisco, CA; [3]Radiology and Biomed. Imaging, [2]UCSF, San Francisco, CA; [4]Otolaryngol-Head & Neck Surg, UCSF Otolaryngology, San Francisco, CA

Abstract: In chronic tinnitus, both intraoperative electrical stimulation and resting-state functional connectivity studies have shown that the caudate nucleus maintains an abnormal relationship with auditory cortex, possibly by acting as a dysfunctional gating mechanism to modulate auditory phantom perception. The specific sub-regions of the caudate that act as this gating mechanism have yet to be defined. Here, we use high-resolution resting-state functional MRI (rs-fcMRI) at both 3T and 7T in patients with tinnitus and/or hearing loss. We hypothesize that abnormal functional connectivity within specific sub-regions of the basal ganglia and the central auditory system will be unique to patients with tinnitus. MRI was performed on a 3.0T or 7.0T MR950 scanner (GE Healthcare) in the same group of subjects. Spontaneous fMRI data (eyes closed) were collected using a gradient echo planar pulse sequence for both 3T and 7T data acquisition. Resting-state fMRI data was spatially preprocessed and analyzed using the CONN toolbox (https://www.nitrc.org/projects/conn/). Seeds were placed in nine specific predefined subdivisions of the caudate. Data from four cohorts were enrolled: 28 patients with tinnitus plus hearing loss (TIN+HL), 12 patients with hearing loss and no tinnitus (HL), 14 patients with tinnitus and no hearing loss (TIN) and 8 healthy controls with no hearing loss or tinnitus (CON). Comparisons were made between groups using unpaired t-tests for each seed implemented in CONN. Nine seeds were placed in both the left and right subdivisions of the caudate. For comparisons between the TIN+HL and HL cohort, only 2/7 subdivisions of the caudate showed significant ($p<0.0005$) increases in functional connectivity isolated to sub-regions of auditory cortex. Both regions fell within the dorsal aspect of the caudate head and anterior body. Both seeds showed increased connectivity with the ipsilateral posterior middle temporal gyrus. Dorsal/posterior subdivisions of the caudate did not show any increases in functional connectivity in the TIN+HL group. Decreased resting-state functional connectivity in the TIN+HL group were identifiable and fell outside of primary or secondary auditory regions. These findings support a growing body of evidence that suggest the basal ganglia is integral to the perception of auditory phantoms. More specifically, increased patterns of resting-state functional connectivity are not found across the caudate, but only within specified subregions. A greater understanding of how specific sub-regions of the caudate are attached to auditory perceptual regions in tinnitus can lead to more targeted strategies for intervention, such as DBS.

Disclosures: L.B. Hinkley: None. **A. Findlay:** None. **D. Mizuiri:** None. **S.W. Cheung:** None. **S.S. Nagarajan:** None.

Poster

575. Auditory Processing: Perception, Cognition, and Action II

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Program #/Poster #: 575.16/CC3

Topic: D.06. Auditory & Vestibular Systems

Support: GACR 16-16729F

Title: Changes in the grey and white matters in the human auditory system due to presbycusis and tinnitus

Authors: *O. PROFANT[1,2], J. SYKA[1], A. SKOCH[3], J. TINTERA[3], V. SVOBODOVA[4], D. KUCHAROVA[4]
[1]Dept. of Auditory Neurosci., Inst. of Exptl. Medicine, Acad. of Sci., Praha, Czech Republic; [2]Otorhinolaryngology, 3rd Fac. of Med. of Charles University, Fac. Hosp. Kralovske Vinohrady, Prague, Czech Republic; [3]MR Unit, Inst. of Clin. and Exptl. Med., Prague, Czech Republic; [4]Otorhinolaryngology and Head and Neck Surgery, 1st Fac. of Med. of Charles University, Univ. Hosp. Motol, Prague, Czech Republic

Abstract: Presbycusis and tinnitus are two of the most common hearing related pathologies. Although both presumably originate in the inner ear, there are several reports about their central components. Central pathologies caused by presbycusis are associated with degraded ability to detect fast temporal changes, related in case of tinnitus to increased spontaneous activity at several levels of the auditory system and in both pathologies to hypofunction of the inhibitory system.
The aim of our project is to identify age, hearing loss and tinnitus related changes within the auditory system and associated structures.
A group of patients with presbycusis and tinnitus (40 subjects), group with presbycusis only (28 subjects) and a group of young controls (19 subjects) underwent audiological examination to characterize the degree of presbycusis and tinnitus. MR morphometry and tractography were acquired using a 3 T Siemens Tim Trio system (Siemens), with a 12-channel head coil. For MR morphometry cortical reconstruction and volumometric segmentation were performed with the aim to evaluate surface and thickness of the grey matter. DWI acquisition of the pathway from the inferior colliculus to auditory cortex was performed by spin-echo EPI sequence. A statistical analysis of fiber density (FD), fiber cross-section (FC) and fiber density and cross-section (FDC) was performed by fixel-based analysis framework (FBA) using mrtrix3 framework. Statistical analysis using R framework was done by linear mixed-effects models with explanatory variables (fixed effects) age, tinnitus, laterality, hearing and random subject-wise intercept.
Significant decrease of cortical thickness occurred in all examined cortical regions (planum temporale, Heschl gyrus (HG), anterior insula, parahipocampal gyrus (PH), primary visual cortex (V1)) as a result of aging. The surface area was significantly affected by laterality (left vs. right hemisphere) in the PH and HG. Tinnitus caused increase in cortical thickness of V1 and PH, however the significance of the tinnitus effect didn't survive the multiple comparisons correction. The analysis of the auditory pathway showed only significant effect of ageing in all three variables (decrease of FD, FC and FDC), whereas hearing loss and tinnitus had no effect. We can conclude that tinnitus and hearing loss have only marginal effect on the structural parameters of the human auditory cortex and pathway from the inferior colliculus to auditory cortex compared to the effect of ageing. Our data also show different effect of ageing on examined cortical regions with a more pronounced decrease of the cortical thickness in the more frontal regions.

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Poster

575. Auditory Processing: Perception, Cognition, and Action II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 575.17/CC4

Topic: D.06. Auditory & Vestibular Systems

Support: NS104911
DC004263

Title: Anticipated ITD statistics built-in human sound localization

Authors: *J. L. PENA[1], R. PAVÃO[2], E. S. SUSSMAN[3], B. J. FISCHER[4]
[1]Neurosci., Albert Einstein Col. of Med., Bronx, NY; [2]Ctr. de Matemática, Computação e Cognição, Univ. Federal do ABC, Sao Paulo, Brazil; [3]Dept of Neurosci., Albert Einstein Col. of Med., Bronx, NY; [4]Seattle Univ., Seattle, WA

Abstract: The variability of natural scenes places perceptual processes in the realm of statistical inference. Perceptual tasks may be optimized if the invariant statistical structure of sensory cues is built into the neural processing. We investigated this question in human sound localization. Localizing sounds in the horizontal plane relies on interaural time differences (ITD). We estimated the ITD statistics from human head-related transfer functions (HRTFs). ITD varied with azimuth following a sigmoid relationship, whose slope was steepest at the center. In addition, ITD was more variable over time for sounds located in the periphery compared to the center, in a frequency-dependent manner. We tested the hypothesis that these statistics are anticipated by the human brain, influencing spatial discriminability and novelty detection. Thresholds for discriminating ITD changes reported by classical studies (Mills, 1958) were predicted by a model that considered both ITD slope and ITD variability. To further test our hypothesis, EEG novelty responses were recorded in human subjects undergoing an oddball stimulation sequence, where repetitive (“standard”) tones of a given ITD were combined with sporadic (“deviant”) tones of a different ITD. By using insert earphones, ITD was shifted with zero variability across time and location. Mismatch negativity (MMN) brain signals were used as an index of discriminability between standard and deviant stimuli. We found that MMNs were weaker for standards in the periphery, where the ITD slope is lower and the ITD variability is higher. Overall, the amplitude of novelty EEG signals was predicted by the difference in ITD between the standard and deviant normalized by the anticipated discriminability of the standard location, indicating that change detection is weighted by expected statistics of the sensory input. These results show that spatial discriminability thresholds and novelty detection are consistent

with a representation of anticipated ITD statistics in the brain, supporting the hypothesis that high-order statistics are built into human perceptual processes biasing behavior.

Disclosures: J.L. Pena: None. **R. Pavão:** None. **E.S. Sussman:** None. **B.J. Fischer:** None.

Poster

575. Auditory Processing: Perception, Cognition, and Action II

Location: SDCC Halls B-H

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Topic: D.06. Auditory & Vestibular Systems

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NSF Grant CRCNS-IIS-1515678
The Hoover Fund

Title: Electrocorticographic responses to vowel sequences in awake and anesthetized states

Authors: *K. V. NOURSKI[1], M. STEINSCHNEIDER[2], A. E. RHONE[1], R. N. MUELLER[1], H. KAWASAKI[1], M. A. HOWARD, III[1], M. I. BANKS[3]
[1]The Univ. of Iowa, Iowa City, IA; [2]Albert Einstein Col. of Med., Bronx, NY; [3]Univ. of Wisconsin - Madison, Madison, WI

Abstract: Elucidating neural signatures of sensory processing across conscious states is a major focus in neuroscience. Clinically relevant conditions of altered awareness include sedation, loss of consciousness (LOC) under general anesthesia, natural sleep and disorders of consciousness. Non-invasive studies in human subjects using the general anesthetic propofol to induce sedation and LOC have shown differential effects on auditory cortical activity, with a greater impact on non-primary and auditory-related areas than primary auditory cortex. High spatiotemporal resolution of electrocorticography can further extend results of non-invasive studies in delineating hierarchical organization of human auditory cortex (e.g., Nourski et al., NeuroImage 2017, 152:78-93). The present study examined changes in cortical responses to vowel sequences during sedation and LOC with propofol. Subjects were adult neurosurgical patients with intracranial electrodes placed to identify epileptic foci. Data were collected prior to electrode removal surgery. Stimuli were sequences of five 100 ms vowels separated by 50 ms silent intervals presented during an awake baseline state and during propofol administration. Subjects were asked to press a button in response to occasional target stimuli. Depth of anesthesia was monitored using the Observer's Assessment of Awareness Scale and bispectral index. Regions of interest included core and non-core auditory, temporo-parietal auditory-related and prefrontal cortex. Activity was measured as averaged evoked potentials (AEPs) and high gamma (70-150

Hz) event-related band power. Vowel stimuli elicited AEPs throughout studied brain areas in the awake state; high gamma activity was restricted to core and non-core auditory cortex. AEPs and high gamma activity within core auditory cortex persisted after LOC. In non-core auditory cortex, propofol administration led to a progressive decrease in the spatial extent and amplitude of AEPs, and increases in onset latency. The spatial extent of AEPs within auditory-related and prefrontal cortex progressively decreased with sedation, and responses were abolished upon LOC. Overall, sensitivity of cortical responses to propofol increased along the ascending cortical processing hierarchy. Loss of responses to sound in auditory-related and prefrontal cortex may represent a biomarker of general anesthesia. The findings serve as a foundation for probing changes in sensory processing associated with general anesthesia induced by other agents, as well as natural sleep and disorders of consciousness (e.g., chronic vegetative state and coma).

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Poster

575. Auditory Processing: Perception, Cognition, and Action II

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Ministry of Education, Culture, Sports, Science, and Technology (Challenging Research (Exploratory) (17K19450))
Ministry of Education, Culture, Sports, Science, and Technology (Grants-in-Aid for Scientific Research on Innovate Areas)
Ministry of Education, Culture, Sports, Science, and Technology (Grant-in-Aid for Scientific research (C) (15K07147))

Title: Experience-dependent tuning of song discrimination in *Drosophila*

Authors: *X. LI, H. ISHIMOTO, A. KAMIKOUCHI
Div. of Biol. Sci., Nagoya Univ., Nagoya, Japan

Abstract: The language skill of human beings is built on the genetic predisposition and further through postnatal learning. Like humans, many mammals and birds have a critical period in youth when hearing the vocal cues of their parents helps them to learn the specific features of their communication sounds. However, wherever in human beings or other animals, the mechanisms remain mysterious. Although the hearing study in *Drosophila* has attracted huge attention in recent years, little is known how auditory experience contributes to perception of

courtship song in fly mating and whether there is also a critical period for it, because for a long time most processes in courtship behavior of *Drosophila* are thought to be innate.>
In this study, we exposed flies to artificial courtship song before behavioral tests and examined whether the perception of inter-pulse interval in *Drosophila melanogaster* was tuned by the auditory experience. We discovered that both male and female fruit flies (*Drosophila melanogaster*) learned to discriminate the species-specific song from early auditory experience and tuned their mating preference in later sexual behaviors. Females who were raised with the auditory experience of their species-specific song later in life rejected conspecific males presented with artificial playback of another species' song. Similarly, males raised with the experience of hearing conspecific song later in life ignored another species' song, which usually increased the mating drive of naïve males. However, song discrimination of both male and female flies was not altered by exposure of another species' song. We further identified the mechanism of experience-dependent acquisition of the song discrimination. This experience-dependent song discrimination relied on GABA synthesis, and the ionotropic $GABA_A$ receptor in a small group of central neurons called pC1 neurons (command-like neurons in mating) gated this tuning. In addition, we found this auditory learning not only occurred in the early adult stage, but also extended to the mature adult stage, suggesting a long critical period for this acquision.>
Here our study gives the hint that simple animals like flies can also tune their perception of specific auditory feature by learning. Our discovery establishes a new and simple system to study how the experience-dependent auditory plasticity is incorporated into higher-order integration center to modulate sensory-motor behaviors at the molecular and cellular levels. A better understanding of how fruit flies learn and discriminate sounds may bridge knowledge gaps in research using humans and other animals.

Disclosures: H. Ishimoto: None. **A. Kamikouchi:** None.

Poster

575. Auditory Processing: Perception, Cognition, and Action II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 575.20/CC7

Topic: D.06. Auditory & Vestibular Systems

Support: NIDCD R03 DC014807

Title: How do neurons overcome developmental hearing loss induced deficits during auditory learning?

Authors: *T. M. MOWERY[1], N. PARAOUTY[2]
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Abstract: Developmental hearing loss leads to deficits in the auditory perceptual abilities of children and these arise from peripheral and central changes along the auditory neuraxis. When peripheral deficits are treated through resolution of ear canal blockage, surgery, hearing aids or cochlear implants, perceptual thresholds often return to control values. On many non-perceptual (cognitive) tasks, some individuals who have recovered from hearing loss (HL) perform at normal hearing (NH) levels while others remain impaired. This suggests that regions down-stream of the perceptual processing centers of the primary auditory neuraxis are sensitive to developmental HL. One such region, which is highly involved in language development, is the auditory striatum. We have recently reported that animals that have recovered from HL continue to show significant changes in cellular properties in the cortex and striatum. Thus we asked how these changes to cellular and synaptic properties affect the learning of an auditory task. We used the gerbil and a brain slice preparation to assess how changes to synaptic excitation, inhibition, and cellular firing rates correlate with behavioral performance in animals as they learn to discriminate two amplitude-modulated tones. We found that the synaptic and cellular changes that occur in NH and HL recovered animals are extremely polarized, but change in such a way that permits learning in both groups of animals. Delays to this learning-induced progression could account for the shallower learning curves observed in both NH and HL animals with impaired acquisition. Precocious onset of this progression could like-wise account for the steeper learning curves observed in animals with faster task acquisition. These results demonstrate a central learning mechanism that emerges during auditory learning, and reveals how the brain can overcome permanent cellular deficits induced by developmental deprivation.

Disclosures: T.M. Mowery: None. **N. Paraouty:** None.

Poster

576. Vestibular Physiology and Anatomy

Location: SDCC Halls B-H

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Program #/Poster #: 576.01/CC8

Topic: D.06. Auditory & Vestibular Systems

Support: Grant-in-Aid for Scientific Research on Innovative Areas
The Takeda Science Foundation
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Kato Memorial Bioscience Foundation
The Nakatomi Foundation

Title: Central mechanism of thermoregulation via the vestibular system in mice

Authors: *C. ABE, Y. YAMAOKA, H. MORITA
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Abstract: The vestibular system is one of the sensory systems which contributes to the sense of balance and spatial orientation. The vestibular system is also participating in the autonomic nervous response, which stimulation to the peripheral vestibular organs induces sympathoexcitation. This response is observed in both rodents and human beings, and we have reported that vestibular system contributes to the arterial pressure response during postural change as one of the feedforward control system (Morita, Abe et al., Sci Rep, 2016). In the other autonomic nervous responses, there is a hypothermic response induced by stimulation to the otolith organs in the inner ear; exposure to the hypergravity environment decreases body temperature by 8 degree Celsius in mice. This response was attenuated by vestibular lesion or genetic deletion of otolith. In order to elucidate the central mechanism of hypergravity-induced hypothermia, we examined the role of Vglut2, Vgat and ChAT positive neurons in vestibular nucleus complex (VNC) on thermoregulation in mice. We used Vglut2-cre, Vgat-cre, and ChAT-cre mice to manipulate each neuron in VNC. AAV-DIO type viral vector was injected in VNC to express photo sensors (channelrhodopsin (ChR2) or archaerhodopsin (Arch)) for optogenetics or hM3D for chemogenetics, which the methods were modified from the previous work (Abe et al., Nat Neurosci, 2017). Unilateral photostimulation of the Vglut2 neurons in VNC induced body tilt to the ipsilateral side, while photoinhibition induced body tilt to the contralateral side. In Vgat-cre mice, opposite response was observed compared with Vglut2-cre mouse. Photostimulation of ChAT neurons did not show any responses. Chemogenetics stimulation of Vglut2 neurons showed hypothermic response with increasing in activity, while Vgat stimulation increased body temperature with decreasing in activity. Deletion of Vglut2 neurons in VNC using AAV2-DIO-taCasp3-TEVp attenuated hypothermia induced by hypergravity exposure. On the other hand, hypothermia was still observed by deletion of Vgat neurons in VNC. Interestingly, chemogenetics stimulation of Vglut2 neurons in VNC 2 days before hypergravity exposure, the hypothermia was attenuated. Taken together, hypothermic response by hypergravity exposure is due to activation of Vglut2 positive neurons in VNC.

Disclosures: C. Abe: None. **Y. Yamaoka:** None. **H. Morita:** None.

Poster

576. Vestibular Physiology and Anatomy

Location: SDCC Halls B-H

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Program #/Poster #: 576.02/CC9

Topic: D.06. Auditory & Vestibular Systems

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

Title: Peripheral mechanism of thermoregulation via the vestibular system in mice

Authors: *Y. YAMAOKA, C. ABE, H. MORITA
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Abstract: The vestibular system is one of the sensory systems to sense the gravity and rotation. Eye movement (vestibulo-ocular reflex) and posture maintaining (vestibulo-spinal reflex) are well known to be controlled via the vestibular system. Autonomic nervous system is also partially dominated by the vestibular system, i.e., stimulation to the peripheral vestibular organs induces sympathoexcitation. This reflex works as a feedforward control system for arterial pressure response in rodents and human beings (Morita et al., Sci Rep, 2016, Abe et al., J Appl Physiol, 2011). Interestingly, the vestibular system participates in the thermoregulation; exposure to the hypergravity environment decreased body temperature (BT) in mice (Fuller et al., PNAS, 2002). This response was attenuated by genetic deletion of the peripheral vestibular organ, suggesting that otolith is important in the afferents for hypothermic response. However, the mechanism of the efferents in this response is still unclear. To examine this, we conducted the experiments focusing on increase in heat loss and/or decrease in heat production during exposure to hypergravity in mice. We used two groups of mice; vestibular lesion (VL) and their sham-operated (Sham) mice. We measured skin temperature using a thermography camera as evaluation for heat loss and temperature of brown adipose tissue (BAT) as evaluation for heat production. Sham mice showed decrease in BT (-6.5 ± 0.5°C), and this response was attenuated by VL (-3.2 ± 0.3°C). Although increase in tail temperature was observed, tail sympathetic denervation did not improve the hypothermic response, suggesting that hypothermia is not due to increase in heat loss. On the other hand, pretreatment with isoprenaline (10 mg/kg i.p.), nonselective β agonist, significantly attenuated the hypothermic response (-1.8 ± 0.5°C). Furthermore, decrease in sympathetic tone seems to be involved in hypergravity-induced hypothermia because hexamethonium administration decreases BT (-5.0 ± 0.4°C) with increase in tail temperature and decrease in BAT temperature. Accordingly, it is possible that hypergravity-induced hypothermia is due to decrease in heat production through the sympathetic nervous system, probably hypometabolism including BAT might be occurred in 2 G.

Disclosures: Y. Yamaoka: None. **C. Abe:** None. **H. Morita:** None.

Poster

576. Vestibular Physiology and Anatomy

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH R00DC012536
McKnight Foundation Scholar
Pew Foundation Scholar
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Title: Anatomical and functional convergence of otolith afferents onto vestibulospinal neurons in larval zebrafish

Authors: *Z. LIU[1], J. ELSNER[1], Y. KIMURA[3], S.-I. HIGASHIJIMA[4], D. G. HILDEBRAND[5], J. L. MORGAN[2], M. W. BAGNALL[1]
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Abstract: Vestibulospinal (VS) neurons influence posture by transforming sensory inputs from vestibular afferents into motor outputs. VS neurons display more complex spatiotemporal tuning than their afferents, suggesting sensory convergence is important for computing head motion signals. However, it has not been possible to directly measure this convergence, limiting our understanding of central vestibular computations. We addressed this question in larval zebrafish, first demonstrating that bilateral ablation of VS neurons resulted in severe deficits of body balance. Therefore, VS neurons serve a postural function in fish as in other vertebrates. Next we developed a technique to intracellularly record sensory responses of VS neurons *in vivo* on a moving table. Recordings from VS neurons in voltage clamp revealed that synaptic transmission from vestibular afferents is mediated by mixed synapses with both chemical (AMPA) and electrical components. The electrical component is usually larger, producing a stereotyped EPSC waveform for each afferent. Therefore we can distinguish inputs from distinct afferents converging onto one VS neuron. Each VS neuron usually received inputs from 2-3 potential afferents. During application of a translational sinusoidal stimulus, EPSCs from vestibular afferents exhibited tuning to specific phases of linear acceleration. EPSCs from most afferents are phase-led relative to peak acceleration, which indicates they encode a mixture of jerk and acceleration, similar to mammalian otolith afferents. Afferent convergence exhibited a range of properties: in some cases, afferents with highly similar tuning converge on one VS neuron; in other cases, directional tuning varied across afferents. Similar results were seen in current clamp measurements of VS membrane potential and firing rate. Serial electron microscopy of vestibular afferents confirmed that on average 3 afferents, out of 17 total, converge onto each VS neuron. By combining EM and electrophysiology, we can reveal a complete map of both connectivity and function from afferents to central vestibular neurons.

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Poster

576. Vestibular Physiology and Anatomy

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Program #/Poster #: 576.04/CC11

Topic: D.06. Auditory & Vestibular Systems

Support: CIHR MOP-93548
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CFI
CIHR fellowship to CM

Title: Spatial tuning for translation in the posterior cerebellar vermis across changes in head-re-body and body-re-world orientation

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Abstract: Many daily tasks (e.g., postural control, reaching, navigation) rely on estimates of body motion with respect to specific body axes (body-centered estimates) and/or with respect to gravity (world-centered). Our vestibular sensors are among the most important sources of self-motion signals. However, they encode motion in head-centered coordinates. To contribute to such tasks, vestibular signals must thus be transformed into body- and world-centered reference frames. Recently, we showed that neurons in the rostral fastigial nucleus (rFN) encode the spatially transformed vestibular signals in 3D required to compute estimates of body motion (Martin et al., 2018). Furthermore, while individual rFN cells reflect a distribution of reference frames, specific aspects of their tuning properties suggest that the rFN reflects a late or "output" stage of the head-to-body reference frame transformation, with the bulk of the computations likely occurring upstream in the cerebellar cortex. Most rFN cells also preferentially encode translation as compared to tilt. This suggests a potentially important role in the head-to-body transformation for regions of the posterior cerebellar vermis (nodulus/uvula, NU, lobules 9 and 10) which have been implicated in computing translation estimates from ambiguous sensory vestibular signals (Yakusheva et al., 2007; Laurens et al., 2013). The goal of this study was to investigate the reference frames in which such translation estimates in the NU are encoded. We recorded NU Purkinje cells in a rhesus monkey during translational motion (0.5 Hz, +/-9 cm) delivered along 13 directions in 3D space. Cell tuning was characterized with the head and body upright and after static reorientation of the head relative to the body in the vertical plane (toward nose- or ear-down) and horizontal plane (leftward). In addition, we characterised the spatial tuning of a subset of cells after static reorientation of the body relative to gravity and after combined head-re-body and body-re-gravity reorientations. The majority of NU cells recorded to date (84%) exhibited spatial tuning across head and body orientations that was consistent with a predominantly head-centered encoding of translation. Our present results are thus compatible with theoretical models (e.g., Green et al., 2004, 2007) proposing that the NU combines spatially-transformed canal signals with head-centered otolith signals to compute a head-centered representation of translation. In addition, they suggest that the bulk of the computations necessary to construct body-centered translation estimates occur elsewhere, likely within parts of the anterior vermis (Manzoni et al., 1999).

Disclosures: C. Martin: None. **J.X. Brooks:** None. **A.M. Green:** None.

Poster

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Topic: D.06. Auditory & Vestibular Systems

Support: CHR 241856
CHR 236330

Title: Vestibular neurons mediating the vestibulo-ocular reflex optimally encode natural self-motion through temporal whitening

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Abstract: Understanding how the brain encodes natural vestibular stimuli has become of great interest since prior studies have demonstrated that neural responses cannot be predicted from responses to artificial stimuli. It is generally accepted that sensory systems have adapted their coding strategies by matching their tuning properties to the statistics of natural stimuli, which results in a neural response that is independent of frequency (i.e., are temporally whitened). The vestibular system encodes head motion in a complex 6D trajectory (3 rotational and 3 translational) and mediates vestibulo-driven reflexes and spatial perception. While the statistics of natural self-motion have been measured recently, how neurons within the vestibular system respond to naturalistic self-motion has for the most part not been investigated to date. Here we investigated how Position Vestibular Pause neuron (PVP) and Floccular Target Neuron (FTN), vestibular neurons that mediate the vestibulo-ocular reflex VOR, respond to naturalistic self-motion. Because of the remarkable properties of the VOR, which requires knowledge about the detailed time course of the head motion, we expected that PVP and FTN neurons would preserve the statistics of natural stimuli in their spiking activities and thus not implement temporal whitening. In contrast, we found that both neural classes displayed temporally whitened responses to naturalistic self-motion. These responses could be well-predicted from the tuning properties to head velocity alone. Thus, our results demonstrate for the first time that both PVP and FTN neurons are tuned such as to optimize information transmission about the detailed time-course that is necessary in order to mediate the VOR.

Disclosures: I. Mackrous: None. **J. Carriot:** None. **K.E. Cullen:** None. **M. Chacron:** None.

Poster

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Program #/Poster #: 576.06/CC13

Topic: D.06. Auditory & Vestibular Systems

Support: CIHR Grant 241856
CIHR Grant 236330

Title: Thalamus coding strategies for representing natural self-motion

Authors: *J. CARRIOT[1], I. MACKROUS[3], G. MCALLISTER[2], H. HOOSHANGNEJAD[4], A. DALE[2], C. MCNICOLL[2], K. E. CULLEN[4], M. J. CHACRON[2]
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Abstract: Self-motion is sensed by the vestibular system, contributing to automatic reflexes and spatial perception. While it is generally accepted that sensory systems have adapted their coding strategies to the statistics of natural signals, how the vestibular system processes natural self-motion is largely unknown because artificial (e.g., sinusoidal) stimuli have been typically used to date. Natural stimuli frequently display complex spatiotemporal characteristics. It is commonly assumed that, through both evolutionary and developmental processes, sensory neurons are adapted to the statistical properties of the stimuli to which they are exposed. This has led to the proposal that sensory systems optimally process natural stimuli by removing redundancy which is commonly referred to as whitening as the neural response then contains equal power at all frequencies (i.e., is “white”). While we have shown that VN neurons optimally encode natural self-motion through temporal whitening, how this information is decoded remains poorly understood. Here, we investigated how neurons within the ventral posterior lateral (VPL) Thalamus, which receive direct input from neurons within the vestibular nuclei (VN) and project to cortical structures respond to natural self-motion stimuli. Our results show that vestibular Thalamic neurons, contrary to VN neurons, do not display whitening. Indeed, their response power spectra were not constant as a function of frequency and instead resemble those of afferents, showing that information as to the head motion’s detailed timecourse is transmitted to cortical structures as required for accurate self-motion perception.

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Poster

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Topic: D.06. Auditory & Vestibular Systems

Support: CIHR Grant 241856
CIHR Grant 236330

Title: Adaptation to the distribution of vestibular stimuli in the thalamus

Authors: *G. MCALLISTER[1], J. CARRIOT[1], J. X. BROOKS[1], K. E. CULLEN[2], M. J. CHACRON[1]
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Abstract: Growing evidence shows that neural sensory systems actively adapt their responses to efficiently encode the full range of changing stimulus distributions. Adaptive encoding has been demonstrated in many sensory systems and species, most extensively in studies of rapid contrast gain scaling in retinal and visual thalamic neurons. Previous studies have found that vestibular stimuli in natural contexts are highly non-stationary and have a large dynamic range, suggesting that such adaptation may also occur in the vestibular system. Here, we investigated adaptation to changes in vestibular stimulus intensity in the thalamus. We recorded extracellular single-unit neural responses in the ventral posterior lateral thalamus of rhesus macaque monkeys, to sinusoidal head rotation with steps in peak rotational velocity (amplitude). We found that the neural response to amplitude steps was strongly nonlinear. For frequencies from 0.5-8Hz, gain increased sub-proportionally when peak amplitude was increased. This change occurred rapidly, consisting of an initial overestimation followed by a gradual reduction in gain. We described the pattern of adaptation by fitting a divisive normalization model that resembles models of adaptive gain scaling in the visual thalamus. Furthermore, we found that the observed adaptation significantly increased mutual information between stimulus and response when compared to a fully linear model, suggesting that this adaptation improves information transmission. In conclusion, we found that adaptive gain scaling does occur in vestibular thalamus neurons in healthy subjects, supporting the view that adaptation is a fundamental aspect of sensory processing. The findings also provide insight into how the posterior ascending vestibular pathway provides an accurate signal to the cortex for reliable perception in varying sensory contexts.

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Poster

576. Vestibular Physiology and Anatomy

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Program #/Poster #: 576.08/CC15

Topic: D.06. Auditory & Vestibular Systems

Title: Complete and irreversible unilateral vestibular loss induces reactive neurogenesis in the vestibular nuclei in adult rats

Authors: *G. RASTOLDO, D. PERICAT, I. WATABE, N. EL MAHMOUDI, C. CHABBERT, B. TIGHILET
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Abstract: Apart from two specific structures: the subgranular zone of the dentate gyrus of hippocampus and the subventricular zone of the lateral ventricles, the adult mammalian brain is considered non-neurogenic. Neurogenesis in other brain regions is limited under normal physiological conditions but could be induced after injury or pathological conditions. This is what happens after unilateral vestibular neurectomy (UVN) in adult cats: our work revealed for the first time the existence of adult reactive neurogenesis in deafferented vestibular nuclei (VN) located in the brain stem. Even more surprisingly, we have shown in an original way that most of the newborn cells are functional and contribute to the recovery of the posturo-locomotor functions in adult cats. We recently switched to the rodent model by replicating the same surgery (UVN) resulting in the same posturo-locomotor and oculomotor syndrome. The objective of this study is to verify whether the reactive neurogenesis observed in the cat model is also expressed in the rodent model after UVN. We used specific markers of cell proliferation (BRDU), stem cells (GFAP and SOX2) and cell differentiation (GFAP: astrocytes, NEUN: neurons, GAD67: GABAergic neurons and IBA1: microglia). The results showed a significant cellular proliferation with a peak of proliferation 3 days after UVN exclusively on the deafferented side in all VN. Most of the newly generated cells survived up to 1 month after UVN and differentiate into astrocytes and microglial cells but also into GABAergic neurons. We also observed SOX2/GFAP-immunoreactive cells in UVN rats and surprisingly in control animals. We observed the same reactive neurogenesis phenomenon in all VN in adult rats. The presence of SOX2 and GFAP co-localization attests to the presence of probably quiescent stem cells in the VN in the intact animal. Our perspectives are: i) to specify the origin of stem cells: birth in vestibular nuclei or migration from brain neurogenic zones, ii) to demonstrate the involvement of this neurogenesis in vestibular compensation and iii) use pharmacological agents that impact on neurogenesis to accelerate the vestibular function recovery.

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Poster

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Program #/Poster #: 576.09/CC16

Topic: D.06. Auditory & Vestibular Systems

Title: Stochastic noise alters the sensitivity of medial vestibular nucleus neurons *in vitro*

Authors: S. STEFANI[1], P. BREEN[3], J. SERRADOR[4], M. SCHUBERT[5], *A. J. CAMP[2]
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Abstract: Background: Stochastic resonance is a phenomenon whereby sensitivity to sub-threshold signals is modified via low frequency noise. Application of stochastic noise has been shown to improve visual, auditory, balance and cardiovascular functions within humans. A key feature of stochastic noise is the low frequency and amplitude of the stimulus- that is, it remains imperceptible to the participant. This means that rather than eliciting a profound habituation or hyperstimulationreflex responses on the back of neuronal activation, stochastic noise presumably exerts its effect by subtlely altering neuronal sensitivity to incoming signals.
Objective: Here we aim to determine how the gain (sensitivity) of individual medial vestibular nucleus (MVN) neurons is modified by the application of stochastic noise.
Methods: All experimental materials and procedures were approved by the University of Sydney Animal Ethics Committee (protocol 2018/1308). All experiments were performed in 3 - 4 week-old male and female C57BL/6 mice. Whole-cell current-clamp recordings of individual neurons in the medial vestibular nucleus (MVN) were made at room temperature from 250 µm tissue slices. Recordings were made in response to a suite of depolarising current steps (10 steps, 10 pA/step) with or without (control) sStochastic noise. The stochastic noise protocol was produced using MATLAB with a maximum amplitude of ± 120 pA. To maintain average background neuronal discharge during stochastic noise, stimulus amplitudes of between 5-20 % of the maximum amplitude were used (i.e. 5 % = ± 6 pA). Spike rate vs current plots were produced and the slope of the line of best fit used to quantify neuronal gain.
Results: In 4/6 MVN neurons stochastic noise produced a significant alteration in neuronal gain when compared with the no noise control condition (all p-values < 0.001). In two of the neurons this difference was expressed as an increase in neuronal gain (46.10 % and 8.50 %) and in two of the cells, neuronal gain was reduced (72.35 % and 28.82 %). However, the neuronal gain of the remaining two neurons was unaffected by stochastic noise.
Conclusion: These results indicate that the sensitivity of MVN neurons are can becan be influenced by the application of stochastic noise. Importantly this preliminary data suggests that the impact of stochastic noise is variable? differential- that is, in some neurons the impact is an

increase in gain while in others it is a reduction. This differential may provide a “normalisation” mechanism to modulate the overall sensitivity of the vestibular system and as such may be useful in the development of therapeutic devices to treat those suffering from balance dysfunction.

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Poster

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Topic: D.06. Auditory & Vestibular Systems

Support: R01 DC01379801 (SMR)
R01 DC008846 (GRH)

Title: Pulsed infrared neural stimulation of vestibular system endorgans evokes sinusoidal vestibulo-sympathetic reflex responses

Authors: D. RICE[1], W. JIANG[1], G. P. MARTINELLI[3], G. R. HOLSTEIN[4], *S. RAJGURU[2]
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Abstract: The vestibulo-sympathetic reflex (VSR) plays a role in modulation of heart rate (HR) and blood pressure (BP) with changes in posture and head position. Previous experiments have demonstrated that the activation of VSR pathway via galvanic electrical stimulation and tilt. In the present study, we have investigated the effects of pulsed infrared radiation (IR) focused on individual vestibular endorgans, either the vertical semicircular canals or the otolith endorgans *in vivo*in a rat model and characterized the resultant physiological modulation of HR and BP.The focused, pulsed IR stimulation allows a detailed characterization of the contributions of individual endorgans to the resultant HR and BP changes. Long wavelength pulsed IR (1863nm) was targeted towards individual vestibular endorgans using custom optical fibers. The cardiovascular responses evoked via the activation of the VSR were measured using a small animal, single-pressure implantable device (DSI Inc.) inserted into the femoral artery.To confirm the site of stimulation and the endorgans affected, the distance of the fiber from target structures and orientation of the beam *in vivo*were determined using micro-computed tomography. Stimulation of the posterior semicircular canals using frequency-modulated IR resulted in significant cardiovascular responses. Overall, the HR dropped between 10 to 40 bpm below baseline (a change of up to 16%) whereas the BP dropped between 5 to 10 mmHg below baseline (a change of up to 11%, n=14). The IR parameter space including irradiance and

modulation frequencies was explored. Light directed at the utricular macula evoked the characteristic upward-torsional movements of ipsilateral eye with a downward rotation of the contralateral eye. However, IR stimulation of utricular macula in the rats failed to evoke changes in HR or BP. In the companion abstract, we present resulting distributions of activated vestibular nuclei neurons following IR of an individual end organs. Combined with previous studies utilizing tilt or galvanic vestibular stimulation, these results are suggestive of selective activation of the vestibular system by pulsed infrared, and an important role of vertical canals in the activation of the VSR pathways. Supported by NIH/NIDCD grants R01 DC01379801 (SMR), R01 DC008846 (GRH).

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Poster

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Topic: D.06. Auditory & Vestibular Systems

Support: NIH/NIDCD grant DC008846 (GRH)
NIH/NIDCD grant DC01379801 (SMR)

Title: Vestibular nucleus neurons that activate vestibulo-sympathetic reflex pathways following single end organ labyrinthine stimulation

Authors: *G. R. HOLSTEIN[1], E. K. CHAPMAN[2], D. L. RICE[4], W. JIANG[5], S. RAJGURU[6], G. P. MARTINELLI[3]
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Abstract: The vestibulo-sympathetic reflex (VSR) pathway can alter blood pressure and heart rate in response to changes in head position with regard to gravity, as occurs when humans rise from a seated or supine position and when quadrupeds rear, climb or burrow. We have previously demonstrated that sinusoidal galvanic vestibular stimulation and tilt can be used to activate central vestibular neurons of the VSR pathway in rats. The activated vestibular neurons were identified by cFos labeling and retrograde tract tracing, and were classified by neurotransmitter phenotype, projection target, and laterality of the projections.
The goal of the present study was to identify the locations of vestibular neurons of the VSR pathway that were activated by pulsed infrared laser stimulation (pIR) of individual vestibular end organs. To achieve this, pIR at 1863 nm, 250 pps baseline pulse frequency, and 200 μs pulse

duration was directed through the round window toward vestibular end organs in rats using polished optical fibers with core diameters of 200 or 400 µm. Changes in blood pressure and heart rate were detected using a telemetric sensor implanted in the aorta via the femoral artery, and recorded using Ponemah software (DSI Inc.; MN). Evoked eye movements were recorded using video-oculography. Cells that were activated by pIR were identified by cFos/DAB immunohistochemistry. Labeled cells were counted in skip-serial sections through the caudal vestibular nuclei that were separated by at least 100 µm. The cell counts from each rat were mapped onto 16 representative rostro-caudal Bregma levels and normalized for comparison across subjects. In some animals, the retrograde tracer FluoroGold was placed in the pre-sympathetic medullary region (RVLM) two weeks prior to pIR stimulation.
Results indicate that blood pressure and heart rate are highly sensitive to unilateral pIR activation of the posterior canal, but not unilateral activation of the utricle. Nevertheless, following unilateral activation of the posterior canal, the highest density of cFos-positive cells is located in a narrow rostrocaudal belt between Bregma levels -11.40 and -11.88, and the highest density of cFos-positive cells resulting from unilateral pIR of the utricle is observed between Bregma levels -11.76 and -12.12. In both stimulus conditions, the highest densities of activated neurons are present in the caudal medial vestibular nucleus. Together with the results of previous studies utilizing tilt or sinusoidal galvanic vestibular stimulation stimuli, the present study suggests that there are subpopulations of VSR neurons in the caudal vestibular nuclei that receive differential end organ input.

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Poster

576. Vestibular Physiology and Anatomy

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Topic: D.06. Auditory & Vestibular Systems

Support: a grant for the fellows of the JSPS No. 17J07245

Title: The effect of dynamic upper limb movements on perception of gravitational direction during prolonged whole-body tilt

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Abstract: Prolonged whole-body tilt, keeping the body tilted for certain time, gradually shifts the perceived direction of gravity toward the direction of body tilt (Wade 1970). The present

study investigated how upper limb movements during body tilt influence the effects of prolonged whole-body tilt in the roll plane on the perception of gravitational direction. Fifteen healthy subjects participated in this study. First, subjects which sat on the tilting chair, were moved toward left-side-down 16 degrees, they were instructed to perform visual vertical (VV) task, in which they adjust a white line presented on the display in front of their head to the perceived direction of gravity. After that, they were asked to perform any of three following action tasks without vision; 1) non-movements, 2) static movements, pointing with their right index finger ahead the center of the eyes and keeping this upper limb position, and 3) dynamic movements, moving their right upper limb up and down along their longitudinal axis for ten times. After the action task, they performed VV task again. We compared subjective visual vertical (SVV) angle between before and after action task for each movement condition. Results show that SVV after action task were significantly tilted toward the direction of body tilt (i.e. leftward) compared with SVV before action for non-movement and static movement conditions. And, in contrast, we found no significant angular difference between SVV before and after the action task for dynamic movement condition. These results suggest that additional spatial cues (i.e. dynamic proprioceptive feedback from muscle spindles and skin receptor, and effect copy) occurred during dynamic upper limb movements contribute to the accurate estimation of gravitational direction.

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Poster

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Topic: D.06. Auditory & Vestibular Systems

Support: NSERC

Title: Vestibular adaptation time constants to mechanical and virtual rotation

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Abstract: The semicircular canals in our vestibular system detect angular acceleration of the head in space, providing us with information for spatial orientation and balance. Activation of primary canal afferents result in oculomotor responses (vestibulo-ocular reflex; VOR) and motion perception. For mechanical rotations, a step change in head velocity generates primary canal afferent activity that decays with a time constant of ~4s. Central multisensory integrative processes, however, prolong responses evoked by such mechanical stimuli. Electrical vestibular stimulation (EVS) may mimic the neuronal activity of the primary vestibular afferents despite

the lack of any associated head motion. Using previously reported transfer functions from animal models, a mathematical conversion model was developed to estimate the equivalent electrical current profile for a given head motion. The goal of this study was to determine whether this electrically equivalent stimulation profile was processed differently in the central neurons eliciting the VOR and perceptual responses. Subjects were seated with their head pitched down in a rotary chair that delivered whole-body yaw rotations. Illusionary yaw rotations were created by electrodes placed bilaterally on the mastoid processes that provided EVS in a binaural-bipolar configuration. Within a darkened room, stimuli were delivered to the seated subject while extraneous somatosensory cues were attenuated with padding. To quantify adaptation time constants, we rotated the chair at a constant velocity (*±10o/s*) over 60s or delivered an electrically equivalent profile with a maximum current amplitude of ±4mA. For perceptual responses, twenty subjects (11 females) were asked to turn a handle corresponding to their perceived position. In a second session, VOR-evoked ocular torsion was recorded using an infrared camera placed in front of the subject. Position signals were processed offline and adaption time constants were determined by fitting the differentiated position signal with an exponential function. Preliminary results based on perceptual responses showed that the time constant under physical stimulation 13.8±2.2s was longer than that evoked by EVS (7.4±1.0s; Wilcoxon Rank-Sum p=0.006). Preliminary observations from the VOR (n=2, 1 female) support our perceptual findings but additional testing is required. Altogether, these results suggest that activation of primary vestibular afferent by EVS are integrated differently from mechanical stimuli in the central vestibular system. Application of EVS to mimic real world motion perception would require further modeling of central processing not yet accounted.

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Poster

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Topic: D.06. Auditory & Vestibular Systems

Support: Wellcome Trust
Gatsby Charitable Foundation

Title: Multisensory signals underlie self-motion representation in the retrosplenial cortex

Authors: *S. KESHAVARZI, C. V. ROUSSEAU, S. LENZI, T. W. MARGRIE
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Abstract: Any neuronal representation of one's location, heading direction or motion with respect to the surrounding scene and objects is generated using internal and external sensory cues. These may include motor, somatosensory, visual and vestibular signals that combine to form a coherent representation of the external world, and the location and motion status of the observer within it. The retrosplenial cortex (RSP) is a multimodal cortical region involved in encoding and storage of spatial information. It receives substantial inputs from the ascending vestibular and head-direction pathways as well as visual cortical areas. However, it is not known whether individual RSP cells signal multimodal information, or whether functionally diverse populations of unimodal cells provide a combinatorial signal. We examined this question by recording responses of RSP cells during passive rotation (yaw) in awake head-fixed mice in the absence and presence of visual cues. Using a custom-built two-photon calcium imaging setup and extracellular recordings with a high-density silicone probe (Neuropixels), we observed neurons in superficial and deep layers of RSP to be modulated by rotation in the dark. Preliminary results showed changes in firing in both excitatory and inhibitory RSP cells evoked by clockwise and/or counter-clockwise rotations. Across the population, the presence of visual cues led to an increased directional tuning suggesting that, at least in some cells, both visual and vestibular inputs underlie the representation of self-motion in the RSP.

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Poster

576. Vestibular Physiology and Anatomy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 576.15/DD6

Topic: D.06. Auditory & Vestibular Systems

Support: NSERC Grant 356026-13
MITACS Accelerate IT10202

Title: You must stop the postural control of balance before you can move

Authors: *R. TISSERAND[1], C. J. DAKIN[2], M. H. F. VAN DER LOOS[1], E. A. CROFT[3], T. J. INGLIS[1], J.-S. BLOUIN[1]
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Abstract: The neural control of transition between posture and movement is dependent upon regulation of reflex-stabilizing mechanisms to enable motion. Optimal feedback control theory postulates that specific postural configurations, such as standing balance, or a movement pattern, such as locomotion, operate under distinct control policies, and that transitions between posture

and movement require disengagement of the current control policy before the engagement of a new one. We investigated this hypothesis by examining the continuity of the vestibular control of balance during transitions between standing balance and locomotion and between two states of standing balance. Sixteen healthy subjects initiated and terminated locomotion, at their preferred walking speed (Experiment 1) or shifted their weight from 50% to 90% on their left leg (Experiment 2), while exposed to a continuous electrical vestibular stimulus (EVS). Ground reaction forces (GRFs) were recorded before, during and after the different transitions. The relationship between the EVS and GRFs was quantified using time-frequency coherence. We observed a coherence null period preceding the onset of anticipatory postural adjustments during both the initiation of locomotion and the weight shift, as well as during the step prior to the termination of locomotion. These results highlight a down-regulation of the balance-correcting mechanisms to enable the transition between posture and movement that is not only related to locomotion. Our results suggest there is a discrete change between motor control policies to disengage the current motor policy to make way for the next, as predicted by optimal feedback theory. Ultimately, we demonstrate that humans must “stop balancing” before they can move and “stop moving” before they can reinitiate standing balance.

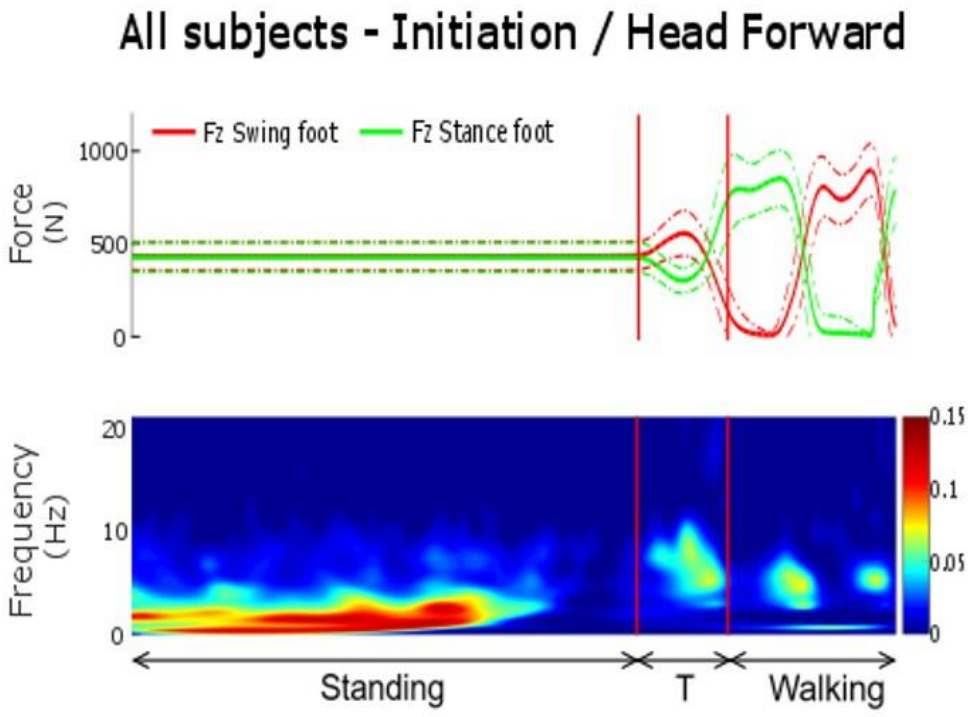

Figure 1: Evidence for a suspension of the vestibulomotor control of balance during locomotion initiation in healthy young adults (n = 10). Graphs represent the average vertical forces ± one standard deviation (top, red and green traces) and the average time-frequency coherence results between vestibular stimulus (EVS) and muscular responses in the mediolateral direction (bottom, colored graph). On the coherence graph, a dark blue color indicates a non-significant coherence. In both graphs, from left to right, the first vertical red line delimits between quiet standing and transition (T) periods and the second vertical red line delimits between transition (T) and walking periods.

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Poster

576. Vestibular Physiology and Anatomy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 576.16/DD7

Topic: D.06. Auditory & Vestibular Systems

Support: JSPS KAKENHI Grant-in-Aid for Young Scientists (A) Grant Number 17H04690

Title: Galvanic vestibular stimulation revisited: A current path account

Authors: *K. AOYAMA[1], N. HAGURA[2,4], E. R. FERRÈ[7,5], T. MAEDA[6,3], Y. IKEGAYA[8,3], H. ANDO[6,3]
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Abstract: Galvanic Vestibular Stimulation (GVS) evokes virtual head motion. It has been used to diagnose vestibular disorders and to experimentally manipulate the vestibular afferents. However, the physiological mechanisms underlying GVS are not yet well understood.
GVS-induced motion has been explained as a vectoral summation of the motion direction synchronously signalled by both vestibular organs. This approach has three critical assumptions. First, the electrical current equally activates both otoliths and canals (*non-specificity*). Second, the activation pattern of the vestibular organs is defined by the polarity of the electrodes stimulating the organs (*polarity dependency*). Third, the overall signal information is summed to calculate the net motion direction (*vector summation*).
Here we show evidence that GVS may activate specific vestibular organs (*specificity*), independently from electrode polarity (*polarity independence*). Five participants were administered with a novel GVS electrodes configuration, in which the current was applied between electrodes on the mastoid and on the neck. Critically, the polarity of the electrodes on mastoids was always identical. According to the traditional theory, GVS will activate both otoliths and canals, and the vectoral summation of the signal should lead to forward and backward sensation. In contrast to this prediction, they perceived upwards or downwards sensations, without any rotation or forward-backward sensations. This indicates otoliths dominant activation was induced by this stimulus. Importantly, they were also administered with the same GVS configuration while facing the ground. In this posture, upward and downward otoliths contribution should be translated to forward and backward postural sway. Participant's postural sway direction was forward and backwards, confirming our prediction. Taken together, our results indicate that depending on the configuration of the electrodes, GVS can specifically activate the otoliths in a polarity-independent manner.
The impedance of the skull bone is well above that of the other tissues. Thus, the path that the GVS current can flow to affect the vestibular organs are restricted. Depending on the electrodes configuration, the current 's path can differ. We propose a novel Current Path Account to explain the physiological effects induced by GVS, where the direction of the current defines the activation pattern of the vestibular organs. We believe that the motion sensation triggered by GVS reflects a specific activation pattern in the vestibular organs, not the biased direction calculated from the non-specific overall activation.

Disclosures: N. Hagura: None. **E.R. Ferrè:** None. **T. Maeda:** None. **Y. Ikegaya:** None. **H. Ando:** None.

Poster

576. Vestibular Physiology and Anatomy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 576.17/DD8

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant NS102157-01

Title: The effects of repetitive subconcussive head impacts on vestibular processing and balance during walking

Authors: *J. B. CACCESE, F. V. SANTOS, M. GONGORA, I. SOTNEK, E. KAYE, F. YAMAGUCHI, J. J. JEKA
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Abstract: Humans require precise integration and modulation of visual, vestibular, and proprioceptive feedback to control balance during walking. Using galvanic vestibular stimulation (GVS), a tool used to probe the vestibular system, our laboratory has demonstrated that repetitive subconcussive head impacts (RSHI) from soccer heading may lead to vestibular dysfunction during quiet stance. However, it is unknown if RSHI disrupt vestibular processing during walking and how vestibular dysfunction affects balance mechanisms during walking. The purpose of this study was to compare changes of balance mechanisms in response to GVS during walking following RSHI. Twenty adult amateur soccer players (10 males and 10 females, 22.3±4.5years, 170.5±9.8cm, 70.0±10.5kg) were randomly assigned to a soccer heading or a control group. Participants in the soccer heading group performed a controlled soccer heading paradigm. All participants underwent balance testing at baseline (PRE), immediately after the soccer heading paradigm (POST-0h), and 24 hours later (Post-24h). During balance testing, participants walked along a foam walkway with the eyes closed under two conditions: with GVS (~40 trials) and without GVS (~40 trials). Outcome measures included mediolateral center-of-mass (COM)-center-of-pressure (COP) separation, and four balance mechanisms: foot placement, mediolateral ankle modulation, hip adduction, and ankle push off. For each balance mechanism, a GVS response was calculated (GVS - mean (without GVS)). Repeated measures ANOVAs were used to compare between group responses across time points, while controlling for concussion history and sex. There were no significant group x time interaction effects for any of the balance measures (COM-COP separation: $F_{2,15}$=2.330, p=0.131; foot placement: $F_{2,15}$=1.448, p=0.266; ankle modulation: $F_{2,15}$=3.405, p=0.060; hip adduction: $F_{2,15}$=2.330, p=0.749). The results of this study suggest that although there may be a disruption in vestibular processing following RSHI, this disruption does not lead to measurable changes in balance during walking. While there were subtle, individual changes in balance mechanisms across time,

these changes may be an indication of the sensitivity of these measures and not of the clinical implications of RSHI.

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Poster

576. Vestibular Physiology and Anatomy

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Program #/Poster #: 576.18/DD9

Topic: D.06. Auditory & Vestibular Systems

Support: NIH R01 NS102157-01

Title: The effects of subconcussive head impacts on vestibular processing and balance during walking

Authors: *F. V. SANTOS[1], J. B. CACCESE[1], M. GONGORA[1], I. S. SOTNEK[1], F. S. YAMAGUCHI[1], J. J. JEKA[2]
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Abstract: Gait is a primordial human function that requires sensory integration of visual, vestibular, and proprioceptive systems. In traumatic brain injury there are profound deficits in sensorimotor function. In addition, previous research has suggested that even mild traumatic brain injury/concussion and repetitive subconcussive head impacts (RSHI) may lead to subtle balance disturbances during standing and walking. We proposed to use galvanic vestibular stimulation (GVS), a technique used to study vestibular contributions to balance, to determine the effect of concussion history and exposure to RSHI on vestibular processing and balance during walking. Twenty adult amateur soccer players (10 females, 22.3±4.5years, 170.5±9.8cm, 70.0±10.5 kg) walked along a foam walkway with the eyes closed under two conditions: with GVS (~40 trials) and without GVS (~40 trials). Peak mediolateral center-of-mass (COM)-center-of-pressure (COP) separation response (GVS - mean (without GVS)) was used as the main outcome measure. Independent variables included self-reported years of soccer participation, age of first exposure to soccer heading, and concussion history. Linear regression models were used to determine if measures of RSHI exposure or concussion history were related to balance response to GVS. The COM-COP separation response was not associated with RSHI exposure or concussion history (age of first exposure to soccer heading, R^2=0.028, p=0.477; years of soccer participation, R^2=0.012, p=0.652; concussion history, R^2=0.139, p=0.105). Although previous research has speculated that there are possible long-term neuropathological consequences associated with RSHI and concussion, including chronic traumatic encephalopathy, our results suggest that years of participation in soccer and a history of concussion are not related to

vestibular processing and balance dysfunction during walking. Moreover, recent literature has suggested that age of first exposure to tackle football leads to later-life cognitive, behavioral, and mood changes. However, we found no evidence of balance dysfunction in those with earlier exposure to soccer heading. Our cohort consisted exclusively of current adult amateur soccer players, and thus, our findings cannot be extended to later-life, retired soccer players.

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Poster

576. Vestibular Physiology and Anatomy

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Program #/Poster #: 576.19/DD10

Topic: D.06. Auditory & Vestibular Systems

Support: DOD CDMRP W81XWH-14-1-0598
DOD CDMRP W81XWH-14-2-0012

Title: Customizing galvanic vestibular stimulation amplitude using postural sway: Sensitivity thresholds are reduced without vision and disrupted proprioceptive feedback

Authors: M. MURARIK[1], S. B. DOUGLAS[1], E. R. STEELE[1], M. M. FEYRER-MELK[1], H. S. LEE[1], J. M. SERRADOR[2], *S. J. WOOD[1]
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Abstract: Neuromodulation using low levels of noisy galvanic vestibular stimulation (GVS) is being explored as a treatment to improve balance function. High intersubject variability in GVS sensitivity suggests that the treatment can be enhanced by customizing the stimulus level to individual sensitivity thresholds. We have developed an objective procedure for determining thresholds using postural sway induced during increasing levels of sinusoidal GVS. One complication is that the target patient population will have varying levels of postural instability prior to and during treatment. The purpose of this study was to compare sensory sensitivity thresholds in 27 healthy subjects across four conditions that represent increasing postural challenge: eyes open on stable surface, eyes closed on stable surface, eyes open on unstable surface, and eyes closed on unstable surface. Inertial motion sensors on the head and torso recorded sway while subjects stood with feet together for 20 sec during sinusoidal 0.1 Hz GVS over a 0.1 - 0.9 mA range in 0.1 mA steps. Both the amplitude of sway as well as the percentage of falls increased from fixed to unstable conditions, and from eyes open to eyes closed conditions. Sinusoidal curve fits were used to characterize sway modulation as a function of the sinusoidal-varying stimuli. Sensitivity thresholds were derived from the lowest stimulus level where the sinusoidal response amplitude exceeded the baseline sway amplitude without GVS

stimulation. Thresholds were significantly reduced during the condition without vision and disrupted proprioception. Sensitivity thresholds determined by this technique are influenced by the feedback available to the participant. Visual and/or proprioceptive feedback may elicit compensatory reflexes that inhibit sway, thus increasing the sensitivity thresholds. These compensatory strategies may be greater in patients with vestibular loss, and therefore need to be considered when determining a threshold-based stimulus level for treatment.

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Poster

576. Vestibular Physiology and Anatomy

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 576.20/DD11

Topic: D.06. Auditory & Vestibular Systems

Support: NASA 80NSSC17K0021
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Title: Neural correlates of vestibular processing during exposure to a spaceflight analog with elevated carbon dioxide

Authors: *K. E. HUPFELD[1], J. K. LEE[1], N. E. GADD[3], I. S. KOFMAN[4], Y. E. DE DIOS[4], J. J. BLOOMBERG[3], A. P. MULAVARA[3], R. D. SEIDLER[2]
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Abstract: Spaceflight negatively affects central vestibular processing and performance of vestibularly-mediated behaviors such as balance and gait. Head-down-tilt bed rest (HDBR) is commonly used as a spaceflight analog to examine effects of body unloading, fluid shifts, and other consequences of spaceflight unrelated to gravitational changes. HDBR paired with elevated atmospheric carbon dioxide (CO_2) to mimic conditions on the International Space Station has been shown to positively influence cognitive performance; however, effects of combined HDBR and elevated CO_2 on vestibular processing have not yet been studied. Here, we examine how a 30-day HDBR intervention with elevated (0.5%) CO_2 influences the neural correlates of vestibular processing in 11 participants (6 males, mean age = 34 years). Over six sessions (twice before, twice during, and twice after CO_2-HDBR) we used fMRI to measure brain activity in response to pneumatic cheekbone taps, a validated method of vestibular stimulation, in addition to assessing balance and mobility. This allowed us to examine immediate and cumulative changes due to CO_2-HDBR and the time course of recovery. We found that frontal,

sensorimotor, temporal, and occipital cortices showed increases in brain activation during vestibular stimulation immediately after starting CO_2-HDBR, with recovery after stopping the intervention. Opposite patterns of immediate decreases in activation after starting CO_2-HDBR followed by recovery were observed in the brainstem. Slower cumulative increases in activation across CO_2-HDBR followed by recovery were seen in occipital cortex. In comparison to another cohort exposed to 70 days of HDBR with ambient air (n = 13; all males; mean age = 29), CO_2-HDBR participants showed multiple regions with a greater degree of activation change from baseline to the end of the intervention, including regions in frontal, parietal, and occipital cortices. CO_2-HDBR participants also showed multiple regions with a smaller degree of activation change from baseline, including in the thalamus, cerebellum, and temporal cortex. These results suggest that CO_2-HDBR may be associated with reduced neural efficiency and/or sensory reweighting in comparison to HDBR alone. Further, the observed differences in the neural vestibular changes between ambient air-HDBR and CO_2-HDBR participants suggest that CO_2-specific effects, such as hypercapnia-induced cerebrovascular reactivity, or the interactive effects of CO_2 and HDBR may uniquely affect central vestibular processing. These findings have implications for better understanding the neural mechanisms of spaceflight-related changes in vestibular processing.

Disclosures: K.E. Hupfeld: None. **J.K. Lee:** None. **N.E. Gadd:** None. **I.S. Kofman:** None. **Y.E. De Dios:** None. **J.J. Bloomberg:** None. **A.P. Mulavara:** None. **R.D. Seidler:** None.

Poster

576. Vestibular Physiology and Anatomy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 576.21/DD12

Topic: D.06. Auditory & Vestibular Systems

Support: Faculty research grant of Yonsei University College of Medicine (6-2016-0040)

Title: Application of virtual reality immersion in postural control assessment

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Abstract: To maintain posture, the visual, vestibular and proprioceptive sensory information are utilized. In dizziness patients, posturography is used to measure variations in center of gravity(COG) to assess postural instability. The VR technology can be applied to create real-life 3D environments where the user can navigate. In this study, we introduce the development of VR immersion contents with varying degrees of visual sway components, and evaluate their validity in assessment of postural control. Three conditions of VR immersion scenarios(VR1-3)

were created and operated on commercially available head mount devices. Ten healthy subjects were instructed to maintain upright posture during exposure to different visual conditions. Posturography data of COG sway during test trials of 30 seconds were collected. Subjective symptoms were measured using visual analog scale and the simulator sickness questionnaire. Mean COG sway velocities were 0.17±0.38 deg/sec and -1.51±0.86 for x- and y-axis directions for VR1, 0.24±0.52 and -1.88±0.75 for VR2, and 0.11±0.53, and -1.94±0.68 for VR3. Mean VAS scores 0.1±0.32, 0.4±1.27, and 2.0±3.09 for VR1-3 respectively. Mean total SSQ scores were 0.37±1.18, 1.5±4.73, and 7.48±11.56 for VR1-3, showing that even healthy subjects showed wide range of VAS and SSQ during VR immersion. Tailored VR immersion scenarios were developed and their applications in assessment of posture control showed that increased visual-vestibular conflict resulted in postural instability. Addition of VR immersion conditions would be helpful to discern minute but significant deficits in patients who experience dizziness but can perform conventional posturography tasks.

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Poster

576. Vestibular Physiology and Anatomy

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 576.22/DD13

Topic: D.06. Auditory & Vestibular Systems

Title: Vestibular contribution to balance control during stair negotiation and locomotion vestibular contribution to balance control during stair negotiation and locomotion

Authors: *C. DAKIN, M. ELWOOD, A. KERN, E. BRESSEL
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Abstract: The vestibular system is an important source of motion and orientation information, and is essential for the control of our posture in space. Generally, the vestibular contribution to the control of posture increases as the balance demands of a task increase, resulting in some tasks seemingly having greater vestibular involvement than other tasks. Here we ask whether this is true for stair negotiation compared to treadmill walking with the long-term goal of understanding how changes in vestibular function with aging may contribute to falls on stair. **Methods:** Fifteen young adults and six older adults walked over a nine-step staircase and on a treadmill (300 steps each for ascent, descent and treadmill) with a cadence of 76 steps/minute (treadmill speed of 0.4m/s) while receiving a small continuous random electric current (0-25hz bandwidth) to their mastoid processes. Electromyography was recorded from eight muscles (soleus, medial gastrocnemius, tibialis anterior, biceps femoris, semitendinosus, vastus medialis, rectus femoris and gluteus medius) and body kinematics recorded from the left leg and trunk. We quantified the relationship between the vestibular stimulus and behavior across conditions using time-

dependent measure of coherence and cross-correlation. **Results:** Preliminary results suggest older adults exhibit greater vestibular influence over muscle activation in the soleus and medial gastrocnemius than young adults during treadmill walking, and in the biceps femoris during stair ascent. Vestibular influence appears to decrease in the soleus, medial gastrocnemius, biceps femoris and semimembranosus during stair descent versus ascent whereas in older adults vestibular influence increases in the tibialis anterior during stance in stair descent. **Conclusions:** Stair negotiation requires changes in how vestibular cues are used to control balance compared to locomotion and much like during locomotion these changes depend on the muscle and phase of the gait cycle. More generally, these results provide a first proof of concept demonstrating the ability to identify subtle changes in vestibular feedback driven control of balance in dynamic and potentially compromising environments.

Disclosures: C. Dakin: None. **M. Elwood:** None. **A. Kern:** None. **E. Bressel:** None.

Poster

576. Vestibular Physiology and Anatomy

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Program #/Poster #: 576.23/DD14

Topic: D.06. Auditory & Vestibular Systems

Support: NIGMS of NIH Grant Number P20 GM103650

Title: Statistical characterization of heading stimuli in natural environments using SLAM

Authors: *C. SINNOTT, T. DANG, C. PAPACHRISTOS, K. ALEXIS, P. MACNEILAGE
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Abstract: Heading is the direction of linear self-motion in head coordinates. It may be estimated based on vestibular signals that provide information about linear acceleration and based on visual optic flow signals that provide information about linear velocity. Prior psychophysical studies have documented significant repulsive biases in perception of both visual and vestibular heading (Cuturi & MacNeilage 2013), meaning that heading azimuth angle is perceived to be more eccentric than the presented stimulus. Theoretical work suggests that such biases may result from a combination of efficient encoding and probabilistic decoding, where both encoding and decoding mechanisms are constrained based on natural stimulus distributions (Wei & Stocker 2015). To our knowledge, these distributions for heading stimuli remain undocumented, so we set out to characterize them. Tracking linear head velocity in natural environments using a head-based system is challenging. Recording of linear head acceleration using an inertial measurement unit (IMU) results in velocity estimates subject to drift, while optic flow analysis of video from a head-mounted camera is subject to ambiguity due to superposition of linear and angular flow and unknown scene scale. To overcome theselimitations we adopted visual-inertial odometry

technology developed for autonomous robots that perform localization and mapping (SLAM). Subjects wore a head-mounted device with calibrated, integrated camera and IMU.The data fusion pipeline yielded robust estimates of linear and angular position (in world-frame coordinates) and velocity (in head-frame coordinates) as subjects moved freely. The distribution of heading azimuth and elevation was peaked near straight ahead, as expected based on natural walking with head facing forward. These highly peaked distributions are qualitatively consistent with predictions of repulsive biases based on efficient encoding and probabilistic decoding.

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Poster

576. Vestibular Physiology and Anatomy

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Program #/Poster #: 576.24/DD15

Topic: D.08. Visual Sensory-motor Processing

Support: Sir Henry Dale Fellowship jointly funded by the Wellcome and The Royal Society (Grant Number 104285/B/14/Z)
H2020-MSCA-IF-2016 747902

Title: A novel apparatus for open- and closed-loop vestibular stimulation in head-fixed mice

Authors: *E. A. RANCZ, B. A. HOROBET, A. P. TRAN-VAN-MINH, Z. YE
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Abstract: Visual virtual reality (VR) is used successfully to study cortical processing in awake, behaving mice. It not only allows for tight control of animal driven visual stimuli, but also has the ability to change the coupling between stimulus and behaviour. However, most visual VR approaches use head-fixed animals, where one important sensory modality, the vestibular system, is taken out of play. Vestibular information is important for many cognitive processes, including spatial navigation. So-called head-direction cells, found in several cortical areas associated with spatial navigation, are primarily driven by vestibular input. Here we present a novel experimental apparatus, in principle compatible with visual VR systems, using a yaw rotational motor which can be used in both open- and closed-loop configuration, allowing animals to navigate in rotational, directional space.
We show that animals adapt to the new environment quickly and behave in a natural way when the motor is engaged. We further show that our approach is compatible with both electrical and optical recording of brain activity at the cellular level. We are currently conducting experiments to establish the degree and nature of recruitment of the head-direction system as well as suitability for behavioural tasks requiring directional information.

We present a novel experimental apparatus, which combines the advantages of head fixation (access to electrical and optical signals from the animal's brain) and rotational vestibular input. It can be used in an open-loop mode to study vestibular sensory representation and processing, while in closed-loop mode allows animals to navigate in rotational space, providing a better substrate for 2D navigation in virtual environments.

Disclosures: E.A. Rancz: None. **B.A. Horobet:** None. **A.P. Tran-Van-Minh:** None. **Z. Ye:** None.

Poster

577. Vision: Retina: Photoreceptors

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Program #/Poster #: 577.01/DD16

Topic: D.07. Vision

Support: CSP Grant 9798
Cariplo Grant 2013.0738
Telethon Grant GGP14022

Title: High-resolution photostimulation strategy using organic light-sensitive nanoparticles to rescue retinal dystrophy

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Abstract: One of the most common forms of degenerative retinal diseases and leading cause of human blindness is age-related macular degeneration (AMD). The objective of this project is to develop an innovative line of research dealing with the use of light-sensitive organic nanoparticles (NPs) as smart materials for bio-hybrid interfaces that can fully integrate with retinal neurons. Recently, we introduced organic conjugated polymers (CPs) as interfaces for neuronal photostimulation. We developed a planar fully organic device composed of a flexible and highly conformable silk substrate covered with photoactive layers of CPs that, once implanted in the subretinal space of Royal College of Surgeons (RCS) rats, was able to rescue light sensitivity and visual acuity (*Nature Materials 2017, 16(6): 681*). With the aim of improving the spatial resolution of the CP-based organic device to target AMD, by scaling down photoactive devices to the cellular size, we engineered and tested subcellular size CP

nanoparticles (CP-NPs) as a "liquid high-resolution prosthesis" that can be implanted in the degenerate macula with a non-invasive injection. CP-NPs were prepared from freshly prepared poly(3-hexylthiophene) (P3HT) using the re-precipitation technique. We found that P3HT-NPs, injected in the eyes of blind RCS rats, covered most of the subretinal space but remained restricted to the outer retina, in place of the degenerate photoreceptors. Interestingly, the analysis of the light-driven behavior revealed a significant light-sensitivity rescue in dystrophic blind RCS rats injected with P3HT-NPs with respect to control glass spheres of the same size. The recovery of both spatial acuity and of the pupillary reflex was also observed in P3HT-NPs treated animals. Our results highlight a potential clinical relevance of this low-invasive approach in retinal degenerative blindness.

Disclosures: J.F. Maya-Vetencourt: None. **E. Colombo:** None. **C.G. Eleftheriou:** None. **A. Desii:** None. **M. Mete:** None. **M. Zangoli:** None. **F. Di Maria:** None. **G. Barbarella:** None. **G. Pertile:** None. **G. Lanzani:** None. **F. Benfenati:** None.

Poster

577. Vision: Retina: Photoreceptors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 577.02/DD17

Topic: D.07. Vision

Support: Foundation for Fighting Blindness
Vision Science Research Program
Krembil Foundation

Title: Targeting Neogenin as a novel therapeutic approach for the treatment of inherited retinal degeneration

Authors: *J. CHARISH[1], H. HARADA[1], X. WANG[1], S. SETHURAMANUJAM[2], G. B. AWATRAMANI[2], R. BREMNER[3], P. P. MONNIER[4]
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Abstract: Retinitis Pigmentosa (RP), or inherited retinal degenerations, are genetically inherited retinal dystrophies characterized by progressive loss of photoreceptor cells and represent one of the most prevalent causes of blindness among working age populations. Much currently remains unknown regarding the underlying mechanisms of photoreceptor death. We have previously shown that the transmembrane protein Neogenin is involved in regulating cell survival in the CNS. Here we show that Neogenin expression is induced in degenerating photoreceptors in two mouse RP models (Rd1 and Rd10). Degenerating photoreceptors can have abnormally high

levels of cGMP and cAMP, and here we demonstrate that 8-Bromo-cAMP administration was sufficient to induce Neogenin expression in-vivo, in wild type mouse photoreceptors, and in-vitro in human photoreceptor surrogate cells. Using targeted in-vivo electroporation, we then demonstrate that i) overexpressing Neogenin in mouse photoreceptors induces cell death and ii) that silencing Neogenin in degenerating Rd1 photoreceptors promotes survival. This suggests Neogenin acts as a previously unidentified pro-death signal in RP. To develop a potential therapeutic approach for targeting Neogenin in RP, we utilized our Neogenin function blocking peptide (4Ig) that is capable of blocking Neogenin's pro-apoptotic activity. Intravitreal injections of 4Ig were administered at the onset of photoreceptor degeneration in Rd1 and Rd10 mice. When assessed at advanced stages of disease progression, 4Ig treatment significantly improved i) rod photoreceptor survival and ii) cone photoreceptor survival. Photoreceptor function was also shown to be significantly improved following 4Ig treatment as demonstrated by i) improved light-evoked retinal ganglion cell recordings, ii) improved scotopic/photopic electroretinogram recordings and iii) improved visual acuity (OptoMotry; CerebralMechanics). Targeting Neogenin therefore represents an exciting new approach for the treatment of RP.

Disclosures: J. Charish: None. **H. Harada:** None. **X. Wang:** None. **S. Sethuramanujam:** None. **G.B. Awatramani:** None. **R. Bremner:** None. **P.P. Monnier:** None.

Poster

577. Vision: Retina: Photoreceptors

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Program #/Poster #: 577.03/DD18

Topic: D.07. Vision

Support: NRF-2017R1D1A1B05028221

Title: Protective effects of zinc and cAMP against A2E-induced toxicity in ARPE19 cells: Possible involvement of lysosomal acidification

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Abstract: Dry age-related macular degeneration (AMD) is characterized by accumulation of drusen and degeneration of photoreceptor cells and retinal pigment epithelial (RPE) cells. It has been proposed that dysfunctional lysosomes in RPE cells contribute to dry AMD pathology by hindering the degradation of shed photoreceptor membranes. We have previously shown that raising intracellular zinc levels can restore lysosomal acidity, and several studies have shown that

raising cAMP levels may restore acidity and degradative functions of lysosomes. In the present study, we examined the effects of zinc and cAMP on lysosomal alkalization and dysfunction in an *in vitro* model of AMD. To induce lysosomal dysfunction in ARPE19 (human RPE cell line), we used A2E (lipofuscin derivative). We quantitatively assessed A2E-induced cell death by measuring the amount of lactate dehydrogenase (LDH) released into the culture medium. In addition, we observed the effects of zinc and dibutyryl cAMP on lysosomal acidity and degradative functions in A2E-treated ARPE19 cells. Lysosomal pH of the cells treated with cAMP or clioquinol (ClioQ, zinc ionophore) was measured by using LysoTracker. Twenty-four hours after A2E treatment, ARPE19 cells exhibited autofluorescence throughout the cell body, and showed significant amount of cell death (69.2 ± 4.9 % LDH release). Addition of clioquinol or dibutyryl cAMP significantly reduced cell death by 20 - 50% in both cases ($P < 0.05$). A2E was seen to accumulate in endosomes and lysosomes, and LysoTracker signals faded, signifying lysosomal alkalization. Moreover, both zinc and cAMP decreased A2E autofluorescence and restored lysosomal pH back to the acidic range. Our results support the possibility that adequate levels of zinc or cAMP may help overcome A2E-induced toxicity in ARPE19 cells that contribute to the pathogenesis of AMD.

Disclosures: J. Choi: None. **B. Seo:** None. **J. Koh:** None. **Y. Yoon:** None.

Poster

577. Vision: Retina: Photoreceptors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 577.04/EE1

Topic: D.07. Vision

Support: NIH T32 EY025202
NIH 1DP2EY022584

Title: A subpopulation of GABAergic intrinsically photosensitive retinal ganglion cells in the mouse retina

Authors: *T. SONODA, T. M. SCHMIDT
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Abstract: The mammalian retina contains three classes of photoreceptors: rods, cones and intrinsically photosensitive retinal ganglion cells (ipRGCs). ipRGCs directly project to over 10 brain areas to mediate a wide range of visual behaviors such as circadian photoentrainment, pupil constriction, and contrast detection. Current evidence points to ipRGCs executing these functions by primarily releasing the excitatory neurotransmitter glutamate and the peptide transmitter pituitary adenylate cyclase-activating peptide (PACAP). Here, we report that a small population of ipRGCs are GABAergic. This population of GABAergic ipRGCs project to the

suprachiasmatic nucleus (SCN) and the intergeniculate leaflet (IGL), which suggests that they are primarily involved in circadian photoentrainment. These results identify a novel inhibitory circuit mediated by retinal ganglion cells in the mouse visual system.

Disclosures: T. Sonoda: None. **T.M. Schmidt:** None.

Poster

577. Vision: Retina: Photoreceptors

Location: SDCC Halls B-H

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Program #/Poster #: 577.05/EE2

Topic: D.07. Vision

Support: CSIR, N Delhi, 37/1593/13/EMR-II
ICMR, N Delhi, IR-480

Title: Effect of long-term iron administration on retinal photoreceptor cells

Authors: *P. KUMAR[1], T. C. NAG[1], T. S. ROY[1], T. VELPANDIAN[1], S. WADHWA[2]
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Abstract: Iron accumulates in many organs with age. Iron overload is a causative factor in several neurodegenerative diseases. In this study, we investigated the effect of long-term, oral iron administration on the photoreceptor cells in rat retina. At 2 months of age, rats were treated orally with ferrous sulphate (500 mg/kg body weight/week), which was continued up to 17.5 months. Electroretinography (ERG), photoreceptor ultrastructural changes and markers of mitochondria (SOD-2 and VDAC1) and autophagy (LC3-II and beclin-1) were examined at different ages (8, 14 and 20 months). In contrast to controls, in iron-accumulated retina, the mitochondria of photoreceptor inner segments were highly disorganized, which also showed a decrease in the expression of SOD-2 and VDAC1 in retinal extracts, in 14 month- and 20-month-old rats. Electron microscopy revealed signs of autophagy in photoreceptor inner segments in both groups, which paralleled with the increased expressions of LC3-II and beclin-1, as detected by immunoblotting. Together with the earlier findings, the present data indicate that photoreceptor damage due to iron accumulation involves not only the outer segments, but also the inner segment mitochondria and that autophagy is induced in photoreceptor inner segments to maintain tissue homeostasis in the iron-accumulated aged retina.

Disclosures: P. Kumar: None. **T.C. Nag:** None. **T.S. Roy:** None. **T. Velpandian:** None. **S. Wadhwa:** None.

Poster

577. Vision: Retina: Photoreceptors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 577.06/EE3

Topic: D.07. Vision

Support: NIH Director's New Innovator Award
Klingenstein-Simons Fellowship in the Neurosciences
Karl Kirchgessner Vision Research Grant

Title: Differential distribution of molecularly distinct M1 intrinsically photosensitive retinal ganglion cells in mouse retina

Authors: *S. LEE, T. M. SCHMIDT
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Abstract: Melanopsin-expressing, intrinsically photosensitive retinal ganglion cells (ipRGCs) are a class of atypical, ganglion cell photoreceptor. The M1 subtype of ipRGCs serves ambient irradiance detectors mediating non-image forming visual behaviors such as circadian photoentrainment and the pupillary light reflex. Despite initial reports of homogeneity within the M1 population, recent reports have suggested that M1 ipRGCs can be molecularly subdivided based on whether they express the transcription factor Brn3b (Brn3b+ M1 and Brn3b- M1). The distribution of RGCs in retina is correlated with what environmental light they encode for visual behaviors. Although reports have shown the distribution pattern of M1 ipRGCs in retina, how Brn3b+ and Brn3b- M1 ipRGCs are distributed has not been determined yet. We therefore performed immunohistochemistry for Brn3b and beta-galactosidase in a whole-mount retina of *Opn4*$^{LacZ/+}$ mouse to identify the location of Brn3b+ and Brn3b- M1 ipRGCs. The retinal location was identified by unbiased poking before enucleation. The number of Brn3b+ M1 ipRGCs are 3-fold lesser than Brn3b- M1 ipRGCs. Brn3b+ M1 ipRGCs are significantly more distributed in the ventrotemporal retina than the dorsonasal retina. In contrast, Brn3b-expressing cell are significantly more found in the dorsonasal retina than in the ventrotemporal retina. Brn3b- M1 ipRGCs are found every region in the retina but with a significantly lesser distribution in the ventronasal retina than dorsonasal retina. Collectively, these results suggest that Brn3b+ and Brn3b- M1 ipRGCs have distinct distribution pattern in the retina, preferring to the ventral and dorsal region, respectively, and that Brn3b expression pattern in M1 ipRGCs does not parallel to typical Brn3b expression pattern in retina.

Disclosures: S. Lee: None. **T.M. Schmidt:** None.

Poster

577. Vision: Retina: Photoreceptors

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Program #/Poster #: 577.07/EE4

Topic: D.07. Vision

Support: NIH grant EY012128

Title: The role of syntaxin 3 in the human retina

Authors: *R. JANZ, S. PUNURU, X. LIU, R. HEMMATI, R. HEIDELBERGER
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Abstract: Syntaxin 3 is a t-SNARE protein, thought to be involved in the trafficking of vesicles in epithelial cells, the exocytosis of secretory granules in mast cells and pancreatic beta cells, as well as the exocytosis of synaptic vesicles in ribbon synapses of the retina. Previous studies in mouse and goldfish have shown that the syntaxin 3 gene expresses two major transcripts by differential splicing named syntaxin 3A and 3B. Syntaxin3B is expressed in photoreceptor and bipolar cells of the retina, while syntaxin 3A is expressed in non-neuronal cells. Patients with mutations in the human syntaxin 3 gene suffer from microvillus inclusion disease (MVID), a disorder of the intestinal epithelium (Wiegerinck et al., 2014). There have also been reports that MVID patients with mutations in the syntaxin 3 gene suffer from vision defects, indicating a role of syntaxin 3 for normal retina function. As a prerequisite for a better understanding of the role of syntaxin 3 in the human retina, we first investigated the expression of the syntaxin 3 gene in different human tissues using RT-PCR analysis. We demonstrated that syntaxin 3B is expressed at high levels in the human retina but only expressed at very low levels in other human tissues. In contrast, syntaxin 3A is expressed at high levels in most human tissues, including small intestine and retina, with the exception of the brain and muscle where the transcript is only expressed at very low levels. Next, we investigated the distribution of syntaxin 3 in the human retina by immunolabeling with antibodies that recognize both syntaxin 3A and 3B. Similar to the pattern found in the mouse and goldfish retina, syntaxin 3 was detected in the ribbon synapses of photoreceptors and bipolar cells. However, in contrast to the mouse where the majority of syntaxin 3 is found in the ribbon synapses with some weak labeling of the photoreceptor inner segments, we detected strong labeling with different syntaxin 3 specific antibodies in the outer segments of the rod and cone photoreceptors. This indicates that in the human retina, syntaxin 3 is probably also involved in trafficking processes in the outer segments of the photoreceptors in addition its role in synaptic vesicle exocytosis at ribbon synapses.

Disclosures: R. Janz: None. **S. Punuru:** None. **X. Liu:** None. **R. Hemmati:** None. **R. Heidelberger:** None.

Poster

577. Vision: Retina: Photoreceptors

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Program #/Poster #: 577.08/EE5

Topic: D.07. Vision

Support: NRF Grant 2017M3A9E2062685

Title: Effect of electrical stimulation on mouse retinal tissues via microelectrode array

Authors: *H. YOO[1], H. YOON HEE[1], H. JEONG[1], S. HWANG[1], S. JUN[1,2]
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Abstract: Recently, the restoration of sight has been enabled by implantable visual prosthetic system for patients blinded by retinal degeneration. The visual prosthetic system, also known as retinal prostheses, delivers electrical stimulation via microelectrode array attached on the surface of retina, evoking action potentials of surviving retinal neurons even though there exists no photoreceptors. Although the retinal prostheses have been applied for clinical applications, there still exist remaining challenges. One of them is the uniform and loose attachment of electrode arrays on to the retinal surface mostly due to the curvature of the retinal surface. To investigate this issue, in this study, as a first step, electrical stimulation is applied to retinal tissues on microelectrode array to simultaneously monitor the evoked activity from multiple locations at different distances between retina and electrode. The distance between the stimulating electrode and the retinal tissue varied to mimic the irregular electrode attachment of implanted artificial retina. It is expected that the results help establishment of safe and effective electrical stimulation parameters in retinal prosthetic devices.

Disclosures: H. Yoo: None. **H. Yoon Hee:** None. **H. Jeong:** None. **S. Hwang:** None. **S. Jun:** None.

Poster

577. Vision: Retina: Photoreceptors

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Program #/Poster #: 577.09/EE6

Topic: D.07. Vision

Support: CSIR Junior Research Fellowship c-2080
DST-318
SERB, (SR/SO/AS-027/2012, TCN)

Title: Changes in photoreceptor synapses and expressions of BDNF and Trk-B in postnatal chick retina exposed to light of variable photoperiods

Authors: *M. MAURYA, T. C. NAG, T. S. ROY
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Abstract: Synaptic ribbons (SR) are unique structural features of photoreceptors that enable them to encode and transmit light response. Alterations in photoperiod and light intensities cause damage to the photoreceptor synapses, though the detailed mechanisms of retinal synaptic degeneration after light stress are unclear. The aim of this study was to understand the effect of bright light and photoperiod on SR length in cone dominated retina. Role of brain derived neurotropic factor (BDNF) and its receptor (Trk-B) was also evaluated. One day-old chicks (*Gallus gallus domesticus*) were acclimated in normal 12 h light –12 h dark cycle (12L: 12D) for 7 days (400 lux), followed by exposure to high intensity light (5000 lux, experimental) and 400 lux (control) at 12L: 12D, 18L: 6D and 24L: 0D conditions. Chicks were sacrificed and their eyes enucleated at 24h and 168h intervals. Transmission electron microscopy (TEM) of SR at 168h revealed that SR length was reduced significantly from 0.374 to 0.348 µm in 12L:12D vs 18L:6D, while the number of cone SR was reduced from 509 to 478 between the two groups and in constant light group, the values were 0.324 µm and 452. Retinal cryosections, immunolabelled with BDNF and Trk-B antibodies showed the significantly high number of BDNF immunoreactive neurons in inner nuclear ($p = 0.03$) and photoreceptor layer ($p = 0.03$) after exposure to 400 lux for 24h duration in 18L: 6D group compared to that in 12L:12D group, whereas after 168h interval, it increased in photoreceptors in 18L:6D ($p = 0.01$) and 24L:0D ($p = 0.01$) groups, compared to that in 12L:12D group. Immunoblotting revealed a reduction in BDNF level in retinas exposed to 5000 lux light for 24h interval under 24L:0D photoperiod ($p = 0.04$). Similarly, a decrease in Trk-B level was also noted in retinas exposed to 5000 lux for 24h duration under 18L:6D photoperiod ($p = 0.01$) and 24L:0D photoperiod ($p = 0.03$). These results indicate altered regulation of synaptic transmission due to changes in ribbon morphology and number. Increased number of BDNF positive photoreceptors and inner nuclear layer cells in 18L: 6D group and in photoreceptors in 24L: 0D group implicate a role for BDNF and its receptor (Trk-B) in neuroprotection against light induced stress in the retina.

Disclosures: M. Maurya: None. **T.C. Nag:** None. **T.S. Roy:** None.

Poster

577. Vision: Retina: Photoreceptors

Location: SDCC Halls B-H

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Program #/Poster #: 577.10/EE7

Topic: D.07. Vision

Title: Histology of the eye of the crepuscular crab

Authors: *J. R. BARRADAS[1], E. VALERO-PACHECO[3], M. ALVARADO[2], P. PACHECO[4], F. ROJAS[5], F. ALVAREZ[6]
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Abstract: Ocypode quadrata is a semi-terrestrial coastal crab, with crepuscular habits, which is active 19 hours a day. It has pedunculated eyes with a 360 ° vision. You can appreciate the flight of insects and capture them in the air. It has been proposed that this crab has compound eyes. However, so far the eyes of this species have not been studied in detail. The compound eyes can be of two types: of apposition, present in diurnal species and of superposition present in nocturnal species. O. quadrata is exposed to light variations and intermittent changes from an aquatic to a terrestrial environment. So it is conjectured that the cellular structure of the eye of this species is of intermediate type to eyes composed of apposition and superposition. In the present work, the cellular structure of the eye of O. quadrata was described by the Histological Technique of Paraffin, Hematoxylin and Eosin Stain, and Scanning Electron Microscopy. The tissue subjected to the Paraffin Technique and Hematoxylin & Eosin Stain. It was processed for longitudinal and transverse histological sections of 10 μm with a crank microtome. The permanent preparations of these cuts were observed, photographed and analyzed by means of an optical microscope with a digital camera. It was identified that the ocular structure of O. quadrata is composed of four layers of tissues: the cornea, the lens, the rhabdomoma and the dendrites. The cornea is divided into three layers: the cuticle, the corneogena cuticle and the distal pigment cells. The cone-shaped lens possesses: interomatidial pigment, retinal cell pigment and retina cells. The Rabdon has: proximal pigment cells, cone cells in process, basal membrane and basal pigment. Finally, the dendrites are responsible for taking the captured information to the tapetum region, where ganglion cells are found responsible for sending information to the brain ganglion through a set of axons that make up the optic nerve. According to the ocular structure observed, it is described as an eye composed of interposition type, considering that there are gradual steps between apposition and superposition.

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Poster

577. Vision: Retina: Photoreceptors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 577.11/EE8

Topic: F.01. Neuroethology

Title: Unique dual rhabdom organization in the fusion stemmata of the firefly (Photuris sp.) larval visual system

Authors: *F. L. MURPHY, A. MOISEFF
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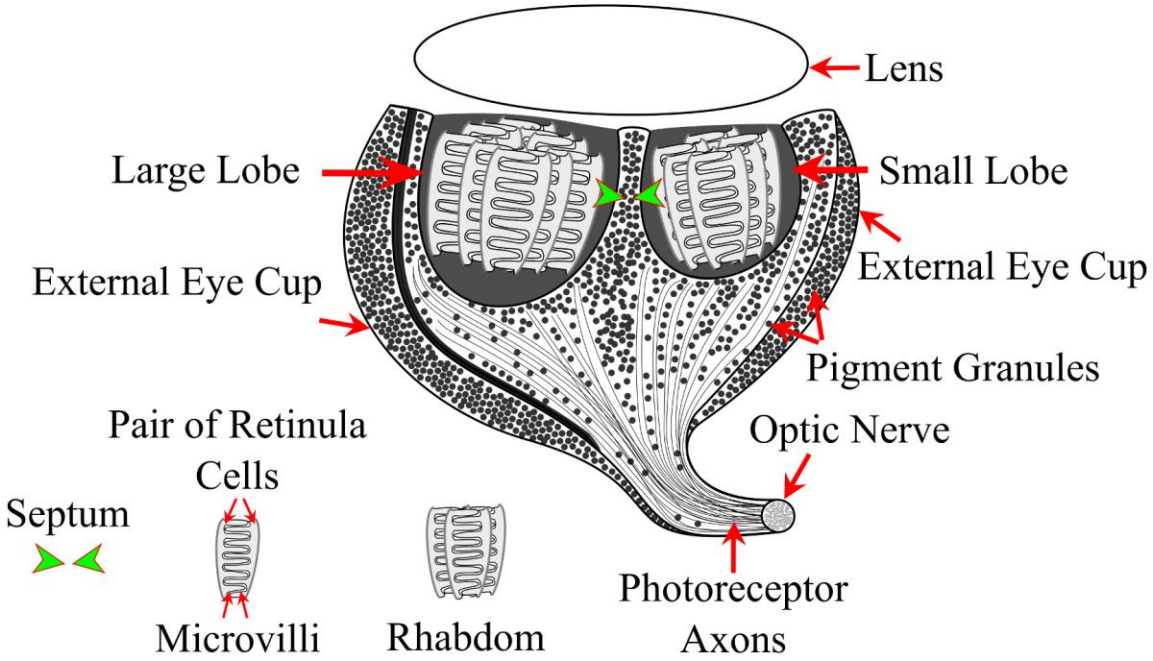
Abstract: Fireflies, as holometabolous insects, have distinctly different visual systems as larvae compared to adults. Adults have a pair of extensively studied compound eyes. Larvae, by contrast, have a pair of stemmata, whose structure and function are unknown. Here, we present the structure of the firefly (*Photuris* sp.) larval stemmata.
Firefly stemmata (i.e., eyes) were located bilaterally on the antero-lateral surface of the head. Each eye had a single, simple lens (diameter ~130µm) and a densely pigmented, asymmetrical eye cup. At its widest point, the diameter of the eye cup was ~150µm, which tapered towards the base of the eye. The optic nerve, originating from the base of each eye, was ~30µm diameter and contained 88 axons (± 0.87, n=4).
Within the eye cup, dense pigmentation surrounded two regions, which we referred to as lobes. Each lobe was asymmetric in size (~256µm; ~189µm cross sectional perimeter of each lobe's superior surface) and devoid of pigment granules. Of particular note, a septum, consisting of a dense band of screening pigment perpendicular to the inferior surface of the lens was oriented along the antero-posterior axis and separated the two lobes.
Ultrastructure of the stemmata revealed that each lobe was a large rhabdom composed of multiple retinula cells. Retinula cells were arranged in pairs where the microvilli of neighboring rhabdomeres interlocked. These photoreceptor (PR) pairs were arranged radially within each rhabdom. The gross anatomy of the visual neurons was accomplished by backfilling of the optic nerve with texas red. 3D reconstruction of texas red labelled neurons revealed that PRs were arranged in vertical columns which extended the depth of each lobe.
The identification of this dual rhabdom system with 88 PRs is consistent with the eye being formed as a fusion-stemmata, an occurrence in holometabolous evolution where multiple ommatidia conjoin forming the larval eye. We believe that the anatomy of the firefly *Photuris* larval stemmata, specifically the rhabdom organization within the dual lobes and PR structure is unique among holometabolous stemmata.



Disclosures: F.L. Murphy: None. **A. Moiseff:** None.

Poster

577. Vision: Retina: Photoreceptors

Location: SDCC Halls B-H

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Program #/Poster #: 577.12/EE9

Topic: F.01. Neuroethology

Support: NIH 1R15EY027112-01A1

Title: The cytoarchitecture of the degenerating eye if the blind cavefish Astyanax mexicanus

Authors: *D. SOARES[1], M. YOFFE[2], Z. TANVIR[2], S. ALI[3]
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Abstract: Astyanax mexicanus is a teleost that has adapted to cave environments approximately 2 mya. Its closest living ancestor is still extant on the rivers outside the caves. During development, cavefish larvae develop retinas and lenses, but as the larva grows the lenses which subsequently undergo apoptosis and the eyes sink into the orbits. Embryonic lens transplantation from a surface larva donor onto a cavefish eye cup rescues the eye. Retinas that have been

rescued are generally presumed to be functional, and that all components are present and normal. Here we use comparative immunohistochemistry, expansion microscopy and transmission electron microscopy to show that the retinal layers is already disorganized. Retinal ganglion cells do not form a tightly organized layer and are intertwined with other cell types. Their projections onto the Optic Tectum follow the same timeline. The plexiform layer has synapses in both forms of the fish, and we have quantified smaller differences. The photoreceptor layer is particularly misshapen, with fewer cells that have much longer outer segments. The discs are not stacked but there are similar numbers of mitochondria in the inner segment. It appears that the cytoskeletal structure of the photoreceptors is malformed. We propose that the retinal organization is already lost in early stages of development and that at closer scrutiny; lens transplantation will likely not completely restore the retina.

Disclosures: D. Soares: None. **M. Yoffe:** None. **Z. Tanvir:** None. **S. Ali:** None.

Poster

578. Visual System: Responses During Behavior

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Program #/Poster #: 578.01/EE10

Topic: D.07. Vision

Support: NIH Grant U01-NS094330
NIH Grant T32-EY007125

Title: Presaccadic modulation of sensory responses in primary visual cortex

Authors: *J. YATES[1], S. H. COOP[3], J. F. MITCHELL[2]
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Abstract: Primates actively sample visual input using rapid eye movements (saccades). This active sampling means that during natural vision, stimuli typically enter the receptive fields of visual neurons because they were brought there by eye movements. This is in contrast to most visual experiments, where visual stimuli appear de novo at locations in visual space. Despite half a century of research on the response properties of neurons in primary visual cortex (V1), we do not fully understand how saccadic eye movements modify the processing and transmission of sensory signals. Here, we studied the representation of visual input in V1 of marmoset monkeys freely viewing visual stimuli. We measured the selectivity of V1 neurons to orientation and spatial frequency, as well as their response gain, immediately before and after saccadic eye movements. We found that saccades produced substantial post-saccadic firing rate modulations in almost all neurons recorded. These modulations resulted from changes in response gain as well as additive increases in spike rate. Importantly, some neurons exhibited pre-saccadic

response gain, implying extraretinal signals can modify encoding in V1 immediately prior to eye movements. Ongoing work is quantifying the nature of this presaccadic gain and its consequences for the cortical representation of visual input.

Disclosures: J. Yates: None. **S.H. Coop:** None. **J.F. Mitchell:** None.

Poster

578. Visual System: Responses During Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 578.02/EE11

Topic: D.07. Vision

Support: Nihon University Multidisciplinary Research Grant (M17-012) (2017)

Title: Visual strategies of elite athletes during attacks

Authors: *M. TAKAYOSE[1], Y. SATO[2], M. FUKAMI[2], H. SATO[3], T. HIRAKI[4], R. KOSHIZAWA[4], S. UMESHITA[5], S. SHIROMA[6]
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Abstract: The information obtained from the visual system contributes to optimal motor control. An athlete must have a visual strategy to obtain valuable information and apply it to achieve an effective performance. Further, elite athletes may employ characteristic visual strategies. However, visual strategies used during dynamic movement has not yet been revealed. The purpose of this study was to clarify the visual strategies of elite athletes from eye movement patterns and cerebral activity recorded while attacking.
The participants were six female elite fencers, including an Olympian, and two male elite boxers. In Experiment 1, the participants in a standing state gazed at a fixation point 3 meters away from them (gaze condition) and then looked at the same point in an unfocused manner (fuzzy condition). In Experiment 2, the participants played competitively in the game style. Eye tracking, electroencephalography (EEG), electromyography (EMG), and a high-speed camera were used to record eye movement patterns, cerebral activity, muscle activity, and body motion during the tasks. Five successful attacks that won a point were analyzed in Experiment 2.
The peak frequency of the EEG power spectrum in cortical visual areas was lower during the fuzzy condition than the gaze condition in Experiment 1. In Experiment 2, beta power during attacks was equivalent to that during the fuzzy condition and lower than that in the gaze condition. The theta power during attacks was equivalent to that during the fuzzy condition and greater than that during the gaze condition. In the attack phase, the eye movement patterns of the

elite athletes showed that they directed their gaze to the specific points of the opponent. Although elite athletes aim their line of sight to important places for successful attacks, the results suggest that they look at the opponent with a peripheral field of view rather than gazing at a particular object or position.

Disclosures: M. Takayose: None. **Y. Sato:** None. **M. Fukami:** None. **H. Sato:** None. **T. Hiraki:** None. **R. Koshizawa:** None. **S. Umeshita:** None. **S. Shiroma:** None.

Poster

578. Visual System: Responses During Behavior

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Topic: D.07. Vision

Support: European Regional Development Fund (ERDF: Center for Behavioral Brain Sciences; J.P. and J.H.)
Marie Curie IEF 624461 (J.P.)
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Patrick Wild Center (N.R.)
Shirley Foundation (N.R.)

Title: Active task engagement and congruent visuomotor feedback enhance experience-dependent network activity in mouse primary visual cortex

Authors: *J. M. PAKAN[1,2,3], E. DYLDA[4], J. U. HENSCHKE[1,2], S. P. CURRIE[4], N. L. ROCHEFORT[4,5]
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Abstract: Constant adaptation to the environment is vital for survival throughout an animal's lifespan. Accordingly, various forms of adult experience-dependent plasticity have recently been demonstrated even in early sensory processing pathways. However, the extent to which neuronal activity is altered when an animal is actively learning to map sensory stimuli to behaviour compared to passively experiencing stimuli, remains controversial. In the primary visual cortex (V1), several recent studies have reported either a stimulus-specific response potentiation to a repetitively presented stimulus (without any associated reward or aversive stimuli), stimulus-specific decreases in the number of visually responsive neurons in both passive and active learning tasks, or conversely, an increase in the number of visually selective neurons to

behaviourally-relevant stimuli during active learning. Part of the inconsistency in these results may stem from the presence or absence of congruence between an animal's self-motion and optic-flow information. In this study, we performed two-photon calcium imaging of layer 2/3 neurons in awake-behaving head-fixed mice to assess population activity in V1 before, during and after both passive and active learning tasks. Mice were able to freely run on a circular treadmill and visuomotor feedback was either matched (motor output congruent with optic-flow information) by utilizing a virtual reality environment, or mismatched by passively presenting visual stimulation at a fixed temporal frequency regardless of the animal's speed. We found that active learning in a visuomotor matched task increased the proportion of neurons that were responsive to a repeatedly presented behaviourally relevant stimulus; conversely, this effect was not seen during passive viewing of a repeatedly presented stimulus. While the accuracy of a decoder to determine stimulus identity from V1 population activity for the visuomotor matched and mismatched conditions was equivalent, we found that overall neuronal activity as well as the average pairwise correlation between neurons was increased during the mismatched condition. Therefore, experience-dependent changes in V1 are facilitated by active task-engagement and visuomotor congruence to efficiently alter the representation of a behaviourally-relevant visual stimulus across learning. Altogether, these results support the view of a dynamic regulation of visual information processing in V1 based on the behavioural and ecological relevance of the sensory input.

Disclosures: J.M. Pakan: None. **E. Dylda:** None. **J.U. Henschke:** None. **S.P. Currie:** None. **N.L. Rochefort:** None.

Poster

578. Visual System: Responses During Behavior

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Program #/Poster #: 578.04/EE13

Topic: D.07. Vision

Support: Nc3Rs David Sainsbury Fellowship

Title: Measuring mouse vision using innate behavioral responses

Authors: *R. STORCHI
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Abstract: Optogenetics and stem cell based treatments provide new opportunities for treating retinal dystrophies [1, 2]. An important step in evaluating treatment effectiveness is represented by behavioural assays of mouse vision. Many commonly used tests are based on sub-conscious, reflex responses [3, 4] whose activity is only indirectly related to perceptual vision. More relevant tests largely rely upon learned associations between visual stimuli and conditioned

stimuli [5] and are inherently throughput because they require long training periods and only allow association with single visual stimuli.
An alternative and more humane approach to assess vision relies on measurements of mouse spontaneous behaviour. These tests rely on the hypothesis that when mice detect a change in their visual environment they naturally change their behaviour. However they are currently low throughput as they rely on very simplified measures of behaviour such as average distance moved [1] or time required to move from a light to a dark area [6].
Here we show that combining a better experimental design with more sophisticated behavioural measures based on changepoint analyses [7] we can obtain reliable high throughput readouts of mouse vision. We designed an open field apparatus to capture mouse behaviour simultaneously with multiple cameras while stimulating the upper visual field and to perform reliable tracking of multiple body parts. We performed three series of experiments designed to capture a large repertoire of innate behavioural responses that allowed us to measure contrast sensitivity and visual acuity. In order to validate the method we repeated the same experiments in visual intact mice and in a mouse model of retinal degeneration (rd1). Results indicate that our method can capture the limit of mouse visual acuity in intact animals and also detect residual cone function in animals affected by severe retinal degeneration.

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DOI:10.1016/j.jneumeth.2015.08.007
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Disclosures: R. Storchi: None.

Poster

578. Visual System: Responses During Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 578.05/EE14

Topic: D.07. Vision

Title: Visuomotor reflexes differ across *Drosophila* species

Authors: I. D'ALESSANDRO, E. J. PARK, *S. M. WASSERMAN
Wellesley Col., Wellesley, MA

Abstract: To generate adaptive behavior, an organism must identify and assign subjective value to salient sensory information. However, what stimuli are deemed salient could change depending upon the local environment. Insects, such as fruit flies (*Drosophila*), for example, rely upon olfactory cues to locate food and oviposition sites. However, not all *Drosophila* species find the same sensory stimuli to be salient. Work done investigating host preferences of four geographically isolated populations of *Drosophila* mojavensis, cactophilic flies that feed and oviposit on necrotic cacti, has revealed olfactory driven behavioral preferences for host cacti specific to the local environment of each population. Similar to olfactory adaptations driven by the variation of host plants across different ecological environments, we wondered whether visual features specific to certain environments could drive divergent visuomotor responses. To examine this, we compared the visuomotor reflexes of D. melanogaster, a cosmopolitan generalist, found in visually dense environments, with D. mojavensis, a cactophilic specialist found in comparatively sparse visual landscapes. We used an electronic flight simulator in which flies are rigidly tethered to a pin and suspended in an LED arena and their steering direction and magnitude measured. Our results reveal the first evidence to suggest variability in visuomotor reflexes across *Drosophila* species.

Disclosures: I. D'Alessandro: None. **E.J. Park:** None. **S.M. Wasserman:** None.

Poster

578. Visual System: Responses During Behavior

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Topic: D.07. Vision

Support: Swiss National Science Foundation BSSGI0_155795

Title: Learning from the past and predicting the future: The role of auditory and retrosplenial cortex input on coding in visual cortex during associative learning

Authors: *A. R. GARNER, G. B. KELLER
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Abstract: How does information from the past affect processing of information in the present in sensory cortex? Primary visual cortex (V1) receives afferent fibers from a number of classically non-visual regions including auditory cortex and retrosplenial cortex, a region known to be involved in associative and contextual learning. Using 2-photon calcium imaging in mice engaged in an auditory-visual classical conditioning paradigm in a virtual reality environment we investigated the functional input patterns in V1 of auditory and retrosplenial afferent axons. We then optogenetically stimulated axons from these regions in V1 to measure which V1 soma could be functionally influenced by the long-range fibers, and compared activity of influenced soma

with the rest of the population during learning. Our results revealed strong visual responses in afferent axons and these responses were modified with learning. Additionally, auditory-influenced and retrosplenial-influenced V1 populations changed activity patterns differentially with learning. Finally, V1 population activity specifically during the visual stimulus could be used to decode whether or not the visual stimulus had been preceded by an auditory stimulus. Our results suggest that long-range input is converted into local processing coordinates and allows coding of sensory stimuli as a function of their relationship to other stimuli and the context in which they are presented already at the level of primary sensory cortex. Moreover, our results suggest that auditory cortex input aids V1 in predictive coding of visual stimuli using auditory cues, while retrosplenial input specifically facilitates coding of stimuli with learned relevance.

Disclosures: A.R. Garner: None. **G.B. Keller:** None.

Poster

578. Visual System: Responses During Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 578.07/FF2

Topic: D.07. Vision

Title: Visual psychophysical measurements in head-fixed mice with classical conditioning

Authors: O. ARROYO, Jr[1], *N. W. OESCH[2]
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Abstract: One of the main goals of retinal neurophysiology is to understand how physiological mechanisms in the retinal contribute to visual processing and perception. Progress has been made by simply inferring how retinal mechanisms contribute to visual perception; however, the ability to directly measure visual behaviors is ideal in the identification of supporting mechanisms. Previously, this has been problematic as visual psychophysics in non-human species traditionally requires extensive training and noisy responding requires many trials to get good psychometric curves. In rodents, the current animal of choice for retinal neurophysiological studies, visual psychophysics has been particularly challenging. Here we developed a simple, classically conditioned visual detection task in head-fixed mice that can be trained in less than 3 days. Water deprived mice learn to associate water reward (US) with a visual stimulus (CS+), and developed an anticipatory licking response (CR) in response to the conditioned CS+. Anticipatory lick responses emerged within one hundred pairings. The anticipatory lick response followed psychometric visual detection curves comparable to prior mouse visual detection behavior tasks, hence providing a sensitive detection measure of the visual stimulus. Multiple aspects of anticipatory licking behavior, such as lick probability, lick rate, delay to lick, and lick rate acceleration are continuously modulated over a range of visual stimulus discriminability.

These multiple measures provide a robust examination of visual stimulus discriminability, and psychometric curves can be determined from a single session of less than 1.5 hours in duration. In addition, the CS+ is readily generalized to other similar stimuli, thereby allowing us to examine a variety of different visual stimuli without extensive retraining. Together, this technique represents a flexible behavioral tool to examine visual perceptual behavior in mice, with relatively little training and high signal to noise minimizing the number of trials needed. The head-fixed preparation allows for tight control of the stimulus inputs and can easily be combined with a variety of in vivo manipulations and ex vivo mechanistic assessments.

Disclosures: O. Arroyo: None. **N.W. Oesch:** None.

Poster

578. Visual System: Responses During Behavior

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Topic: D.07. Vision

Support: ISF grant 961/14 to ID

Title: Neural variability quenching increases with learning

Authors: *A. ARAZI[1,2], I. DINSTEIN[3,2,1]
[1]Dept. of Brain and Cognitive Sci., [2]Zlotowski Ctr. for Neurosci., [3]Dept. of Psychology, Ben Gurion Univ., Beer Sheva, Israel

Abstract: Background: Neural responses to an identical sensory stimulus vary across trials. This trial-by-trial variability is relatively large before stimulus presentation and significantly reduced (i.e. quenched) after stimulus presentation. Greater magnitudes of variability quenching were previously reported in trials where weak sensory stimulus was accurately detected, in trials with shorter reaction times, and in subjects with better perceptual thresholds. These studies suggest that reduced neural variability following stimulus presentation is associated with better perceptual performance. However, other studies have reported that individuals with larger overall moment-to-moment variability throughout the entire experiment exhibit faster learning of new motor skills and better cognitive performance. These studies have suggested that more variable neural networks can move flexibly and explore different states more effectively. Here, we examined the magnitude of neural variability as subjects learned to perform an orientation discrimination task with improved accuracy and speed. **Methods:** Twenty-seven subjects performed a forced-choice orientation discrimination task while their neural activity was recorded with EEG. In each trial, a circle with black and white stripes appeared on the screen and subjects were asked to report whether the stripes were oriented to the right or left. The angle of orientation changed across trials using a staircase procedure such that performance was set to

70% accuracy. Subjects completed 10 blocks of 120 trials and we quantified the mean angle, reaction time, and trial-by-trial EEG variability, for each of the blocks. **Results:** Subjects exhibited significant improvement in orientation angle and reaction time between the first and last block of the experiment demonstrating that they learned the task. The magnitude of neural variability quenching was significantly larger in the last block as was the magnitude of pre-stimulus variability. **Conclusions:** Subjects improved their performance throughout the experiment, exhibiting lower discrimination thresholds and shorter reaction times. This improvement was accompanied by an increase in pre-stimulus trial-by-trial variability and an increase in variability quenching, suggesting a possible link between perceptual learning and the magnitude of neural variability.

Disclosures: A. Arazi: None. **I. Dinstein:** None.

Poster

578. Visual System: Responses During Behavior

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Program #/Poster #: 578.09/FF4

Topic: D.07. Vision

Support: EY024662

Title: All-optical stimulation and imaging in macaque V1 reveals neural and behavioral masking effects of optogenetic stimulation in a threshold detection task

Authors: *S. C.-Y. CHEN, G. BENVENUTI, M. P. WHITMIRE, Y. CHEN, W. S. GEISLER, E. SEIDEMANN
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Abstract: To understand the neural basis of perception, we need tools for measuring and manipulating neural population responses in behaving animals. Optical-genetic methods provide a powerful tool for achieving this goal, but the use of these techniques in behaving macaques, an important animal model for studying human perception, has been limited. Here we used rAAVs to co-express a red-shifted opsin (C1V1) and a calcium indicator (GCaMP6f) in excitatory neurons in macaque V1. We then used widefield imaging to measure GCaMP6f response to visual stimuli and to C1V1 optogenetic stimulation. Robust response was recorded to 0.6 mW/mm^2 stimulation, a level much lower than previously reported. Even at this low light level, stimulation-evoked response could be larger than visually-evoked response to optimal stimuli. We hypothesized that the stimulation evoked-activity will interact in V1 in a sublinear way with visual responses, thereby reducing neural and behavioral sensitivity in visual detection tasks. To test this hypothesis, we applied optogenetic stimulation while a monkey detected a small Gaussian target (0.33° FWHM) at a retinotopic position corresponding to a co-expression site

(ecc. ~1.5°). The monkey indicated the presence of the target (50% of the trials) with a saccade to the target location. We compared the monkey's performance in separate blocks with stimulation (in all trials) and with no stimulation. Visual and optogenetic stimulation lasted up to 250 ms, and were terminated as soon as the monkey initiated a saccade. Our behavioral and neural measurements were consistent with our hypothesis. Across several experiments using light intensities between 0.6 to 2.2 mW/mm^2, we found that the monkey's detection threshold with stimulation was significantly higher than without stimulation; this masking effect increased with light intensity. Stimulation reduced hit rates but had no effect on false alarm rates, which were near zero. Similarly, we observed that the detectability of the target-evoked calcium response decreased in the presence of optogenetic stimulation. We repeated the experiment with the target placed about 1° away at a location corresponding to a V1 site expressing only GCaMP6f. At this site (~4 mm from the co-expression site), we recorded a small stimulation-evoked response but found no behavioral or neural effects on target detectability. Overall, our results reveal neural and behavioral effects of sublinear summation in V1, and represent a first step toward an all-optical platform for manipulating population activity in behaving macaques and studying the effect of these manipulations on visual processing and behavior.

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Poster

578. Visual System: Responses During Behavior

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Topic: D.07. Vision

Support: Wellcome Trust Grant 200501/Z/16/Z
BBSRC BB/M009513/1

Title: A framework to interpret population activity of neurons tuned to multiple signals: Visual speed and self-motion

Authors: *A. B. SALEEM[1], E. A. B. HORROCKS[1,2], I. MARESCHAL[2]
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Abstract: Neural populations commonly encode multiple signals. The implications of this encoding on a downstream area interpreting one of the signals remains unclear. Indeed, how do we interpret neurophysiological and psychophysical experiments where responses to a single signal are studied in isolation? To gain an insight into this, we developed a new modelling framework. We focused our analysis on visual coding, which is often studied under stationary

conditions, although animals spend large periods of time moving around in a naturalistic setting. In addition, an increasing number of studies find that visual cortical neurons are strongly influenced by self-motion signals (Busse et al., 2018). We therefore applied our framework to the paradigm of visual speed encoding during self-motion.
We generated populations of 50 Poisson-spiking model cells with tuning for both visual speed and self-motion speed. Based on previously reported data from mouse visual cortex, we modelled cells to respond to different weighted combinations of visual and self-motion speed (Saleem et al., 2013). We then trained separate spike count based Bayesian decoders on visual speed using population spiking generated under either a coupled condition, where visual speed and run speed observed a fixed linear relationship, or an uncoupled condition, where visual speed and run speed varied independently. We then tested their ability to discriminate visual speeds based on decoding spiking activity generated under a range of conditions.
Within our framework we find that visual speed discrimination is strongly affected by test condition. Specifically, we find that performance is reduced under stationary conditions, which is consistent with reports in human psychophysics (Durgin et al., 2007). Conversely, visual speed discrimination performance is stable for textures moving at different distances from the observer, which alters the relationship between run speed and visual speed or gain. Interestingly, we find that the population biased towards equal weightings of visual speed and run speed reported (Saleem et al., 2013) performs visual speed discrimination better in most cases compared to a population with a uniform distribution of weightings. This was true when tested under the conditions where run speed and visual speed are linearly coupled, but not when stationary. We also find that a decoder trained on the coupled condition performs better at all gains tested except when stationary.
We conclude that our model provides an accessible framework for interpreting population activity of neurons tuned to multiple signals, and generates predictions which can be tested experimentally.

Disclosures: A.B. Saleem: None. **E.A.B. Horrocks:** None. **I. Mareschal:** None.

Poster

578. Visual System: Responses During Behavior

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Topic: D.07. Vision

Support: KAKENHI Grant 17K14941

Title: V1 layer 6 corticothalamic feedback encodes behavioral state by complementary activity of two neuronal populations

Authors: *S. AUGUSTINAITE, B. KUHN
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Abstract: Layer 6 (L6), the deepest lamina of cerebral cortex, is one of the key structures regulating behavior state related information processing within cortex and various subcortical areas. However, very little is known about the functional significance of different L6 circuits in vivo. Here, we focus on primary visual cortex L6 feedback projections to visual thalamus (dorsal lateral geniculate nucleus, dLGN) which regulate visual signal transmission from retina to cortex. After injecting fluorescent microspheres into dLGN and AAV.CAG.flex.GCaMP6f into cortex, calcium imaging of retrogradely marked L6 corticothalamic (CT) neurons was performed in vivo with 2P microscopy in a head-fixed Ntsr1-cre mouse. The neuronal activity from the same neurons was recorded for several hours and / or repeatedly recorded during different days while presenting full-screen drifting gratings and monitor mouse activity state with electrocorticogram, pupil size and locomotion speed recordings. This allowed us to study the corticothalamic feedback during different behavior states, ranging from full alertness to sleep. We found that the strength of feedback to lateral geniculate nucleus depends on state: neuronal activity is stronger during more active / alert behavior. Moreover, feedback is composed of two complementary signals mediated by two different neuronal populations: visual stimulus (i) activated or (ii) suppressed CT neurons. Visual stimulus activated neurons respond to a particular orientation / direction stimulus while remaining quiet during other orientation / direction stimuli and the dark periods, that is, in the absence of visual stimulation. Visual stimuli suppressed neurons, on the contrary, are active during the dark periods, but get inhibited with visual stimulation. Encoding behavioral state by this complementary neuronal activity, corticothalamic feedback can regulate thalamocortical transmission in a state - related manner continuously, in the presence or absence of visual input. The functional role of the feedback, however, might be different. Visual signal processing might get facilitated by visual stimuli activated CT neurons, while visual stimulus suppressed CT neurons might prime dLGN neurons to a certain behavioral state - related activity level in the absence of visual stimuli.

Disclosures: S. Augustinaite: None. **B. Kuhn:** None.

Poster

578. Visual System: Responses During Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 578.12/FF7

Topic: D.07. Vision

Title: Pathological cortical regions in patients with refractory epilepsy display normal physiological responses during cognitive tasks

Authors: *S. LIU, J. PARVIZI
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Abstract: High frequency oscillation (HFOs, 80 - 500 Hz) are known to be a reliable biomarker for the delineation of pathological epileptic foci in patients with refractory epilepsy. By contrast, high frequency broadband (HFB, 70 - 180 Hz) signal is a reliable biomarker for the local physiological responses in a given cortical site during cognitive and behavioral tasks. To date, however, it remains unclear whether epileptic cortical tissue, with abundant intrinsic HFO activity, is also capable of generating normal functional HFB responses during a cognitive task. A systematic comparison of temporal and spectral properties of epileptic HFO and functional HFB is also lacking. Here, we recruited three patients with anatomically similar electrode coverage in the lateral occipital and posterior ventral temporal cortex. Patients participated in the same visual cognitive task. We mapped the occurrence of HFOs during rest and during the experimental task and computed the stimulus-locked HFB responses during the experimental task. We discovered that, in all three subjects, the epileptic brain sites with abundant pathological HFOs were capable of generating physiological functional responses during the presentation of visual stimuli. In addition, we noticed a clear difference in the profile of pathological and cognitively-induced physiological high frequency signals. The average duration of high-band poweraugmentation for HFOs was 82 ms whereas that of HFB was significantly longer (501 ms, $P < 0.05$). The spectral width of spontaneous HFOs was 32 Hz, substantially smaller than that of task-driven HFBs (92 Hz, $P < 0.05$). A significant change in the slope of power spectral density was found only in HFO ($P < 0.05$) but not in HFB activities. Further, visually induced HFOs were temporally discordant with HFBs induced by the same tasks ($P < 0.001$). Our findings clearly demonstrate that brain structures involved with epileptogenicity may elicitnormal physiological responses to cognitive stimuli. The pathological HFOs and task induced HFBs that are originated from the same cortical tissue exhibit different temporal and spectral characteristics, and do not coincide in time. Since the use of intracranial EEG in human cognitive neuroscience has mainly been restricted to the clinical circumstances of patients with drug resistant epilepsy, investigating the connection and distinctions between HFB and HFO has its practical implications, and should shed light on the cognitive reserve function of epileptic neuronal populations.

Disclosures: S. Liu: None. **J. Parvizi:** None.

Poster

578. Visual System: Responses During Behavior

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Topic: D.07. Vision

Support: DFG Grant EXC 1086

Title: The functional role of human early visual areas during blinks and saccades as revealed by intracranial EEG

Authors: *M. J. KERN
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Abstract: Introduction: In natural viewing conditions, eye movements like blinks and saccades are ubiquitous, they take place roughly three times per second in natural viewing conditions, even so they are hardly consciously perceived. However, little is known about how the early visual areas of the human cerebral cortex operate during such natural performed events and how information processing in these areas is reflected in neural population activity. Hence, the aim of this study was to characterize the brain activity pattern in early visual areas during blinks and saccades.
Methods: In the present study we used intracranial EEG from implanted electrodes covering early visual areas in human cerebral cortex of four patients to investigate blink- and saccade-related brain activity during natural, non-experimental viewing conditions. In this way, a large number of eye movements (~3000 per patient) could be used without additional burden on the patients. Intracranial EEG is an optimal candidate for this kind of study since it offers a temporal resolution in millisecond-time scale and is less susceptible to ocular artifacts compared to standard EEG (Ball et al., 2009). The spectral composition of the recorded ECoG signals was calculated using a complex Morlet wavelet.
Results: We clearly show that both blinks and saccades were accompanied by a biphasic broadband gamma decrease-increase pattern in all studied visual areas (V1, V2, V3d, V3v, V4d, V4v and Fusiform Gyrus). In contrast to blinks, saccades additionally elicited a late, narrower-banded gamma increase starting after eye movement offset. Astonishingly, a significant decrease in gamma power was observed even before eye movement onset, especially notable during saccades in V1.
Conclusions: Since the timing of the gamma suppression is in line with psychological studies and in case of saccades starts even before eye movement onset, we think that this strongly indicates active top-down mechanisms from higher brain areas. The subsequent gamma power increase that starts around eye movement offset may reflect an amplified re-uptake of visual information, supporting uninterrupted visual perception. Finally, the late gamma power increase after saccades may reflect the greater amount of visual information that has to be processed compared to blinks.
References: Ball, T., Kern, M., Mutschler, I., Aertsen, A., Schulze-Bonhage, A., 2009. Signal quality of simultaneously recorded invasive and non-invasive EEG. NeuroImage 46, 708-716. https://doi.org/16/j.neuroimage.2009.02.028

Disclosures: M.J. Kern: None.

Poster

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Program #/Poster #: 578.14/FF9

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Simons Collaboration on the Global Brain

Title: Predicting perceptual decisions using visual cortical population responses and choice history

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Abstract: Our understanding of the neural basis of perceptual decision making has relied largely on relating fluctuations in single neuron responses to perceptual decisions, on a trial-by-trial basis. We sought to extend our understanding of perceptual decision making in two ways. First, we asked how our ability to predict animals' decisions would be improved by considering small simultaneously-recorded neuronal populations rather than individual units. Second, we asked how predictions would be improved by taking into account the animals' choice and reward histories. It is well known that perceptual decisions can be strongly affected by these factors, but their influence is seldom considered when relating neuronal responses to decisions.
We trained two macaque monkeys to perform a fine orientation discrimination task while we recorded from small neuronal populations in early (V1) and midlevel (V4) visual areas using multi-electrode arrays. Responses of individual V4 neurons were weakly predictive of decisions, but only in the post-stimulus fixation period and only in one animal; in V1, only a few neurons showed significant decision-related activity. To relate population activity to decisions, we trained a linear classifier. The classifier predicted choice slightly better than the best single unit in the recorded population and revealed limited, but more robust choice-related information. Including choice- and reward-history information in the model had a modest influence on performance, except when the recorded populations contained little decision-related information. We conclude that fluctuations in small neuronal population responses in early and mid-level visual cortex are only weakly related to perceptual decisions, even when choice and reward histories are taken into account.

Disclosures: A.I. Jasper: None. **S. Tanabe:** None. **A. Kohn:** None.

Poster

578. Visual System: Responses During Behavior

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Topic: D.07. Vision

Support: FP7 - ITN - Initial Training Networks

Title: Suppression & sparsification of visual responses during perceptual learning

Authors: *P. THAMIZHARASU, C. V. TOGT, E. RUIMSCHOTEL, I. F. PICA, L. D. KRAKER, C. LEVELT
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Abstract: In order to understand how neurons in primary visual cortex change their activity patterns during perceptual learning, we developed a two-alternative forced choice behavior paradigm for head-fixed mice allowing us to chronically monitor calcium responses of the same neurons in visual cortex by two-photon microscopy. During the task, the mice learn to discriminate between different visual stimuli and respond by licking a left or right lick spout in order to receive reward. Once the mice learn the task, the visual stimuli are partially changed forcing the mice to relearn the task. We observe that neurons in V1 start to anticipate the visual stimulus and reward with training. Improvements in behavioral performance were closely associated with reduced number of visually responsive neurons. In fact, V1 becomes suppressed upon visual stimulation after training. These effects are partially reversed with relearning. Our findings suggest that scarification improves coding efficiency in V1 but interferes with learning new associations.

Disclosures: P. Thamizharasu: None. **C.V. Togt:** None. **E. Ruimschotel:** None. **I.F. Pica:** None. **L.D. Kraker:** None. **C. Levelt:** None.

Poster

578. Visual System: Responses During Behavior

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Title: Two complementary population coding schemes in primate V1 contribute to scale-invariant pattern discrimination

Authors: *G. BENVENUTI[1], Y. CHEN[2], W. S. GEISLER[2], E. SEIDEMANN[2]
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Abstract: Humans can discriminate fine differences in orientation of visual patterns over a wide range of spatial scales. How does our brain carry out such a challenging, scale-invariant computation? Neurons in primary visual cortex (V1) are selective to the orientation of visual stimuli within their receptive fields (RFs) and are organized in cortical columns, based on their orientation preferences. Therefore, the brain might be expected to carry out orientation discrimination by comparing the orientation-specific columnar response patterns in V1. However, at every visual field location, V1 neurons have a limited range of RF sizes. As a consequence, the quality of the discrimination information at the columnar level and at the level of single-cells will decrease rapidly once the scale of the oriented stimuli on the retina exceeds this range of RF sizes. Thus, a simple decoder of the orientation column or single-cell responses cannot easily explain why behavioral discrimination thresholds are relatively constant across spatial scale. To investigate this puzzle, we used voltage-sensitive dyes to image V1 responses over an area of 8x8 mm^2 while two monkeys carried out a fine orientation discrimination task with oriented Gabor stimuli. We found that, like humans, monkeys' orientation discrimination performance is relatively scale-invariant. We then determined the orientation discrimination performance of the columnar responses for a wide range of spatial frequencies (SFs). As expected, we found that the orientation discrimination performance of the columnar responses is relatively constant for medium and high stimulus SFs, but drops substantially for low SFs, unlike behavioral performance. However, we also found a surprising coarse-scale signal that corresponds to the projection of the luminance layout of low SF stimuli to V1's retinotopic map. This homeomorphic and distributed representation, which carries high quality orientation information through variations in the level of population activity across the retinotopic map, can explain the behavioral performance at low spatial frequencies. We conclude that two separate decoders, one operating at the fine orientation column scale for medium and high SFs, and one operating at a larger retinotopic scale for low SFs, are likely to contribute to ours and monkeys' striking scale invariant pattern discrimination capabilities.

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Poster

578. Visual System: Responses During Behavior

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Support: Wellcome Trust 205093
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Wellcome Trust / Royal Society 200501

Title: Visual responses are more robust during navigation than passive viewing

Authors: *E. M. DIAMANTI, K. D. HARRIS, A. B. SALEEM, M. CARANDINI
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Abstract: Neurons in visual cortex are involved in the processing of visual inputs, but during behavior their responses are also modulated by task-related factors. Yet, little is known on whether sensory processing remains the same across conditions of increased behavioral complexity, from passive viewing of simple visual stimuli to navigation in visually rich environments. Are visual responses during passive viewing as strong as during navigation ? Do the same cells respond across all conditions?
We used 2-photon calcium imaging to record neural activity across primary visual cortex and 6 higher visual areas. Head-restrained mice either passively viewed drifting gratings or ran along a corridor in virtual reality (VR). The VR corridor contained two landmarks (a vertical grating or a plaid) repeated after 40 cm. We ran the VR sessions in two modes: closed-loop, where the speed of the virtual corridor matched the animal's run speed; open-loop, where previous closed-loop visual scenes were played back to the animal regardless of its running speed.
In closed-loop mode, neurons responded strongly to the landmarks in the corridor. Based on a measure of variance explained, these responses were highly reliable for most cells. In open-loop, however, there were fewer responsive cells, and the reliability of their responses was markedly reduced. The reduced responsiveness observed in open-loop could not be explained by differences in running behavior. In addition, cells did not simply become silent in open-loop mode: many cells maintained their selectivity to the visual landmarks, but the variance explained by their response profile was lower than in closed-loop. Responses in VR were not well predicted by responses to grating stimuli: for example, an independent population of neurons responded to vertical drifting gratings when mice viewed stimuli passively, than when the same gratings appeared as landmarks in VR.
We conclude that visual processing during active navigation is more reliable than during passive viewing, and involves a neuronal population that is not driven by passive grating stimuli. These

findings suggest that the difference in sensory responses between active behavior and passive viewing is beyond a mere modulation by task-related factors.

Disclosures: E.M. Diamanti: None. **K.D. Harris:** None. **A.B. Saleem:** None. **M. Carandini:** None.

Poster

578. Visual System: Responses During Behavior

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Topic: D.07. Vision

Support: EY024072

Title: Effects of single-cell stimulation in macaque V1 on performance in a threshold detection task

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Abstract: Cortical microstimulation has played a critical role in establishing causal links between sensory processing, network activity and perceptually guided behavior. Such microstimulation alters the activity of large populations of neurons, however, and it remains unclear how single sensory neurons contribute to behavior. Even if perception is based on a large population of neurons in sensory cortex, the response of single cells can be amplified by the highly interconnected local cortical network, particularly when the sensory input is weak or ambiguous, thereby affecting behavior. To assay the impact of activity in single neurons on network activity and perceptual judgments, we stimulated single neurons in V1 of three macaque monkeys while they performed a threshold detection task. Single cell stimulation was performed using patch electrodes either in the whole cell (n=15) or loose patch (n=14) configuration. We hypothesized that placing the animal in a threshold detection task would increase the impact of the activity of single V1 neurons. The animal was required to report whether a small, low contrast and briefly presented Gabor target appeared at one of two possible locations by making a saccade toward the target location. In half of the trials (randomly selected) with target at zero and low contrast levels (~3%), depolarizing current that coincided with the timing of the visual target (0.1-0.2 nA for whole cell, and 1-3 nA for loose patch, respectively) was injected to evoke action potentials (between 21-240 spikes/s). If single-cell stimulation has a large effect on the animal's perception, we would expect to observe a bias in the animal's choice toward the location of the cell's receptive field or a change in the reaction time. Single-cell stimulation, however, did not induce a discernible shift in the animal's psychometric function or change the latency of saccades toward either of the target locations even when the contrast of the target was

0% and even when the cell was highly sensitive to the target. Our results suggest that the impact of the activity of single cells on network activity in macaque cortex is relatively small, that the role of single V1 neurons in perceptual tasks is limited, and that perceptual judgements are based on the concerted action of large population of neurons.

Disclosures: N.J. Priebe: None. **B. Li:** None. **E. Seidemann:** None.

Poster

578. Visual System: Responses During Behavior

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Program #/Poster #: 578.19/FF14

Topic: D.07. Vision

Title: Behavioral state and experience modify natural behavioral responses to visual stimuli in mice

Authors: *R. IJEKAH, J. HOY
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Abstract: The ability to use vision to rapidly identify and respond to suddenly appearing biologically relevant stimuli is vital to survival and highly conserved across species. Recent studies of ethological visual behaviors such as predator avoidance and prey capture in mice have helped in understanding the neural basis of this type of visual processing in mammals. Here, we follow up on our original studies of visually-guided prey capture in the mouse to show that mice respond in an ethologically appropriate way towards simple virtual stimuli with prey-like features. In particular, C57BL/6J mice approach, orient without approach, or freeze in response to virtual stimuli presented in the lower to middle visual fields depending on the size and speed of the stimuli. Intriguingly, the sizes and speeds of virtual stimuli that reliably evoke each of these behaviors vary as a function of prey capture experience and hunger state. We show for the first time in the mouse, how specific internal states and experiences systematically modulate natural, visually-guided behavioral responses to simple stimuli. These observations suggest the animal's state influences both the salience and valence of visual stimuli presented in this context. Accordingly, we hypothesize that the cells and circuits which encode the behaviorally relevant stimulus features will also exhibit such state-dependent modulation. Our current and planned work investigates this possibility and should rapidly shed light on mechanisms underlying experience-dependent changes in selecting behavioral choices.

Disclosures: R. Ijekah: None. **J. Hoy:** None.

Poster

578. Visual System: Responses During Behavior

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Topic: D.07. Vision

Support: NIH Grant EY11747

Title: Human detection of occluding targets is near optimal for natural scenes

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Abstract: A fundamental visual task is separating relevant signals from background clutter. The natural-signals hypothesis suggests that perceptual systems exploit regularities in the statistical structure of natural scenes to solve this problem. Here, we study a novel class of target stimuli that fully occlude the background directly underneath the target. Despite the high prevalence of occlusion in nature nearly all studies on detection in humans and primates focus on additive targets. We provide psychophysical measurements for occluding target detection for a large range of background conditions and retinal eccentricities. We also describe a principled model for detection of occluding targets in naturalistic stimuli.
The psychophysical results were summarized by measuring eccentricity thresholds (retinal eccentricity for 70% correct detection) for four different occluding targets presented in natural backgrounds at different distances from the fovea. The luminance and contrast of the targets was fixed, and precise experimental control of the statistics (luminance, contrast and pattern similarity) of the natural backgrounds was obtained using a recently developed method known as constrained scene sampling. For luminance we found that performance was worst when the luminance of the background was close to the mean luminance of the target; whereas, performance declined with increasing background contrast and similarity.
To model the results we developed an ideal observer in which detection was limited by the approximate sampling density of ganglion cells in the human retina. To measure the scene statistics used by the model we first filter natural scene patches with and without the target by the optics of the human eye. We then simulate the output of the retinal ganglion cells by blurring and downsampling the image to match their sampling density at a given eccentricity. Next we decompose the information relevant for target detection into a luminance, boundary, and pattern components. The variances and covariances of the components are measured for a large set of backgrounds and retinal eccentricities. Finally, performance of the optimal classifier is measured in the set of background and eccentricity conditions for which we have measured human psychophysical responses. After applying a single scale parameter (efficiency), the model

thresholds were in close accordance with human thresholds. We conclude that much of the variation in performance for detecting occluding targets across the visual field arises from the stimulus uncertainty induced by the statistical structure of natural scenes and the limitations of retinal sampling.

Disclosures: R.C. Walshe: None. **W.S. Geisler:** None.

Poster

578. Visual System: Responses During Behavior

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Title: Hemodynamic response function (HRF) used to predict brain imaging responses from spiking switches sign and functional form between task-engaged and drowsy states

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Abstract: When interpreting hemodynamics-based brain imaging such as fMRI, the HRF is key to making predictions from modeled inputs and thereby relating the measured hemodynamics to the inputs. The HRF is taken as a proxy for neurovascular coupling to local neural activity. It is typically assumed to remain locally consistent although the coupling can change strength between drowsy and alert states (Schölvinck et al. 2010). Here we tested if the HRF remained consistent when switching between alert engagement in a task and states of drowsiness with eyes closed.
We recorded intrinsic-signal optical images (specifically, blood volume) with concurrent multi-unit spiking (MUA) from macaque primary visual cortex (V1). The animals performed a predictable, periodic fixation task for juice reward. This task elicits a powerful task-related hemodynamic response, entraining to task timing independent of visual stimulation (Sirotin and Das, 2009). Recording sessions extended over multiple hours in total darkness other than the

small (~2 arc min) fixation cue. Recordings thus included segments when the animal was actively engaged in the task, interspersed with segments when he shut his eye and appeared to drift asleep.
While the animal was alert and engaged in his task, the measured hemodynamics was dominated by the task-related response; the MUA showed weak task-linked fluctuations. During drowsy segments the hemodynamics showed large phasic fluctuations in local blood volume while the MUA showed large, multi-second bursts of activity. We used multilinear regression ('deconvolution': Dale 1999) to estimate the HRF over a moving window (typically 150 sec) traversing the entire session including alert and drowsy segments. The 'drowsy' HRF resembled a standard causal HRF kernel predicting an increase in local blood volume following the spiking, with typical times to peak and peak width. The 'alert' HRF was distinctly different, with an acausal temporal profile reflecting the periodic task timing, and a reversed sign predicting local decrease of blood volume following spiking. Cross validation between the two epochs was poor: the mean of the 'drowsy' HRF kernels gave consistently good predictions (quantified by Pearson's r) over the drowsy segments, but gave incorrect phase-reversed predictions in the alert segments. Neurovascular control thus likely involves very different neural mechanisms in drowsy vs. alert engaged states. These results should have considerable bearing on our understanding of the HRF, and the interpretation of fMRI in terms of local neural activation.

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Poster

578. Visual System: Responses During Behavior

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Title: Effect of eye movement on orientation tuning of neurons in visual cortex - a modeling study

Authors: *M. HOBBI MOBARHAN[1], I. E. AASEBØ[1], M. B. RØE[1], K. K. LENSJØ[1], G. T. EINEVOLL[3], T. HAFTING-FYHN[2], M. FYHN[1]
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Abstract: A characteristic feature of neurons in primary visual cortex (V1) is their strong response to visual stimuli of a particular orientation (orientation selectivity). During natural

behavior animals move their eyes, but it remains unclear how these eye movements affects basic properties of the receptive fields. Most studies of visual response properties are performed in head-restrained or anesthetized animals, and it has been shown that the eye movements of freely moving rats are more complex and fundamentally different with regularly disconjugate and often asymmetrical movements. By experiments alone, it is problematic to determine the specific influence of eye movements on the receptive fields of simple cells in V1, because the activity is confounded by a lot of non-visual input. Thus, in order to determine how eye-movements (including torsional rotations of the pupil) affect the orientation tuning of typical V1 receptive fields, we used a computational model. In particular, we use experimentally measured eye movements in freely exploring rats (Wallace et al. 2013) to construct a drifting grating stimulus embedding the eye movements. This stimulus is then convolved with a Gabor-like receptive field consisting of two elongated ON and OFF subfields, and passed through a nonlinear function to estimate the firing rate of the neurons. This simple model for receptive fields of V1 simple cells predicts high degree of orientation tuning in spite of eye movements. This prediction is in accordance to recordings from the V1 of behaving rats where most units show impaired orientation tuning during movement while a small subset of units in layer VI retain a remarkable stable orientation tuning. However, a shift in preferred orientation was observed for movements involving torsional rotations of the pupil. This suggests that an explanation of the experimentally observed reduction in orientation tuning during movement, requires model mechanisms beyond linear receptive fields combined with a static nonlinearity.

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Poster

578. Visual System: Responses During Behavior

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Topic: D.07. Vision

Support: RCN 217929
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Title: Stable orientation tuning in the freely moving rat: Movement-robust orientation selective neurons in the deep layers of the primary visual cortex

Authors: *M. B. RØE[1], I. E. AASEBØ[1], M. HOBBI MOBARHAN[1], K. K. LENSJØ[1], G. T. EINEVOLL[3], T. HAFTING-FYHN[2], M. FYHN[1]
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Abstract: A hallmark of neurons in the primary visual cortex (V1) is their orientation selectivity. However, it remains elusive how orientation tuning is affected during natural movement as recordings have mostly been from restrained animals. In the present study, we implanted tetrodes in the deep layers of V1 and conducted extracellular recordings of single units in awake rats. The animals moved freely in an enclosure surrounded by monitors presenting visual stimuli. In accordance with previous findings, most orientation-tuned units showed a reduced or disrupted orientation selectivity during movement compared to sessile behavior. However, a subpopulation of units sustained a remarkably stable orientation selectivity also during movement. These movement-robust orientation selective (MROS) units were predominantly located in layer 6 (L6), and maintained their preferred orientation across multiple recording sessions in freely moving and sessile states. To examine the effect of head-rotation on orientation tuning, the awake animal was placed on a remotely controlled tilting platform, creating a misalignment between stimulus and head-angle. As predicted, the sharp tuning of the MROS to its preferred orientation remained stable during continuous change of the platform angle. Interestingly, a shift in preferred orientation was observed when the platform, and thus the animals head, was fixed at a specific angle over time. The stability of the MROS units may be partly due to lower inhibitory surround in receptive fields of deep layer neurons. Moreover, the MROS units likely receive inputs from vestibular or oculomotor systems, for instance via the recently reported pathway from retroplenial cortex to V1 that convey vestibular-mediated head-motion information onto V1L6 neurons. Taken together, the functional properties of these units suggests that specialized receptive field properties and compensatory mechanisms such as counter eye-rolling or vestibular input maintain stable orientation tuning during passive and natural behavior.

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Poster

579. Visual Cortex: Functional Architecture and Circuits II

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Topic: D.07. Vision

Support: NIH Grant EY027157
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Title: Luminance enhances ON/OFF asymmetries in primary visual cortex by increasing the excitation/suppression ratio of the stimulus response

Authors: *R. MAZADE, J. JIN, C. PONS, J. ALONSO
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Abstract: ON and OFF thalamic afferents segregate in primary visual cortex making cortical neurons ON-dominated or OFF-dominated. It is currently unknown if the responses of ON- and OFF-dominated neurons are differently affected by luminance range, which can vary by more than two orders of magnitude in natural scenes. Here, we demonstrate that luminance strengthens both the excitatory response evoked by preferred stimuli and the response suppression evoked by preferred and non-preferred stimuli in both types of neurons. However, because the excitatory response is strengthened more, visual responses become stronger, faster, and more transient, and OFF-dominated neurons become faster and more sustained than ON-dominated neurons. We performed horizontal penetrations through cat primary visual cortex with multielectrode arrays and mapped cortical receptive fields with static grating stimuli, while varying the maximum luminance with neutral density filters (0.024 to 239 cd/m^2). Receptive-field polarity was measured as the (ON-OFF)/(ON+OFF) maximum responses (-1: OFF-dominated, +1: ON-dominated) and the response temporal profile as the average temporal response to the ten preferred grating stimuli. Cortical receptive fields showed a pronounced bi-modal distribution for contrast polarity with a dip centered at zero ($p<0.001$, Hartigan test), allowing us to split them into two groups. Our results demonstrate that the excitatory responses of ON- and OFF-dominated neurons to preferred stimuli increase with each luminance log-unit by ~8.5 spk/s (ON: $R^2=0.81$, $p<0.001$; OFF: $R^2=0.84$, $p<0.001$). In contrast, the response suppression increases only by ~1.3 spk/s for preferred stimuli (ON: $R^2=0.69$, $p=0.003$; OFF: $R^2=0.79$, $p=0.001$) and ~3.0 spk/s for non-preferred opposite-phase stimuli (ON: $R^2=0.88$, $p<0.001$; OFF: $R^2=0.91$, $p<0.001$). The increase in the response strength reduced the response latency per luminance log-unit by ~7.0 ms for OFF- ($R^2=0.97$, $p<0.001$) and ~6.0 ms for ON-dominated neurons ($R^2=0.98$, $p<0.001$). In addition, the increased excitation/suppression ratio reduced the response duration per luminance log-unit by ~9.0 ms in ON- ($R^2=0.96$, $p<0.001$) and ~7.5 ms in OFF-dominated neurons ($R^2=0.93$, $p<0.001$). As a result, OFF-dominated responses became faster (OFF vs ON latency at 239 cd/m^2: 49.5 ± 0.52 vs 54.0 ± 0.58 ms, $p<0.001$, Wilcoxon test) and more sustained (OFF vs ON width at 239 cd/m^2: 25.4 ± 0.59 vs 22.5 ± 0.76 ms, $p<0.001$, Wilcoxon test) than ON-dominated neurons. We conclude that luminance speeds up stimulus detection and enhances the temporal differences between darks and lights by increasing the excitation/suppression ratio of cortical responses.

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Poster

579. Visual Cortex: Functional Architecture and Circuits II

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Support: ZIA000069

Title: High-accuracy decoding of complex visual scenes from neuronal calcium responses

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Abstract: The brain contains billions of neurons defined by diverse cytoarchitectural, anatomical, genetic, and functional properties. Sensory encoding and decoding are popular research areas in the fields of neuroscience, neuroprosthetics and artificial intelligence but the contribution of neuronal diversity to these processes is not well understood. Deciphering this contribution necessitates development of sophisticated neurotechnologies that can monitor brain physiology and behavior via simultaneous assessment of individual genetically-defined neurons during the presentation of discrete sensory cues and behavioral contexts. Neural networks are a powerful technique for formulating hierarchical representations of data using layers of nonlinear transformations. Here we leverage the availability of an unprecedented collection of neuronal activity data, derived from ~25,000 individual genetically-defined neurons of the parcellated mouse visual cortex during the presentation of 118 unique and complex naturalistic scenes, to demonstrate that neural networks can be used to decode discrete visual scenes from neuronal calcium responses with high (~96%) accuracy. Our findings highlight the novel use of neural networks for sensory decoding using neuronal calcium imaging data and reveal a neuroanatomical map of visual decoding strength traversing brain regions, cortical layers, neuron types, and time. Our findings also demonstrate the utility of feature selection in assigning contributions of neuronal diversity to visual decoding accuracy and the low requirement of network architecture complexity for high accuracy decoding in this experimental context.

Disclosures: R.J. Ellis: None. **M. Michaelides:** None.

Poster

579. Visual Cortex: Functional Architecture and Circuits II

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Title: Interrelated gradients of orientation, spatial frequency, and ON/OFF selectivity in primary visual cortex

Authors: *E. KOCH, J. JIN, Q. ZAIDI, J. ALONSO
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Abstract: The cat primary visual cortex has a map for different stimulus features whose topographic relations remain poorly understood. Here we demonstrate a systematic relationship among spatial frequency resolution, orientation selectivity and ON/OFF response balance in the cortical map. We then show, with computational modeling, that these relations originate from the cortical clustering of ON and OFF thalamic afferents. We performed tangential penetrations in cat visual cortex with linear multielectrode arrays (32 recording sites separated by 0.1 mm). We frequently found systematic gradual changes in spatial resolution (spatial frequency cutoff) with cortical distance. Spatial resolution was strongly correlated with cortical map location ($r=0.464$, $p<0.0001$, $n=239$ units, 7 penetrations, 4 animals) being high in iso-orientation domains and low in pinwheel centers. Because iso-orientation domains tend to cross the border between ocular dominance columns, the binocular border often had high spatial resolution. Because iso-orientation domains also tend to cross the border between ON and OFF domains, monocular iso-orientation domains at the ON/OFF border also had high spatial resolution. Importantly, spatial resolution was low for pinwheels near the binocular regions indicating that it is more closely associated with high orientation selectivity than binocularity. Finally, the ON/OFF response balance of the receptive field was correlated with orientation selectivity ($r =0.391$, $p < 0.0001$, $n = 153$ units, 12 animals), being more balanced in cortical regions with narrowly tuned neurons than broadly tuned neurons. A simple computational model demonstrates that a cortical gradient for ON/OFF response balance can reproduce the relationships between orientation and spatial frequency measured experimentally. In the model, thalamic afferents project within a cortical sheet of 1x2 mm that has two ocular dominance columns, two OFF and two ON domains. The afferents compete to find the cortical region with the best-matched retinotopy, ocular dominance and ON/OFF polarity (axon separation: 50 microns, arbor spread: 0.5 - 1 mm). We show that the resulting clustering of ON and OFF afferents produces ON and OFF domains with low orientation selectivity and low spatial resolution at the center of ocular dominance columns. It also produces ON/OFF balanced cortical domains at the binocular border and ON/OFF border. We conclude that the structure of cortical orientation and spatial frequency selectivity gradients emerges from the segregation of thalamic afferents by eye input and ON/OFF polarity in primary visual cortex.

Disclosures: E. Koch: None. **J. Jin:** None. **Q. Zaidi:** None. **J. Alonso:** None.

Poster

579. Visual Cortex: Functional Architecture and Circuits II

Location: SDCC Halls B-H

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Program #/Poster #: 579.04/GG5

Topic: D.07. Vision

Support: EY027361

Title: Human amblyopia increases perceptual dark dominance

Authors: *C. PONS, R. MAZADE, J. JIN, M. DUL, Q. ZAIDI, J.-M. ALONSO
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Abstract: Visual information reaches the cerebral cortex through four parallel pathways that originate in ON and OFF retinal ganglion cells from the contralateral and ipsilateral eyes. During brain development, depriving one eye of visual input weakens its impact on visual cortex (Wiesel and Hubel, 1963), a process that is thought to equally affect ON and OFF pathways. Our results indicate that this assumption needs to be reconsidered. We have previously shown that optical blur reduces the visual salience of lights more than darks (Pons et al., 2017) and, therefore, it should reduce ON cortical responses more than OFF (Komban et al., 2014). Based on these results, we hypothesized that sustained optical blur during brain development should weaken ON cortical pathways more than OFF, thus permanently increasing perceptual dark dominance in visual salience. To test this hypothesis, we recruited 18 human subjects diagnosed with amblyopia in one eye and normal visual acuity in the fellow eye. Visual acuity was measured with a Snellen chart and was 20/25 or better for the fellow eye and up to 20/400 for the amblyopic eye. Subjects were asked to count as fast as possible the number of light or dark targets (1, 2 or 3) embedded in binary noise and perform this task monocularly using the refraction that provided the highest visual acuity for each eye. Consistent with our hypothesis, amblyopia affected the visual salience of light targets more than dark. On average, the dark-light difference in performance was ~3 times larger for the amblyopic eye than the fellow eye (dark-light difference in percent correct: 11.46 ± 1.15% for amblyopic eye; 3.87 ± 0.50% for fellow eye, $p < 0.001$, two-sided Wilcoxon tests, n=18 subjects). The average light-dark difference in reaction time was also ~1.3 times larger for the amblyopic eye (reaction time: 1.61 ± 0.10 sec for amblyopic eye; 1.25 ± 0.07 sec for fellow eye, $p < 0.001$; two-sided Wilcoxon tests, n=18 subjects). The dark dominance in visual salience not only increased with amblyopia but was strongly correlated with the reduction in visual acuity ($R^2=0.75$, $p < 0.001$). Unlike for visual salience, however, the average dark-light difference in grating orientation discrimination at high spatial frequency was not significantly higher for the amblyopic eye (14.26 ± 3.93% for amblyopic eye, 9.13 ± 4.24% for fellow eye, p=0.36, two-sided Wilcoxon test, n=7 subjects). These results can all be explained by a computational model that uses greater luminance/response saturation for ON than OFF pathways. We conclude that the ON cortical pathway is more vulnerable to amblyopia than the OFF pathway, a finding that could have implications for future amblyopia treatments.

Disclosures: C. Pons: None. **R. Mazade:** None. **J. Jin:** None. **M. Dul:** None. **Q. Zaidi:** None. **J. Alonso:** None.

Poster

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KU Leuven: C14/17/109

Title: Sub-millimeter resolution fmri reveals interdigitated body- and disparity-selective columns within macaque parietal cortex

Authors: *X. LI[1], Q. ZHU[1], W. VANDUFFEL[2,3,1]
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Abstract: The primate parietal lobe contains a heterogenous population of neurons and the first electrophysiology studies already suggested that different types of neurons may be grouped into distinct interdigitating functional modules (Mountcastle et al. 1975). However, unlike early visual cortex, the columnar organization of parietal cortex is surprisingly under-investigated. Here, we studied the mesoscopic functional organization of parietal cortex in awake rhesus monkeys using high resolution fMRI: ~0.6mm isotropic voxels with implanted phased-array coils at 3T (Janssens et al. 2012; Li et al. 2017). In a passive-viewing expt. 1, binocular disparity-defined radial sine-wave gratings and their size-matched monocular counterparts were presented to activate disparity-biased neurons. Two other experiments were conducted to identify body- and face-selective cortical clusters at the same resolution. In expt. 2, achromatic images from 10 different categories, as in Popivanov et al. (2012), were presented. Only common activations across the 3 contrasts (achromatic monkey bodies vs. size-matched fruits, objects and faces, respectively) and scan sessions were labeled as body-selective. Face patches were defined in the same way, but using fruits, objects and bodies as controls. In expt. 3, a completely different set of colorful face, body and object stimuli with different shapes and sizes (diameter ~ 24° of visual angle, matching that of the disparity stimuli, instead of < 15° of visual angle in expt. 2) were used. We tested the reproducibility of the category-selective activations across different stimulus sets of expt. 2 and 3. Our results show highly reproducible alternating patterns of disparity- and body-selective activations within cytoarchitectonically defined LIP (Lewis and Van Essen 2000) across different sessions and subjects (and different stimulus sets for body patches). Intriguingly, the body- and disparity-selective activations interdigitated, with multiple body patches located between disparity-selective activations. The results suggest a columnar organization of LIP

neurons for processing bodies (body parts) and disparity. Future studies are required to examine whether other columnar structures exist in the parietal lobe, besides the body and disparity columns.

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Poster

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Program #/Poster #: 579.06/GG7

Topic: D.07. Vision

Support: NIH Grant EY05253

Title: Expanding the luminance range increases ON/OFF response asymmetries in visual cortex

Authors: *H. RAHIMI NASRABADI, J. JIN, R. MAZADE, C. PONS, Q. ZAIDI, J. ALONSO
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Abstract: The visual cortex has two parallel pathways that signal local luminance increments (ON) and decrements (OFF) in visual scenes. ON cortical responses to light increments show a more pronounced saturation with luminance contrast than do OFF cortical responses to light decrements. This greater ON luminance response saturation is important because it reduces the spatial resolution of light targets, an effect that is thought to decrease on mid-gray backgrounds (Kremkow et al., 2014, Pons et al., 2017). The effect of mid-gray backgrounds was previously measured with standard monitors of ~200 cd/m^2 maximum luminance and could be due to an increase in background luminance, a reduction in luminance range or a combination of both. To distinguish among these possibilities, we measured ON and OFF luminance response functions using an LCD monitor of ~1,000 cd/m^2 maximum luminance (TRU-Vu, SRMH-15-AR series), which allowed us to use three luminance ranges (300, 600 and 1000 cd/m^2) and multiple combinations of background and target luminance for each range. ON and OFF luminance response functions were fit with Naka-Rushton functions to estimate the luminance that generated the half-maximum response (L_{50}) and the exponent of the function. Our results demonstrate that the L_{50} is consistently lower for ON than OFF responses across different luminance ranges and backgrounds (normalized ON and OFF L_{50} for 300, 600 and 1000 cd/m^2 luminance range averaged across backgrounds: 0.29/0.41, 0.28/0.41, 0.22/0.46; normalized ON and OFF L_{50} for 100, 400 and 600 cd/m^2 backgrounds at 300 cd/m^2 range: 0.28/0.41, 0.27/0.40, 0.31/0.43, $p<0.0001$, Wilcoxon tests). The difference between ON and OFF L_{50} increased by 92% when the luminance range was expanded from 300 to 1000 cd/m^2 (0.13 vs. 0.25, $p<0.0001$, Wilcoxon test), but remained roughly constant when the luminance range did not change (300 cd/m^2) and only the background luminance increased (0.13, 0.13, 0.12 for 100, 400 and 600

cd/m^2 background luminance, p>0.1, Wilcoxon tests). Expanding the luminance range from 300 to 1000 cd/m^2 also increased the maximum ON and OFF responses by ~4 spk/sec per 100 cd/m^2 and reduced the exponent by ~0.15 per 100 cd/m^2. Conversely, increasing the background luminance from 100 to 600 cd/m^2 (300 cd/m^2 luminance range) reduced the ON and OFF maximum response by ~4 spk/sec per 100 cd/m^2 and increased the exponent by ~0.17 per 100 cd/m^2. We conclude that expanding the luminance range from common laboratory values (~ 200 cd/m^2) to more natural values (~ 1,000 cd/m^2) enhances the ON-OFF differences in luminance/response saturation, maximizing spatial resolution for darks and low-contrast discrimination for lights.

Disclosures: H. Rahimi Nasrabadi: None. **J. Jin:** None. **R. Mazade:** None. **C. Pons:** None. **Q. Zaidi:** None. **J. Alonso:** None.

Poster

579. Visual Cortex: Functional Architecture and Circuits II

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Program #/Poster #: 579.07/GG8

Topic: D.07. Vision

Support: EY05253

Title: Functional organization of cortical maps for ocular dominance and light-dark polarity in primary visual cortex

Authors: *S. NAJAFIAN, J. JIN, Q. ZAIDI, J. ALONSO
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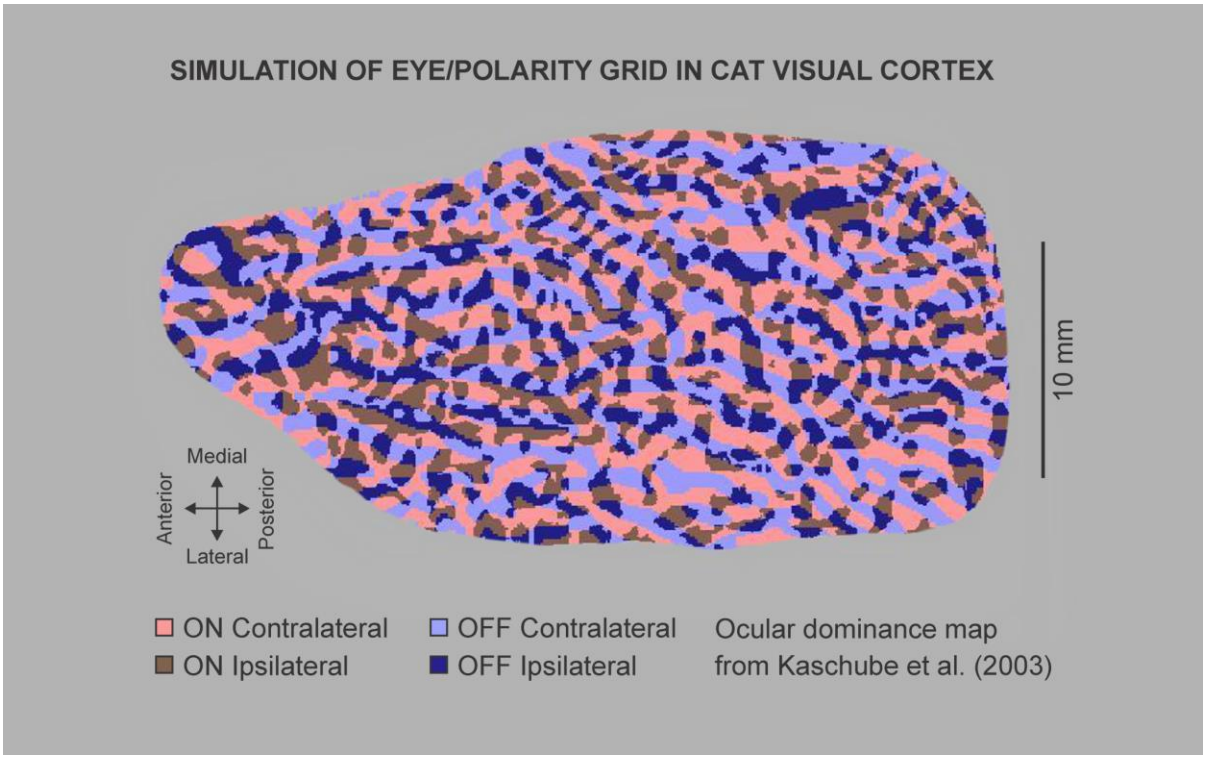
Abstract: The cat primary visual cortex has a map of retinal stimulus position (retinotopic map) that is split into four copies, two for each eye and two for each contrast polarity. These four copies are arranged in a grid (Kremkow et al., 2016) that aligns the eye and polarity axes with the axes of lowest and highest retinotopic gradient, probably to maximize the binocular retinotopic match needed for binocular vision and the light-dark retinotopic mismatch needed for processing stimulus orientation (Kremkow and Alonso, 2018). Here, we investigate the two-dimensional organization of the eye-polarity grid with computer simulations. We used a multivariate-normal-distribution filter to simulate the cortical spread of attraction-repulsion interactions that sort thalamic afferents by eye input during development. We then varied the geometry of the filter across cortical regions to reflect local variations in retinotopic gradients. The filter was convolved with a cortical patch of random binary noise representing the unsorted thalamic afferents (1: contralateral, 0: ipsilateral). By systematically varying the filter parameters (e.g. elliptical geometry with different spreads along major/minor axes), we generated a database of ocular dominance patches resembling those found in nature. We then took published ocular

dominance maps from different animals (cats, monkeys and humans), divided each map into 3 x 3 mm patches, and used our database to find the filters that best reproduced the geometry of each patch (i.e. best match in average width, length and orientation of ocular dominance stripes). This simulation generated a map of local retinotopic gradients that was used to generate the map for light-dark polarity and the eye-polarity grid for each animal (Figure 1). The predicted eye-polarity grids of cats, monkeys and humans shared the same general geometry but differed in the width and length of the eye-polarity stripes. We are currently investigating how the eye-polarity grid changes when OFF thalamic afferents occupy more cortical territory than ON thalamic afferents.



Disclosures: S. Najafian: None. **J. Jin:** None. **Q. Zaidi:** None. **J. Alonso:** None.

Poster

579. Visual Cortex: Functional Architecture and Circuits II

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JSPS Postdoctral Fellowship

Title: Multimodal functional mapping of posterior parietal cortex in mice

Authors: *R. HIRA, L. B. TOWNSEND, I. T. SMITH, S. L. SMITH
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Abstract: Posterior parietal cortex (PPC) in rodents plays a role in cognitive functions including navigation, multisensory integration, working memory, and decision making. However, its location has not been precisely determined in mice. PPC is bordered by the primary somatosensory cortex (S1), but other boundaries remain unclear. Higher visual areas (HVAs) could overlap with PPC in whole or in part. To precisely determine the location of PPC in mice, we performed a series of functional, multimodal intrinsic signal optical imaging experiments. First, we mapped HVA locations relative to cranial landmarks including lambda, which are commonly used for stereotaxic targeting. We found that, relative to cranial landmarks, the mouse-to-mouse variability in the locations of HVAs are large (mean ± S.D.: 10.0 % ± 12.1% overlap in HVAs across six adult mice). In comparison, when mapped relative to each other (e.g., using the centers of two HVAs as registration points), the locations of HVAs were more consistent across mice (46.4 % ± 16.8% overlap in HVAs across six adult mice). Thus, precise targeting of HVAs requires functional mapping in individual mice. Second, we used tactile stimulation of the tail, trunk, and ear to map parietal areas ~300 μm anterior from the anterior border of the Antereomedial (AM) HVA, and adjacent to the Anterior (A) and Rostrolateral (RL) HVAs. This mapping identified a cortical area between S1 and anterior HVAs, which we refer to as the Anterointermediate (AI) area. AI is likely a component of PPC. Third, we developed a multimodal mapping protocol to rapidly locate these areas adjacent to AI, based on simultaneously mapping HVAs and subregions of S1. The resulting maps provide functional landmarks for targeting PPC in mouse experiments. These findings also highlight the precise relative locations of cortical areas, despite variable relationships to cranial landmarks. We propose that subregions of mouse PPC have distinct integration roles such as tactile-visual, auditory-visual, visual-motor, and cognitive integration, similar to findings in primate PPC.

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Poster

579. Visual Cortex: Functional Architecture and Circuits II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 579.09/GG10

Topic: D.07. Vision

Title: A model for the development and dynamics of visual orientation selectivity

Authors: G. NGUYEN, *A. W. FREEMAN
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Abstract: Aims. Orientation selectivity, a major feature of primary visual cortex, depends on convergent input from on- and off-centre subcortical pathways. A cortical column receives input from highly overlapping on- and off-populations, and it is unclear how these inputs segregate into the separate on- and off-subfields of a simple cell receptive field. Orientation selectivity is refined by intracortical inhibition, but the mechanisms and extent by which inhibition shapes orientation tuning are still controversial. Our aim was to describe a model that, first, explains the segregation of on- and off-inputs to the cortex and, second, shows how inhibition contributes to the orientation tuning, contrast invariance and orientation mapping seen in real cortex. **Methods.** The model consisted of an array of subcortical channels, each of which included a photoreceptor, bipolar cell, ganglion cell and geniculate cell. These on- and off-channels converged onto two networks of cortical neurons, one excitatory and the other inhibitory. The inhibitory network then converged onto excitatory neurons. Each neuron in the model was modelled as a first-order low-pass temporal filter and represented by a nonlinear differential equation. Time courses of neuronal impulse rates were obtained by solving all equations simultaneously. Geniculocortical synaptic weights were initially all equal and then set through an iterative process representing visual development. The stimuli were drifting gratings with a range of orientations, and a synapse's strength was increased only if it enhanced the impulse rate of the target cortical neuron. **Results.** Cortical impulse rates were initially low because of destructive interference between neighbouring on- and off-inputs. The Hebbian development process favoured one sign of input over the other at any point in the visual field, resulting in the segregation of on- and off-inputs. As cortical contrast sensitivity grew so did inhibition. This favoured the response at the preferred orientation, shaping orientation tuning. At the end of development, orientation tuning bandwidth in some neurons was as sharp (15° half-width at half-height) as that seen in primate and carnivore primary visual cortex. This same *iceberg effect* also resulted in contrast invariance: increasing contrast increased inhibition, keeping tuning curves slim. Finally, maps of preferred orientation in the model are shown to have similar characteristics to measured maps. **Conclusion.** A model containing both Hebbian geniculocortical connections and feedforward intracortical inhibition can reproduce a number of the essential features of cortical orientation selectivity.

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Poster

579. Visual Cortex: Functional Architecture and Circuits II

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Topic: D.07. Vision

Support: NIH R01 EY027383
NIH R01 EY022090

Title: Thalamic and feedback connections to mouse V1 differentially drive excitation and inhibition within distinct subnetworks

Authors: *R. D'SOUZA[1], P. BISTA[1,2], A. BURKHALTER[1]
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Abstract: The lateral posterior nucleus (LP) is a higher order thalamic structure that is involved in the contextual modulation of cortical visual representations (Roth et al., 2016). In mouse primary visual cortex (V1), axons originating in LP selectively target layer 1 (L1) and L5, which are also principal targets of feedback axons from higher cortical areas. Moreover, the dorsal lateral geniculate nucleus (dLGN), in addition to projecting to its primary target L4 in V1, also sends afferents into L1. It is unclear how diverse thalamic and cortical areas exert their respective influence on V1 function through the L1 neuropil. It was recently shown that projections from the dLGN and the higher visual lateromedial area LM, to L1 of V1, terminate preferentially in a non-uniform pattern of repeating regions termed 'patches', which interdigitate with regions termed 'interpatches' that are selectively avoided by these afferents (Ji et al., 2015). Given this organization, one way in which inputs from diverse sources could differentially regulate circuit output in V1 is through pathway-specific recruitment of excitatory and inhibitory actions within functionally and spatially distinct modules. To test this hypothesis, we mapped synaptic inputs from each of dLGN, LP, and LM to parvalbumin-expressing (Pv+) and pyramidal cells in L2/3 of V1 in Ai9 X Pvalb-Cre mice. Anterogradely labeled LP axons showed a remarkable propensity to target the interpatch zones in L1 of V1, thereby largely interdigitating with dLGN and LM axons. Pv+ dendrites and axon terminals in L1 showed a preference for localizing in patches, but Pv+ perisomatic baskets were denser in L2/3 modules aligned with interpatches. Channelrhodopsin2-assisted mapping in acute cortical slices showed that the inhibition/excitation (I/E) balance, defined here as the average ratio of the total EPSC recorded in a Pv+ cell to that recorded in a neighboring (< 50 μm) pyramidal cell upon presynaptic optogenetic stimulation, was pathway-specific. dLGN axonal inputs generated an I/E balance that was strongly tilted towards inhibition, unlike LP inputs which did not show any such bias in either patches or interpatches. LM axons showed a bias for recruiting inhibition in interpatches but not in patches, and exhibited an overall weaker tilt towards inhibition compared to dLGN inputs. Together, these results demonstrate fine-scale specificity with which diverse inputs to L1 control computations in V1. We propose that long-range excitatory afferents exploit the bimodular organization in V1, by differentially engaging the I/E balance within spatially segregated subnetworks, in order to modulate V1 at microcircuit-level precision.

Disclosures: R. D'Souza: None. **P. Bista:** None. **A. Burkhalter:** None.

Poster

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Wellcome Trust Grant 205093
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Title: Excitatory and inhibitory presynaptic networks supporting orientation selectivity in primary visual cortex

Authors: *L. F. ROSSI, K. D. HARRIS, M. CARANDINI
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Abstract: The response selectivity of neurons in the primary visual cortex (V1) stems from the integration of excitatory and inhibitory inputs from hundreds of presynaptic neurons across cortical layers. It remains unclear how this presynaptic network supports a neuron's orientation preference, and what differences exist between the inhibitory and excitatory presynaptic networks.

We targeted individual pyramidal L2/3 neurons in mouse V1, and identified their presynaptic network through single-neuron initiated monosynaptic tracing with an EnvA-dG-DsRed rabies virus. Mice expressed GCaMP6 in all excitatory neurons, so that we could perform two-photon microscopy to reconstruct the postsynaptic neuron and its local presynaptic network, and to measure their retinotopy and orientation tuning while the animal was passively viewing and free to run on spherical treadmill. For each postsynaptic neuron, we identified 132±41 (s.e., n = 13 mice) presynaptic partners across L1-5. On average, at least 49% of these presynaptic partners were excitatory, expressed GCaMP6 and displayed activity dependent fluorescence changes. The remaining cells were putatively inhibitory, as they did not express GCaMP6.

Presynaptic networks were not distributed uniformly in cortical space, but were elongated: in retinotopic space, this elongation matched the orientation preference of the postsynaptic neuron. Within this arrangement, there were striking differences between excitatory and inhibitory neurons: inhibitory neurons were concentrated locally around the postsynaptic cell, while excitatory neurons were distributed over a wider area. In retinotopic space, this distribution favored locations coaxially aligned with the orientation preference of the postsynaptic neuron. Differences extended also to the distribution across layers: inhibitory neurons constituted a majority in L2-3, while excitatory presynaptics dominated input from L4-5.

These results demonstrate that presynaptic networks support the orientation preference of L2/3 neurons through distributed, retinotopically elongated excitation balanced by local, dense,

retinotopically elongated inhibition. Moreover, they suggest feedforward excitation and local inhibition play a dominant role in the computation performed by L2/3 neurons.

Disclosures: L.F. Rossi: None. **K.D. Harris:** None. **M. Carandini:** None.

Poster

579. Visual Cortex: Functional Architecture and Circuits II

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Program #/Poster #: 579.12/GG13

Topic: D.07. Vision

Support: NSF Grant 1734854
Swartz Foundation Grant

Title: Modeling feedback for a large set of visual stimuli in a detailed, spiking model of macaque V1

Authors: *C. L. CHARIKER[1], L.-S. YOUNG[1], R. SHAPLEY[2]
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Abstract: A major challenge in understanding and modeling local networks of neurons in the cerebral cortex is trying to understand the network's interaction with its sources of feedback current. We report here a new approach to modeling feedback in a modified version of a previously constructed, biophysically detailed, spiking network model of macaque V1 input layer 4Cα (Chariker et. al. JNS 2016). The model consisted of ~41,000 excitatory and inhibitory integrate-and-fire cells in several hypercolumns of 4Cα. Model neurons had conductance based AMPA, GABA, and NMDA recurrent synapses, as well as sparse feedforward input from LGN and feedback from layer 6. While the original model successfully exhibited and made predictions about a number of important V1 phenomena simultaneously (e.g., orientation/spatial-frequency selectivity, simple/complex cells, gamma rhythms, firing rate distributions), the feedback component (layer 6) was only modeled for a specific set of visual inputs (background or full contrast drifting gratings ranging over several orientations and spatial frequencies). In order to study V1 phenomena involving a more general set of stimuli, we introduced and benchmarked a rate-dependent direct coupling of the feedback layer 6 to 4Cα. The feedback coupling scheme respects known anatomical features in the Layer 6-4Cα feedback circuit. The parameters of the coupling were chosen to replicate contrast response data in V1. With these parameters, we also studied V1 phenomena involving a wider range of visual stimuli (drifting gratings varying in contrast, orientation, spatial frequency, and temporal frequency). The phenomena included contrast invariance, spatial contrast sensitivity, and temporal frequency tuning. The new scheme of modeling feedback was judged to be successful based on how well the model matched experimental data. The particular detailed, mechanistic nature of the implementation of the

model allowed us to analyze the dynamical mechanisms underlying a wide range of visual cortical function.

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Poster

579. Visual Cortex: Functional Architecture and Circuits II

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Support: Chateaubriand Fellowship of the Office for Science & Technology of the Embassy of France in the United States
European Union H2020 Programme (H2020-Adhoc-2014-20) under grant agreement no.720270 (The Human Brain Project SGA1)

Title: Specific intracortical connectivity rules reconcile push-pull and broad inhibition in V1 simple cells

Authors: *M. TAYLOR[1], R. BENOSMAN[2], D. CONTRERAS[1], A. DESTEXHE[3], Y. FREGNAC[3], J. ANTOLIK[2,3]
[1]Neurosci., Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA; [2]Inst. de la Vision, Paris, France; [3]UNIC-CNRS, Gif-sur-Yvette, France

Abstract: Previous experimental studies have produced seemingly contradictory evidence on the nature of interaction between excitation and inhibition in simple cells of cat primary visual cortex (V1). Studies using drifting sinusoidal gratings have shown that both excitation and inhibition are modulated by the phase of the stimulus, and this modulation is anti-correlated, implying so called “push-pull” organization of the intra-cortical connectivity with respect to the functional properties of the neurons. Studies using flashed bar stimuli have shown that only excitation is locked to the phase (position) of the stimulus, while inhibition remains broad and phase unspecific across space.
Here we reconcile these experimental results by constructing a model of the granular layer of V1, with high biological fidelity, that exhibits both behaviors to the two different stimuli. We show that a moderate bias of excitatory neurons to synapse onto other neurons with correlated receptive fields (RFs), and a weak bias of inhibitory neurons to synapse onto other neurons with anti-correlated RFs can explain the V1 spike, Vm, and conductance dynamics under these different stimulation paradigms.
We explore a parameter space of RF-correlation-based bias for excitatory and inhibitory synapses and find surprisingly that a wide range of connectivity parametrizations give rise to experimentally observed spike and Vm dynamics in simple cells, suggesting that elementary

functional properties such as orientation tuning and its invariance to contrast, as well as the push-pull structure of RFs can arise robustly across a variety of connectivity schemes. However, the underlying conductance dynamics change dramatically across this parameter space: very different conductance dynamics can underlie the same functional properties. Experimental conductance data restrict the acceptable parameters to moderately biased excitatory connectivity and weakly biased inhibitory connectivity.
This work demonstrates that in complex dynamical systems such as cortex, it is dangerous to use restricted results from few different experimental conditions to make far-reaching conclusions on the nature of the underlying neural system. Only systematic, comprehensive treatment of the primary visual cortex, that incorporates a broad range of established constraints from different experimental designs, can lead to reconciliation of the often seemingly contradictory diversity of experimental findings, and in turn to accurate characterization of the system under study.

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Poster

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Topic: D.07. Vision

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1U01NS099720-01
NIH, EY022577

Title: Receptive field size and spatial phase organization in macaque V1 with two-photon imaging

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Abstract: Recent studies in macaque primary visual cortex (V1) have shown that maps of spatial frequency (SF), ocular dominance, orientation, and retinotopy, all co-exist to efficiently represent the visual scene (Nauhaus et al ‘16; *Efficient receptive field tiling in primate V1*). However, to establish a complete model of the functional organization of classic receptive field properties, along with corresponding coding limitations, we must also characterize the cell-by-cell organization of spatial phase and receptive field size. Here, we performed 2-photon imaging

in macaque V1 to accomplish this task. We used both synthetic (OGB) and genetically-encoded (GCaMP6) calcium indicators. Responses were measured to static sinewave gratings and sparse noise.
To begin, we find that RF width is homogeneous in each region-of-interest (ROI) - whereas the SF preference can systematically change by multiple octaves within a 500 μm field-of-view, the map of RF width (deg) is relatively flat when placed on a similar octave scale. Furthermore, we find that RF width is a poor predictor of preferred SF (cyc/deg). Our recordings are limited to upper layer 2/3, where the population has phase tuning curves with relatively low amplitude (i.e. they are more "complex" than "simple"). Overall, the results are consistent with prior studies showing that "complex cells" have a RF size that is poorly coupled to preferred SF (Movshon et al J. Physiol '78; Hubel and Wiesel '68 J. Physiol).
Next, our data shows that spatial phase is clustered in V1 - in each ROI, the population has a significant bias for the preferred phase of a given orientation and SF in the sinewave grating stimulus ensemble. We are continuing to investigate how this clustering relates to retinotopy and if it allows for complete tiling at each location of the visual field. In summary, we are continuing to identify a more complete model of the V1 functional architecture. Such a model is necessary for a descriptive understanding of the V1 population code. Furthermore, a complete model of V1 maps may provide important clues into the mechanisms that give rise to feature tuning in the cortex.

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Poster

579. Visual Cortex: Functional Architecture and Circuits II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 579.15/GG16

Topic: D.07. Vision

Support: Rutgers University – Newark Chancellor's Seed Grant

Title: Role of neuropilin-2 in the establishment of a functional neuronal connectivity in the mouse primary visual cortex

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Abstract: The establishment of cortical circuits during development is under the control of multiple signaling systems. One of these signaling systems is class 3 Semaphorins, more

specifically Sema3F. In the absence of the Sema3F signaling system during development will result in thalamocortical axons from the somatosensory and motor thalamic relay nuclei misproject to the visual cortex. As the presence of thalamocortical misprojections were only investigated during development, we ignore if abnormal thalamocortical wiring still exists in the adult. However, we know that the proximal part of the apical dendrite of layer 5 pyramidal neurons located in the adult primary visual cortex (V1) of mice knock-out for Sema3F or its obligate receptor Neuropilin-2 (Nrp2) present an abnormally high density of excitatory spines. These findings led to the hypothesis that Sema3F signaling plays a key role in the development of a functional network in V1 by pruning ectopic thalamocortical synapses. However, the identity of the presynaptic inputs rescinded under the control of the Sema3F signaling system as well as the gain of function achieved through this pruning were never established. To determine the identity of the neurons targeting erroneously the proximal dendrite of V1 L5 neurons in the absence of the Sema3F signaling**,** we injected either a retrograde tracer in V1 or an anterograde tracer in different thalamic relay nuclei of wild type (WT) and Npn-2 null mice. Then, using calcium imaging in awake mice, we compared the orientation tuning and the receptive field structure of V1 neurons of WT and Npn-2 null mice. Finally, we tested the effect of the absence of Sema3F signaling on visual perception by comparing the performance of WT and KO mice at visual discrimination tasks. We found that: [1] non-visual-specific thalamocortical inputs are still present in the V1 of adult Npn-$2^{-/-}$ mice; [2] the computational properties of L5 V1 neurons are negatively affected by the absence of Nrp-2 during development; [3] the receptive field structure of V1 neurons in Nrp2 null mice is degraded compared to WT; [4] Nrp2 KO mice showed lower visual discrimination ability compared to WT. Our results suggest that Sema3F signaling is implicated in the establishment of the thalamocortical connections in V1 and plays an important role in the emergence of the computational properties of V1 neurons.

Disclosures: H. Khdour: None. **T.S. Tran:** None. **P. Polack:** None.

Poster

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Topic: D.07. Vision

Support: NRF-2016R1C1B2016039
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Title: Spatial organization of simple and complex cells in the primary visual cortex

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Abstract: Selective response for various types of visual features is a hallmark of neurons in the primary visual cortex (V1), and great effort has been devoted to the classification of neurons depending on their functional tuning. As suggested in the pioneering study of Hubel and Wiesel (Hubel & Wiesel, 1968), one of the most studied criteria is the classification between simple and complex cells, where simple cells detect the edge in the visual stimulus, and the complex cells encode more integrated information. Conventionally, simple and complex cells have been considered two distinct classes of V1 neurons, based on the bimodal distribution of the modulation ratio (F1/F0; Skottun et al., 1991). On the contrary, later studies have suggested that these two types of cells are not clearly separated and can be considered as variations along a continuous spectrum (Mechler & Ringach 2002, Priebe et al., 2004). However, it is still unclear what induces such variation of the modulation ratio across V1 neurons. Here, we propose that the modulation ratio of V1 neurons is constrained by the spatial separation between ON and OFF retinal afferents to the neuron. Previously, we suggested that the spatial organization of ON and OFF retinal ganglion cells (RGCs) constrains the orientation preference of a V1 neuron, and the moiré interference between ON and OFF RGC mosaics can seed the hexagonal arrangement of orientation columns (Paik & Ringach, 2011). By extending this notion, we suggest that the distance between ON and OFF retinal receptive fields may constrain the modulation ratio of V1 cells. If ON and OFF retinal receptive fields are close to each other and highly overlapped, the connected V1 neuron shows nonlinear response dynamics of a complex cell. As ON and OFF retinal receptive fields become further apart from each other, the subregions of V1 become more separated, resembling the receptive field of a simple cell. Since the distance between ON and OFF retinal receptive fields periodically varies across the moiré interference, our model suggests a quasi-periodic arrangement of simple and complex cells. We observed such periodic variation of overlap between ON and OFF subregions in the animal data (Kremkow et al., 2016). We further predict that the spatial distribution of complex cells is correlated with the local structure of orientation maps because both structures are seeded by the common retinal organization. Overall, we suggest that the various functional tuning properties are determined by the retinal afferents, resulting in spatial correlation between different properties across the cortical surface.

Disclosures: G. Kim: None. **J. Jang:** None. **S. Paik:** None.

Poster

579. Visual Cortex: Functional Architecture and Circuits II

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Program #/Poster #: 579.17/HH1

Topic: D.07. Vision

Title: Cellular and circuit mechanisms underlying processing of binocular visual information in visual cortex

Authors: *S. HONNURAIAH, H. H.-Y. HUANG, G. TESTA-SILVA, W. M. CONNELLY, G. J. STUART
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Abstract: The binocular region of primary visual cortex plays a critical role in processing visual information received from the eyes. Recent work from our lab and by others demonstrates that this binocular visual information is integrated sub-linearly in layer 2/3 pyramidal neurons in binocular visual cortex. To better understand how binocular information is integrated in layer 2/3 of binocular visual cortex here we combine optogenetic and electrophysiological methods to identify putative binocular and monocular neurons in vitro and characterize their active, passive and morphological properties. We have identified two distinct populations of layer 2/3 pyramidal neurons in binocular visual cortex, one that receives long-range monosynaptic excitatory input from the contralateral visual cortex (putative binocular neurons) and one that does not (putative monocular neurons). In addition, we find that input from the contralateral visual cortex strongly excites a subset of fast-spiking, putative parvalbumin positive (PV) interneurons, activating feed-forward inhibition that could drive sub-linear synaptic integration in layer 2/3 pyramidal neurons. While we found no differences in passive and morphological properties of putative binocular and monocular layer 2/3 pyramidal neurons, the active properties of putative binocular neurons were significantly different from putative monocular neurons. Specifically, the slope of the input/output (f/I) curve generated during somatic current injection was lower in putative binocular layer 2/3 pyramidal neurons, leading to reduced action potential firing. These data suggest that binocular layer 2/3 pyramidal neurons are intrinsically less excitable than monocular neurons. This difference indicates that binocular layer 2/3 pyramidal neurons may have different cellular integration rules from monocular neurons during synaptic integration. Using a morphologically realistic active model of layer 2/3 pyramidal neurons, we demonstrate that differences in axonal potassium channels likely underlie the difference in input/output (f/I) curves of putative binocular and monocular neurons. In conclusion, we provide evidence that distinct populations of both excitatory and inhibitory neurons are involved in processing binocular visual input in binocular visual cortex. Furthermore, we show that these different neuronal populations have different active properties. These findings provide insight into the cellular and circuit mechanisms used by the cortex to process binocular visual information.

Disclosures: S. Honnuraiah: None. **H.H. Huang:** None. **G. Testa-Silva:** None. **W.M. Connelly:** None. **G.J. Stuart:** None.

Poster

579. Visual Cortex: Functional Architecture and Circuits II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 579.18/HH2

Topic: D.07. Vision

Support: OPTOVISION RES-169-7579

Title: Mapping cortico-cortical network activity with fmri elicited by optogenetic stimulation of primate v1

Authors: *M. ORTIZ-RIOS, M. HAAG, B. AGAYBY, F. BALEZEAU, M. C. SCHMID
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Abstract: Optogenetics in nonhuman primates have become an increasingly important resource to understand brain function and its relationship to behaviour in health and disease. However, approaches to influence behaviour using this method remain challenging in primates, in part due to a lack of detailed knowledge about how optogenetic stimulation affects neuronal activity across large-scale brain networks. Here we use fMRI to map the effective connectivity induced by optogenetic stimulation of primary visual cortex (V1). We injected 25.5 µl of *AVV9-hSyn-hChR2(H134R)-eYFP* across multiple depths and sites into the opercular part of V1 (at 5-7° visual eccentricity representation) of two monkeys resulting in an estimated virally transfected area of 12-20 mm^2. Opto-fMRI experiments were performed in a 4.7T vertical scanner and began at least eight weeks after construct injections. High-resolution images were taken from awake monkeys that remained seated inside the scanner in total darkness. LED stimulation (460 nm) was applied via a 1.5 mm diameter optical-fiber placed over the dura of V1; e.g. without penetrating brain tissue. Stimulation was performed in blocks of 30 seconds alternated with 30-second-long blocks without stimulation. To assess the effect of light stimulation on the BOLD response we used multiple power amplitudes (110mW, 90mW, 70mW as measured at the fiber tip), frequencies (40Hz, 10Hz and 5Hz) and intensity matched red light (625 nm) for control analyses. To assess the BOLD response modulation, we used a model-free assessment of the signal using the coherence measured between the BOLD response and the optical stimulation rate (e.g. 1/60 sec). In visual cortex, blue, but not red light resulted in coherent modulation of areas V1, V2, V3 and MT. The local BOLD response amplitude in V1 was incrementally modulated with increasing power and frequency. The distal cluster in MT/MST was mainly driven at the highest amplitudes and frequencies (e.g. 110-90mW, 40-10Hz). We also observed strong modulation in ventromedial V1 however with a phase lag, perhaps indicative of a negative BOLD response. Outside the occipital lobe, we also found reliable activation in the dorsal genu of the caudate nucleus and moderate modulation in the right ipsilateral frontal eye field (FEF) region most likely reflecting poly-synaptic modulation. Our results of V2 and MT activation are consistent with monosynaptic input to these areas via V1 layer 4B neurons (Nassi & Callaway, 2009) and as such provide a new perspective onto how optogenetic V1 stimulation might influence saccadic eye movements by eliciting perceptual changes (Jazayeri al., 2012) that involve large-scale cortical networks.

Disclosures: M. Ortiz-Rios: None. **M. Haag:** None. **B. Agayby:** None. **F. Balezeau:** None. **M.C. Schmid:** None.

Poster

579. Visual Cortex: Functional Architecture and Circuits II

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Program #/Poster #: 579.19/HH3

Topic: D.07. Vision

Support: Max Planck Society

Title: Mapping functional synaptic weights with *in vivo* spine imaging and correlated ultrastructural anatomy

Authors: *B. SCHOLL[1], C. THOMAS[2], D. GUERRERO-GIVEN[2], N. KAMASAWA[2], D. FITZPATRICK[1]
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Abstract: Understanding how single neurons integrate synaptic inputs from myriad sources to generate somatic responses remains a challenge. Further adding to this challenge are recent studies revealing significant functional diversity in populations of synaptic inputs onto individual cortical cells. While organization motifs have been identified in the form of dendritic clustering of inputs aligned to the somatic output and local synaptic clustering of distinct properties, how this relates to unitary synaptic strength and whether clustered inputs result from individual axons remains unknown. To address these issues, we combined *in vivo* two-photon calcium imaging and serial block-face scanning electron microscopy (SBFSEM) to correlate ultrastructural and functional properties of the same subcellular features of individual cells. Here we focus on measuring orientation selectivity and ocular dominance of individual dendritic spines on the basal dendrites of cells sparsely labeled with GCaMP6s in ferret visual cortex. Following morphology-based correlation, we were able to quantify postsynaptic and presynaptic characteristics including spine morphology such as spine head volume, PSD length, bouton size, and vesicle pool size of functionally-identified spines. Furthermore, SBFSEM allows for reconstruction of presynaptic axons to examine whether functionally-defined clusters of spines are axon-coupled or have separate inputs. Comparing synaptic functional properties and ultrastructural properties, we find that spines are commonly connected to a single, exclusive axonal bouton, and exhibit a diversity of sizes (volume, PSD length) regardless of the degree of co-tuning with the soma orientation preference. Interestingly, larger presynaptic boutons form synapses onto spines with similar eye preference to that of the soma. Continued development and use of *in vivo* synaptic imaging and SBFSEM will be key to unravel how synaptic inputs and their functional properties are integrated within the dendritic tree of individual cortical cells.

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Poster

579. Visual Cortex: Functional Architecture and Circuits II

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Topic: D.07. Vision

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Paris-Saclay Idex Icode
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Title: Propagation of "network belief" in the primary visual cortex: Synaptic contribution of the horizontal intrinsic connectivity

Authors: *M. PANANCEAU, B. LE BEC, C. DESBOIS, X. TRONCOSO, Y. FREGNAC
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Abstract: Contextual long-range interactions involved in perceptual binding are generally thought to depend on cortico-cortical feedback (Gilbert and Li, 2013) and attention (Lamme & Roelfsema 2000). In contrast, the contribution of lateral diffusion mechanisms intrinsic to V1 is less known (review in Frégnac & Bathellier, 2015) We explore here the role of the "horizontal" long-distance intra-cortical connections in the dynamic emergence of facilitatory and predictive responses in the primary visual cortex (V1) of the anesthetized cat. .
A previous study of the spatiotemporal features of the subthreshold receptive field (RF) of V1 cells (Gerard- Mercier et al. 2016) showed that 1) synaptic responses to flashed 3-4° Gabor patches can be elicited from the far periphery (up to 15°) and most remarkably, exhibit a coherent organization reminding the grouping bias of the "perceptual association field" for collinear contours (Field et al, 1993) and 2) that presentation of centripetal apparent motion (AM) iso-oriented Gabor patches (GPs) induces a facilitatory modulation along the cell's preferred orientation axis.
Intracellular experiments were designed to further characterize the spatio-temporal coherence requirements of this facilitatory effect. We then used 6-stroke apparent motion (AM) concentric sequences of GPs at saccadic-like speeds (~200°/s) centered on the subthreshold RF. The motion path extended up to 25° into the periphery. The response to the RF center stimulation alone was compared to the one induced by the AM sequences, which were either centripetal or centrifugal, with GP collinear or cross-oriented to the motion path. Control conditions included randomized order of the GPs presentation and the change of the AM speeds. Sequences restricted to the silent periphery of the RF were also tested to reveal possible filling-in responses.
Our results shows that the contextual sequence originating from the far periphery has a strong

boosting effect on the evoked discharge, resulting in a significant phase advance (5-20 ms) of the synaptic response. The supra-linearity of the boosting is specific to centripetal collinear AM at saccadic speeds and could not be induced by either the centrifugal AM or random sequences or at low speed. These results are consistent with our hypothesis that “Gestalt-like” interactions are triggered when the visual input carries a sufficient spatiotemporal coherence matching the properties of the underlying V1 connectivity. We propose that horizontal connectivity participates to the propagation of a network-based belief, resulting in some kind of "prediction" process travelling through the V1 network.

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Poster

579. Visual Cortex: Functional Architecture and Circuits II

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Topic: D.07. Vision

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FP7/2007-2013)/ERC grand agreement no 339244-FUSIMAGINE

Title: Functional mapping of the primary visual areas in awake non-human primates with ultrafast ultrasound imaging

Authors: *F. ARCIZET[1], K. BLAIZE[2], M. GESNIK[3], H. AHNINE[4], T. DEFFIEUX[5], P. POUGET[6], F. Y. CHAVANE[7], M. FINK[3], J.-A. SAHEL[4], M. TANTER[8], S. A. PICAUD[9]
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Abstract: Functional organization of primary visual areas in non-human primates has been massively studied using different imaging techniques as fMRI, optical imaging (calcium, VSDi) or electrophysiological methods with single electrodes or multielectrodes arrays. In the domain of vision, all these techniques allow studying functionality of visual cortex at different scale levels. However, mesoscopic and microscopic examinations of cortical activity in visual cortex have rarely been done simultaneously because of technical limitations. Common imaging techniques as fMRI present low temporal resolution although optical imaging techniques as VSDi are limited to the surface exploration. New functional ultrasound (fUS) imaging technique, which consists of recording cerebral blood volumes variations, overpasses several of these

limitations. Indeed, with this method, we obtained functional images with an high spatial resolution (~100µm) and an high temporal frequency (1Hz) even at deeper cortical locations (~1,5cm) that cannot be reached with other optical imaging methods (calcium imaging or VSDi), for example through the cortical sulci. In this study, the animals were performing a passive fixation task in which different types of visual stimuli were flashed briefly on a computer screen while we were recording functional ultrasound images of their visual cortex. We showed that using this innovative technique, we were able to reconstruct retinotopic and ocular dominance maps of primary visual areas (V1/V2/V3) with an high spatio-temporal resolution in awake behaving monkeys. Indeed, the results showed evidence of the retinotopic organization of the calcarin sulcus in V1, V2 and V3 areas. This technique also allows revealing the presence of ocular dominance bands in V1 cortex. Together, these results provide a novel perspective of using functional ultrasound imaging to study deep cerebral activity in different brain areas at the columnar or layer level.

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Poster

579. Visual Cortex: Functional Architecture and Circuits II

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Title: Prediction of future input explains lateral connectivity in primary visual cortex

Authors: *E. FRISTED, Y. SINGER, A. J. KING, M. F. IACARUSO, N. S. HARPER
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Abstract: Neurons in primary visual cortex (V1) show complex tuning properties[1] and functional specificity of local synaptic connectivity, whereby neurons with strong response correlations and similar spatial receptive field structure are more likely to be connected and to form strong connections[2][3]. However, it is not clear what is the functional significance of this

relationship between tuning properties and connectivity. We hypothesize that the connectivity of neurons in V1 is optimized to predict the immediate future of visual inputs from recent past inputs. To test this hypothesis, we implemented a temporal prediction model[4] in a one hidden layer recurrent neural network (RNN), which was trained to predict the next frame in movies of natural scenes from the recent past frames. Tuning properties were determined by measuring the network's response to drifting grating stimuli and natural visual scenes, and network connectivity was obtained from the recurrent weight matrix. The excitatory units of the network developed tuning properties resembling those of mouse V1 neurons[5], and were sparsely connected. The network showed functionally specific connectivity, whereby excitatory units were more likely to connect and form strong connections if they had similar orientation or direction tuning. Furthermore, connection probability between units increased with their response correlation to natural visual scenes. Finally, units with both simple- and complex-like tuning properties emerged in the network. Having validated the model, we used it to generate testable predictions of the lateral connectivity of inhibitory units in V1. Our results provide support for the hypothesis that the lateral connections in V1 are optimized for prediction of future visual input.

References

[1] Hubel & Wiesel (1962) J Physiol 160: 106-154. [2] Ko et al. (2011) Nature 473:87-91. [3] Cossell et al. (2015) Nature 518:399-403. [4] Singer et al. (2017) bioRxiv 224758. [5] Niell & Stryker (2008) J Neurosci 28:7520-7536.

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Poster

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Topic: D.07. Vision

Support: MIT Grant EY025437

Title: Mapping thalamic projections onto L2/3 pyramidal neurons of visual cortex

Authors: *A. BALCIOGLU
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Abstract: Cortical pyramidal neurons receive thousands of excitatory inputs from cortical, as well as subcortical sources. The relative numbers of these inputs, and their location across the arbor, and in relation to each other, determines the impact of each given source on action potential firing. Where inputs are situated on the arbor in terms of the apical vs basal tufts,

distance from the soma, branch points, and how they are distributed or clustered at these locations will significantly influence how they are integrated locally per dendritic segment, as well as across the entire cell. Unfortunately, we have little information regarding the mapping of different afferent inputs sources onto cortical pyramidal neurons. Using a thalamus specific Cre driver line crossed to a conditional synaptophysin Tdtomato (syn-Td) reporter line to specifically label thalamic afferents, in combination with a methodology for sparse labelling of cortical neurons and their excitatory synapses, we asked the following question: Do excitatory projections coming from thalamus differ in their distribution on L2/3 pyramidal arbors, as compared to intracortical excitatory inputs? L2/3 pyramidal neurons in pups expressing thalamic syn-Td were labeled by *in utero* electroporation with eYFP as a cell fill and teal-PSD95 to mark stable excitatory synapses. After cranial window implantation at 6 weeks, the entire cell volume was imaged *in vivo* at 8 weeks using multi spectral, high resolution, two-photon microscopy. PSD95-labeled excitatory synapses on imaged neurons were scored as either thalamic (syn-Td positive) or cortical (syn-Td negative) with a custom-written 4D point-tracking system implemented in Fiji. We found that: 1) The number of thalamic inputs onto the L2/3 neurons was much higher than expected considering L2/3 is not the primary recipient layer of thalamic innervation. 2) There was no preference for apical vs. basal distribution of thalamic inputs. 3) Thalamic innervation was not uniform across cells, with different cells receiving different degrees of innervation. 4) Distribution of thalamic vs. cortical afferents across individual dendritic branches was non-random. In conclusion, cortical L2/3 pyramidal neurons receive extensive cell type specific and structured thalamic innervation.

Disclosures: A. Balcioglu: None.

Poster

579. Visual Cortex: Functional Architecture and Circuits II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 579.24/HH8

Topic: D.07. Vision

Support: NSERC Discovery Grant

Title: Modelling contour integration in deep artificial neural networks

Authors: S. KHAN[1], A. WONG[1], *B. P. TRIPP[2]
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Abstract: Contour integration is a phenomenon in V1 where stimuli from outside a neuron's classical receptive field have an influence over its responses. In particular, responses are enhanced if a preferred stimulus within the classical receptive field is part of a larger contour. Contour integration is thought to be mediated by intra-area lateral and higher-layer feedback

connections. Past computational models have tested potential mechanisms and replicated observed neurophysiological data. However, past studies have done little to explore the role of contour integration in the perception of naturalistic scenes.
In this research, we explore the integration of a computational model for contour integration into a deep artificial neural network, for the task of object classification, in order to investigate the relationships between low-level contour integration phenomena and complex perceptual functions. The computational model is trained to match empirical enhancement gains for several different contour configurations, including variations in contour length, spacing, and curvature. Unlike previous computational models, no predefined lateral connection structure is assumed, and the networks learns the patterns and weights of sparse lateral connections. These connection structures are compared with observed horizontal connections in the V1 cortex. Importantly for scaling the model to networks that perform sophisticated naturalistic tasks, the model works with learned feed-forward kernels, rather than idealized Gabor kernels. Future work will explore the effects of parameter variations on various perceptual contexts, such as recognition of partially occluded objects.

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Poster

579. Visual Cortex: Functional Architecture and Circuits II

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Topic: D.07. Vision

Support: MTA-DE Neuroscience Research Group
NAP2

Title: Functional topography and synaptic targets of a new cell type in the primary visual cortex of the cat

Authors: *Z. F. KISVARDAY, M. SRIVASTAVA, C. ANGEL, S. MOHAMMED, A. NADHEM
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Abstract: Traditionally, neurons possessing spiny dendrites such as pyramidal, star-pyramidal and spiny stellate cells are considered excitatory in nature whereas smooth dendritic neurons are inhibitory. Here we report a smooth dendritic, i.e. spine free cell type in layer 6 of the adult visual cortex which shows, however, a host of morphological features typical for excitatory neurons. The neuron was selected from a large pool of intracellularly labelled cells based on its unique morphological features such as large soma size, numerous long and barely branching dendrites which were emitted in all directions from the cell body. The main axon entered the

white matter but also gave off long-range horizontal axons which extended up to 2.8 mm in layers 5/6. 3D-reconstruction of the cell revealed that the axon provided chiefly en passant boutons (n=1192) which were rather uniformly distributed without obvious spatial clustering. Dendritic length, surface and volume measurements revealed that these parameters are at least 3 times larger than corresponding parameters of any known neuron types in the cat visual cortex. Quantitative electron microscopy of the labelled boutons from projection zones representing proximal and distal parts of the axon showed that for both categories the postsynaptic targets are chiefly dendritic spines and dendritic shafts of other excitatory neurons while GABA immunopositive dendrites represent a minority of the targets. Superimposing the axonal field on the orientation map obtained with intrinsic signal optical imaging showed that the majority of connections prefer oblique orientations rather than iso-orientation. The data obtained for this novel cell type suggest that its likely role is integration of a broad range of cortical inputs.

Disclosures: Z.F. Kisvarday: None. **M. Srivastava:** None. **C. Angel:** None. **S. Mohammed:** None. **A. Nadhem:** None.

Poster

579. Visual Cortex: Functional Architecture and Circuits II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 579.26/HH10

Topic: D.07. Vision

Support: Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS)—AMED
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Title: Mesoscale and cellular scale calcium imaging of the primary and secondary visual cortex in marmoset monkeys

Authors: *T. MATSUI, T. HASHIMOTO, T. MURAKAMI, M. UEMURA, K. KIKUTA, T. KATO, K. OHKI
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Abstract: Primate neocortex analyzes visual scenes with a hierarchical neuronal network consisting of multiple interactive functional structures spanning broad range of spatial scales,

such as cortical areas, columns and single cells. To understand how such cortical entities interactively process visual scenes, it is necessary to record neuronal responses at multiple spatial scales in a seamless manner. We are currently developing such a method applicable to primates using a combination of wide-field and two-photon calcium imaging (see for details Uemura et al., in this meeting). In this study, we report preliminary application of the method to marmoset monkeys. Neurons in the primary and secondary visual cortex (6.5 x 6.5mm field-of-view) were transduced with adeno-associated viruses carrying genetically encoded calcium indicator optimized for primates (see Sadakane et al., 2015 and Uemura et al., in this meeting). We then performed wide-field and two-photon calcium imaging in the same animal to seamlessly cover multiple spatial scales. Wide-field imaging revealed the orientation map in both V1 and V2 whose boarder was identifiable by the change in the size of the orientation columns. V2 was also identifiable by an alternating pattern of band-like regions with high and low orientation-tuning, likely corresponding to the cytochrome oxidase stripes. Subsequent two-photon imaging revealed orientation columns and pinwheels organized at the single cell level in V1 and V2. In V2, we also observed clustering of cells with low orientation-tuning and high orientation-tuning, consistent with the alternating pattern observed with wide-field imaging. Thus the present results demonstrate the usefulness of calcium imaging to understand the primate visual cortical network at multiple spatial scales.

Disclosures: T. Matsui: None. **T. Hashimoto:** None. **T. Murakami:** None. **M. Uemura:** None. **K. Kikuta:** None. **T. Kato:** None. **K. Ohki:** None.

Poster

580. Vision: Processing of Contrast, Form, and Color

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 580.01/HH11

Topic: D.07. Vision

Support: ARL CAST 076910227001
ARL-74A-HR53

Title: Contextual effects of high dynamic range (HDR) luminance flankers on orientation discrimination

Authors: *C. P. HUNG[1,3], A. V. HARRISON[2], A. J. WALKER[1,4], M. WEI[1,4], B. D. VAUGHAN[1]
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Abstract: Computational models of visual search behavior include luminance normalization based on spatial context and laboratory conditions of ~100:1 luminance contrast ratio (standard dynamic range, SDR). How do feature and luminance context interact under real-world contrast ratios of up to 10^6:1? Recent reports of brightness perception have revealed non-linear effects of luminance normalization at contrast ratios over 1000:1 (high dynamic range, HDR), expanding the perceived shadings of gray at the mode of the luminance distribution. We hypothesize that, because visual neurons encode both luminance and orientation, luminance and orientation processing may interact non-linearly during visual recognition. We tested whether flanker effects on target orientation discrimination is stronger for the brightest flankers or for flankers that are similar to the target in luminance. We measured EEG, eye tracking, and visual recognition behavior under a two-alternative forced choice (2AFC) task and under rapid serial visual presentation (RSVP, 0.5-2 Hz). Stimuli consisted of 45- and 135-deg Gabors presented on a 5 × 5 grid of luminance patches (100:1 patch contrast, 10000:1 peak contrast including Gabors). The target was a contrast blend of Gabors at the two orientations, and subjects indicated via keypress the orientation with the higher contrast. In one condition, the co-oriented flanker patches were the brightest patches, and in the other condition, the co-oriented flanker patches were similar in luminance to the target patch. Dependent variables include behavioral response time and accuracy, stimulus and ocular-locked EEG amplitude, latency and frequency, and pupil size. Our results show that luminance-orientation context has a significant bias on orientation discrimination that is opposite across the two conditions. The bias is more robust for the second condition, favoring a luminance-similarity contextual effect. We are modeling the effect of luminance and shape interaction in visual search by manipulating the level of interaction between luminance adaption and local orientation based filtering. Standard models of visual search assume shape based filtering and luminance adaptation happen largely in parallel and independently, but nonlinear luminance adaption may directly affect the filtering of shapes with regard to the background.

Disclosures: C.P. Hung: None. **A.V. Harrison:** None. **A.J. Walker:** None. **M. Wei:** None. **B.D. Vaughan:** None.

Poster

580. Vision: Processing of Contrast, Form, and Color

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 580.02/HH12

Topic: D.07. Vision

Support: SFB 1280

Title: Establishing optogenetics in pigeons on a histological, physiological and behavioral level

Authors: *N. ROOK[1], J. TUFF[1], S. ISPARTA[1], O. MASSECK[2], S. HERLITZE[3], R. PUSCH[1], O. GUNTURKUN[1]
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Abstract: One of the major goals of neuroscience is to understand the function of specific cell types in micro- and macro-brain circuitry, in health, and disease. To study the function of neuronal networks, methods that are able to control neuronal activity precisely are indispensable. This ambitious goal was first achieved with optogenetics, allowing researchers to activate or silence specific networks with high temporal and spatial resolution through the integration of artificial light-sensitive ion channels into the cell membrane. Given those advantages it is not surprising that optogenetics has been established in a wide range of species such as rodents and primates in the last years. In contrast to the revolution optogenetics has brought to rodent research, only few studies have been reported in birds and none so far in pigeons. However, pigeons are a very valuable research organism as they offer a model with excellent visual capabilities for comparative research. Therefore, establishing optogenetics in pigeons is inevitable for future research. One crucial step of optogenetics is the integration of the light-sensitive proteins into the cell membrane of neurons mainly via viral vectors and cell type-specific promoter systems. A lot is known about those parameters in rodents, but this data cannot simply be transferred to another organism since viral transduction and transgene expression have been found to vary extensively across species. Therefore, in a first study, we compared the transduction efficiencies of three adeno-associated viral vector (AAV) serotypes AAV1, AAV5 and AAV9 either with the neuron-specific hSyn promoter or with the non-selective CAG promoter. We found that AAV1 was the most efficient serotype regardless of the utilized promoter system. Therefore, this construct was used for the following physiological and behavioral experiments. With simultaneous electrophysiological recordings and optical stimulation in the anesthetized pigeon, we could show that blue light at 480 nm reliably evokes action potentials in ChR2 expressing cells. Finally, stimulating ChR2 expressing cells in the entopallium of the pigeon brain (visual area) during a visual discrimination task significantly reduced the performance of the pigeons. Thus, this study successfully established optogenetics in pigeons offering a tool box for further optogenetic experiments in birds

Disclosures: N. Rook: None. **J. Tuff:** None. **S. Isparta:** None. **O. Masseck:** None. **S. Herlitze:** None. **R. Pusch:** None. **O. Gunturkun:** None.

Poster

580. Vision: Processing of Contrast, Form, and Color

Location: SDCC Halls B-H

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Program #/Poster #: 580.03/HH13

Topic: D.07. Vision

Support: Tata Trusts grant

Title: Age dependence of visual gamma oscillations elicited by Cartesian gratings in human EEG

Authors: DINAVAHI V P S MURTY, S. RAY
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Abstract: Gamma rhythms have been proposed to be abnormal in various neuropsychiatric disorders such as schizophrenia. Since these rhythms could be important for developing non-invasive and early diagnostic tools for such diseases, it is necessary to study their dependence on various demographic variables like age and gender. Recent studies have observed two different gamma oscillations (slow: 20-40 Hz and fast: 40-70 Hz) in human occipital cortex that are induced by visual gratings in EEG/MEG recordings. In this study, we explored the relationship between these oscillations and age, using full-screen static Cartesian gratings in 41 young-middle aged subjects (aged 20-48 years, mean age: 29.3 ± 6.2 years, females: 14) and 44 elderly subjects (aged 51-85 years, mean age: 64.3 ± 7.6 years, females: 18) with no known history of neuropsychiatric illness.

We found that ~80% of the subjects showed a change in power from baseline by 0.5 dB or more in at least one of the two gamma rhythms. Interestingly, we found that stimulus-induced change in fast, but not slow gamma power decreased significantly with age in both males and females. Further, females had significantly higher change in fast, but not slow gamma power compared to males in both young and elderly age groups. Moreover, the total number of bipolar electrodes in centro-parietal to occipital regions showing gamma activity decreased with age for fast gamma for both genders, but not for slow gamma.

Apart from gamma, we also tested responses of two other oscillations: steady-state visual evoked potentials (SSVEP) using gratings counterphasing at 16 Hz that generated a peak at 32 Hz, and suppression in alpha (8-12 Hz) power. Similar to slow gamma, SSVEP at 32 Hz did not differ with age, both in terms of power change or scalp distribution. However, suppression in alpha power to static gratings tended to decrease with age as in the case of fast gamma, although the results were weaker and not significant for males.

Our preliminary analyses show that both slow gamma oscillation and SSVEP in slow gamma range are more stable across age and gender compared to fast gamma or alpha suppression. These characteristics may make these oscillations desirable candidates for biomarker testing in diseases of the brain especially in elderly, like in the case of Alzheimer's disease.

Disclosures: Dinavahi V P S Murty: None. **S. Ray:** None.

Poster

580. Vision: Processing of Contrast, Form, and Color

Location: SDCC Halls B-H

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Topic: D.07. Vision

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China Postdoctoral Science Foundation 2016M602754

Title: The cortical dynamics underlying contour integration in human visual system

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Abstract: The human visual system efficiently extracts local elements from cluttered backgrounds and integrates these elements into meaningful contour perception. Contour integration is a critical step before object recognition, in which contours often play an important role in defining the shapes and borders of the to be recognized objects. Previous investigations have suggested that both striate and extrastriate visual areas are involved in the process of contour integration. However, the cortical dynamics of these areas in the human brain during contour integration is less understood.
The present study used fMRI to investigate the temporal evolvement of contour integration at different levels of the visual processing hierarchy. Specifically, using a sandwich masking paradigm, we combined psychophysics and fMRI to reveal how low-level (V1, V2, V3) and higher-level (V3B, LO) visual areas responded during the process of contour integration. In fMRI session, the Gabor filed with or without a contour embedded was presented with a duration of 33, 67, 133, 267 ms and was temporally sandwiched between masking stimuli. Human participants were required to indicate whether the contour was present or not. By contrasting BOLD responses between contour and non-contour (i.e. CI index) for all stimuli duration condition, we measured the psychophysical time course of contour integration and its underlying cortical dynamics.
The results showed that both low- and higher-level visual areas were involved in the process of contour integration, but they responded at different activation patterns. First, the first stimuli duration at which the visual areas revealed significant CI activation was earlier in LO than in other areas (LO: 33 ms; others: 67 ms). Second, we found that the CI index in higher visual areas (LO, V3B) increased as the stimulus duration increased, which was consistent with behavior

results. These results suggested that LO is critical involved in the processing of contour integration. However, the CI index in early visual areas (V1, V2) was significant at two separate stimulus duration: an early activation (67 ms) and a late reactivation (267 ms). This activation and reactivation pattern is consistent with previous EEG-fMRI study (Mijović et al., 2014). These results suggest the feedback from higher visual area to lower visual area is involved to contour integration. Our findings fit well with the incremental grouping theory (Roelfsema, 2006), in which a feedforward sweep generates a coarse template in higher visual areas with large receptive fields before the processing of detail information in lower visual areas with small receptive field through feedback mechanisms.

Disclosures: Y. Li: None. **Y. Wang:** None. **S. Li:** None.

Poster

580. Vision: Processing of Contrast, Form, and Color

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Program #/Poster #: 580.05/HH15

Topic: D.07. Vision

Support: 2016/23/N/HS6/00829

Title: Late positivity component as neural correlate of post-perceptual processing in different version of visual backward masking task

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Abstract: The goal of the presented study is to disentangle electrophysiological correlates of visual awareness from other task-related processes through manipulating the level of processing of visual stimuli. Brain activity reflecting conscious perception should only be correlated with subjective visibility (measured with ratings on Perceptual Awareness Scale, PAS) and be independent of the perceptual task. Two experiments (E1 and E2) with a visual backward masking task were conducted. The design of both experiments was within-subject; each participants performed 420 trials with stimulus, location and condition order randomized. Thirty healthy participants (12 male, M=24.21) took part in E1. In each trial a stimulus (either a line or a letter, depending on condition) was shown randomly in one of four possible locations (2º from fixation). Stimuli, displayed for 16 ms, were followed by a fixed mask. Then, participants were asked to perform 2AFC task, either with line slope identification or letter classification. After each trial participants rated the visibility of stimulus on the PAS scale. In E2 36 healthy students participated in the experiment (15 male, M=23.41). This time, stimuli was the same in each condition (masked digit, either red or blue, presented for 50 ms). Similar to E1, every subject performed a 2AFC task (either discriminating the color or magnitude of the digit) and assessed

subjective visibility of it on the PAS scale. In both experiments EEG data were collected with 64-channel BioSemi System and preprocessed according to the same routine: filters: 0.1Hz-30Hz band (plus 50Hz notch filter); ICA Ocular Correction; visual inspection; re-referencing to average; baseline window: -200ms to 0ms pre-stimulus. Amplitude of two components: Visual Awareness Negativity (VAN: 140-240 ms, channels PO7, PO8, PO4, PO3, O2, O1) and Late Positivity (LP, 380-480 ms, Pz) was analyzed with weighted regression mixed model.In E1: for VAN component time window the mean amplitude correlated with condition. The mean amplitude in LP window correlated with PAS rating and condition (with no significant interaction between condition and PAS). In E2 the mean amplitude of VAN correlated with PAS rating in both conditions. The mean amplitude in LP window correlated with PAS rating in the magnitude judgement condition, but not in the color judgement condition.Both VAN and Late Positivity were observed, but their role was different depending on task that was performed. VAN amplitude was sensitive to PAS Ratings in E2, but not in E1. The pattern of LP was also different in E1 and E2. Therefore, our results contest the interpretation of LP and VAN as neural correlate of visual awareness.

Disclosures: M. Koculak: None. **K. Gociewicz:** None. **M. Wierzchon:** None. **M. Binder:** None.

Poster

580. Vision: Processing of Contrast, Form, and Color

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 580.06/HH16

Topic: D.07. Vision

Title: Two-photon imaging evidence for neuronal responses to superimposed cross-gratings in macaque V1

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Abstract: The responses of V1 neurons to a grating are suppressed by a superimposed orthogonal grating. This cross-inhibition effect suggests divisive normalization, a crucial computation that determines the non-linearity in neuronal contrast response functions. Previous studies investigated cross-inhibition through single-/multi-unit recording and functional brain imaging (e.g., fMRI). Here we used two-photon calcium (GCaMP5) imaging (Li et al., Neuron, 2017) to study this effect again, with the advantage to record simultaneously the responses of hundreds of neurons at single-neuron precision, and with relatively unbiased neuron sampling. We recorded the responses of superficial layer (2/3) V1 neuron (150 and 400-μm depths) at 4-5^{o}

eccentricity in an awake, fixating monkey. The stimuli were either a single Gabor or two orthogonal Gabors forming a plaid. Each Gabor drifted at 2-cycles/sec, with 6 orientations (30^o apart) and three SF ranged from 2-5 cpd. The contrast was either 0.32 (near the saturation contrast) or 0.08. Two types of V1 neurons responded to single and plaid gratings. The first type (~70%) was orientation-tuned, and the peak responses to a grating were overall suppressed by an orthogonal grating. The responses to a 0.32-contrast grating ($R_{0.32}$) were similarly reduced by an orthogonal 0.32-/0.08-contrast grating (median Cross Modulation index CM = ((R_{cross}-$R_{0.32}$)/((R_{cross}+$R_{0.32}$)) = -0.39/-0.43). $R_{0.08}$ were more suppressed by an orthogonal 0.08-contrast grating (median CM=-0.41), than by a 0.32-contrast grating (median CM=-0.23). A small portion of neurons showed enhanced $R_{0.08}$ by an orthogonal grating of either contrast (CM>0). We did not observe the winner-take-all effects, in which only $R_{0.08}$ would be suppressed by a 0.32-contrast orthogonal grating, but not $R_{0.32}$ by a 0.08-contrast grating. The second-type (~30%) showed previously unreported behaviors. These neurons showed weak and orientation non-selective responses to a 0.32-contrast grating, but strongly responded to 0.32+0.32 plaid gratings (median CM=0.58). The two types of neurons showed similar peak response strengths to their preferred single or plaid gratings. Our results provide more detailed and unbiased estimates of the cross-inhibition effects for first-type V1 neurons. The previously reported winner-take-all effect is not observed, which we suspect may result from higher stimulus contrasts placed in the saturation region of neurons' contrast response functions. The second-type V1 neurons' responses cannot be revealed via traditional single-unit recordings that first map the RFs with oriented stimuli, and then assess the responses to plaid gratings.

Disclosures: S. Guan: None. **N. Ju:** None. **S. Zhang:** None. **S. Tang:** None. **C. Yu:** None.

Poster

580. Vision: Processing of Contrast, Form, and Color

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 580.07/HH17

Topic: D.07. Vision

Support: IBS-R015-D1

Title: Location-specific attentional modulation of neural representation of color in the human LGN

Authors: *S. PARK[1], S. HONG[2,3], Y. LEE[1,4], W. SHIM[1,4]
[1]Ctr. for Neurosci. Imaging Research, IBS, Suwon, Korea, Republic of; [2]Psychology, Florida Atlantic Univ., Boca Raton, FL; [3]Ctr. for Complex Systems and Brain Sciences, Florida Atlantic Univ., Boca Raton, FL; [4]Biomed. Engin., Sungkyunkwan Univ., Suwon, Kyunggi-Do, Korea, Republic of

Abstract: Previous work on the nonhuman primate visual system shows a distinction between two chromatic channels, parvocelluar (L-M) and koniocellular (S-(L+M)) in the lateral geniculate nucleus (LGN). Using fMRI in conjunction with encoding methods, we investigate how neural representations of L-M opponent and S-cone opponent colors are modulated by spatial attention in the human LGN. On each block, observers viewed expanding or contracting equiluminant concentric ring patterns with colors varying on either the L-M or S-(L+M) axis only or colors varying on both color axes. To manipulate spatial attention, participants were instructed to detect specific letters in an RSVP sequence presented at the foveal 0.5° or to detect a circular probe presented at an eccentricity of 5° from the central fixation point. The probe was defined by sudden chromatic contrast reduction in a circular region. We constructed an inverted encoding model of color (Brouwer & Heeger, JNeurosci, 2009) and reconstructed population-level color-selective response profiles in the LGN using a leave-one-run-out procedure. The result showed the effect of spatial attention on color-selective responses in the LGN. The color selectivity was more prominent when spatial attention was deployed to the foveal visual field, compared to when attention was directed to the parafoveal visual field. Crucially, however, the color-selective response to S-cone opponent colors was stronger when the parafoveal visual field was attended, whereas the color response to L-M opponent colors was stronger when the foveal visual field was attended . The distinct patterns of attentional modulation of the neural responses to L-M opponent and S-cone opponent colors may arise from the cone distribution on the retina, where S-cone populations spread more across parafovea compared to L and M cones, which are heavily concentrated in the central fovea (Ahnelt et al., 1987). This location-specific attentional modulation was absent in the early and high-level visual cortical areas (V1, V2, V3, V4v, and VO). Our results indicate that neural representations of colors in the human LGN are affected by spatial attention and that color-selective responses to L-M and S-cone can be distinctively modulated by location-specific attention.

Disclosures: S. Park: None. **S. Hong:** None. **Y. Lee:** None. **W. Shim:** None.

Poster

580. Vision: Processing of Contrast, Form, and Color

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 580.08/II1

Topic: D.07. Vision

Support: Whitehall Foundation

Title: Spatio-temporal processing of rod and cone inputs to mouse V1

Authors: *I. RHIM[1,2], G. COELLO-REYES[1,2,3], I. NAUHAUS[1,2,3]
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Abstract: Classic paradigms for parsing parallel inputs to visual cortex vary three stimulus dimensions: space, time, and color. Although channels in the mouse retina are also tuned to these dimensions, only achromatic spatio-temporal tuning based on rod inputs has been carefully investigated at the level of the cortex. Rod inputs are routed through relatively sluggish and spatially broad circuitry in the retina. The notion that cortical studies are based on rod inputs can be gleaned from the fact that visible wavelengths were able to drive all regions of the visual field; S-opsin dominates the cone mosaic in the ventral 2/3 of the retina and is only sensitive to UV wavelengths.
In our previous work, we showed that the dorsoventral opsin gradient in the mouse retina causes an "opsin map" in V1 and higher visual areas that correlates with the map of vertical retinotopy (Rhim et al., J. Neurophysiol. 2017). Our ongoing calcium imaging experiments measure the same opsin map in cortex as a means to determine graded levels of rod saturation, as a function of baseline luminance - as luminance falls, robust responses remain, yet the cone-opsin map is systematically lost. We will present data from this paradigm to show that standard in-vivo mouse vision experiments measure rod processing. We are also using this paradigm to measure differential contributions of rods and cones to spatio-temporal processing in V1. In addition to wild-type mice, rod-deficient KO mice (Gnat1 -/-) are being used in this series of experiments for a guaranteed pure cone condition. Under mesopic and photopic conditions, we are testing the hypothesis that color tuning is inseparable from spatio-temporal tuning, consistent with classical notions of color vs. form and motion. Our current results are that spatial frequency tuning is inseparable across both color direction and retinotopic location in mouse V1.

Disclosures: I. Rhim: None. **G. Coello-Reyes:** None. **I. Nauhaus:** None.

Poster

580. Vision: Processing of Contrast, Form, and Color

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Program #/Poster #: 580.09/II2

Topic: D.07. Vision

Support: NIH Grant EY026031-02

Title: Neuronal specialization for object size detection by higher order visual projection neurons in *Drosophila*

Authors: *C. STAEDELE, M. A. FRYE
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Abstract: Discerning an object from the visual panorama is essential for animal survival and a complex task that requires sophisticated neural algorithms. An important part of distinguishing objects from panoramic background is the extraction of salient features of objects such as size,

edges, contrast, and color. Feature-selective neurons seem to play a key role in this task and have been identified in several animals. Yet, how they detect and encode different attributes of objects such as object size is only partially understood. Despite possessing a low-resolution eyes and brains with fewer than a million neurons, *Drosophila melanogaster* shows superb capabilities for size-based classification of visual objects in that vertically elongated bars and small two-dimensional objects elicit behaviors of opposite valence. Our work focuses on Lobula Columnar (LC) neurons, a class of 22 anatomically similar visual projection neuron types in the fly lobula that have recently been suggested to be involved in higher order feature detection. We found that three out of 22 LC neuron classes show size-based responses to visual objects. Using two-photon optical imaging, we found that one is tuned to small two-dimensional objects (LC11), another is selective for elongated one-dimensional bars (LC15), and a third combines selectivity for both small objects and bars (LC12). None are excited by motion of the visual panorama. We hypothesized that this difference in size tuning is based on a unique channel composition and transcriptional profile of each LC type, plus different synaptic connectivity. Thus, we characterized the receptive field properties and found that all three LCs are tuned differently to e.g. vertical and horizontal object size, direction, motion, and contrast. Interestingly, blocking GABAergic inhibition abolished object selectivity in all three LC types and facilitated responses to panoramic gratings. We are currently testing the contribution of feed-forward inhibition to the size-based categorization of visual objects.

Disclosures: C. Staedele: None. **M.A. Frye:** None.

Poster

580. Vision: Processing of Contrast, Form, and Color

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Program #/Poster #: 580.10/II3

Topic: D.07. Vision

Support: 1DP2-EY025439
The Whitehall Foundation

Title: Contribution of inhibitory cell types in mouse primary visual cortex to perceived contrast

Authors: *R. BLAZING, J. R. SIMS, R. KIRK, M. FOWLER, A. MCKINNEY, M. JIN, L. L. GLICKFELD
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Abstract: Visual perception results from the concerted activity of functionally and morphologically diverse populations of neurons in the visual cortex. It is unclear, however, how the computations of distinct inhibitory cell types contribute to specific aspects of perception. The activity of parvalbumin-expressing (PV+) inhibitory interneurons regulates the gain of local

pyramidal cells in a manner that mimics changes in stimulus contrast (Atallah et al., 2012; Nienborg et al., 2013). In addition, the exogenous activation of PV+ cells in the primary visual cortex (V1) increases the threshold for detecting changes in contrast. Thus, we hypothesized that PV+ interneurons in V1 uniquely and specifically control perceived contrast. To test this hypothesis, we trained mice expressing the light-sensitive ion channel, Channelodopsin-2 (ChR2), in either PV+ or somatostatin-expressing (SOM+) interneurons in V1 on a head-fixed, two-alternative forced choice (2AFC) contrast discrimination task. In this task, the mouse is presented with two circular gabor stimuli of different contrasts in opposite visual hemi-fields, and must turn a wheel to indicate the higher contrast stimulus. We obtained a psychophysical readout of contrast discrimination by changing the relative contrast of the two stimuli. Regression analyses confirm that mice are performing the task by relying on the contrast ratio of the two stimuli, and not on the absolute target contrast. To determine the effect of the activity of specific interneuron classes on perceived contrast, we used blue light to stimulate ChR2-expressing PV+ or SOM+ cells in V1 during presentation of the visual stimuli. We found that activation of both PV+ and SOM+ interneurons in left V1 biased choice in the 2AFC task towards the left stimulus, indicating a decrease in the perceived contrast of the contralateral stimulus. Thus, we show that in the mouse, the contribution of V1 to contrast perception is not regulated by cell-type-specific mechanisms, but rather is generally mediated by inhibition. To assess whether the activity of these unique cell types specifically mediates the perception of contrast, we are currently training mice on a size discrimination task in which we vary the relative size of two same-contrast stimuli. We expect that if changes in PV+ and SOM+ activity specifically control perceived contrast, the effects of activation of these cell populations on behavior in the size task will be analogous to decreasing stimulus contrast. Together, these experiments will serve to elucidate the cortical computations underlying the perception of discrete visual features.

Disclosures: R. Blazing: None. **J.R. Sims:** None. **R. Kirk:** None. **M. Fowler:** None. **A. McKinney:** None. **M. Jin:** None. **L.L. Glickfeld:** None.

Poster

580. Vision: Processing of Contrast, Form, and Color

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 580.11/II4

Topic: D.07. Vision

Title: Can the inclusion of a specialized sensory-preprocessing stage lessen the training requirements and improve the generalization behavior of a deep network?

Authors: *A. S. RIOS[1], G. C. MEL[2], V. AKOPIAN[1], L. ITTI[1], B. W. MEL[1]
[1]USC, Los Angeles, CA; [2]Stanford Univ., Palo Alto, CA

Abstract: Deep neural networks, which purport to represent the ventral visual processing stream, perform well at large scale object recognition benchmarks, yet exhibit several un-biological performance characteristics:
• They require massive quantities of human-labeled data
• Learning depends on high-precision mathematical operations
• Trained networks are highly susceptible to "adversarial" inputs
• Learning new material causes catastrophic forgetting of old material
Thus, modern neuromorphic software and hardware systems, while technically impressive, fall short of reproducing the remarkable learning capabilities and learning style of the human brain. The simplest theory to explain this constellation of undesirable traits is that modern neural network architectures lack the proper representational biases that would allow them to efficiently solve the biologically relevant problems given to them, particularly the specialized early sensory processing stages. In order to perform well on benchmark tests, therefore, these generic networks must be given too many parameters in too many layers, which leads to the demand for huge quantities of labeled data, and the need for high-precision gradient calculations during learning. At the behavioral level, these oversized and inefficiently parameterized models are slow to learn, are fooled by inputs that would never fool a biological vision system, and have difficulty incorporating new knowledge without destroying previously stored information [1,2].
Given the importance of contours for object classification [3], we hypothesized that pre-processing images with a commercial-grade contour detector that nominally replicates V1 functionality would simplify the learning task, such that attaining any given level of classification performance would require significantly less training data and time, and the resulting network would be less susceptible to egregious classification failures, catastrophic forgetting, etc. Our preliminary results indicate that the contour preprocessing does indeed significantly reduce network training requirements. Evaluation of the robustness of classification behavior under various challenges is ongoing.
[1] Goodfellow, IJ, Shlens, J, and Szegedy, C. "Explaining and harnessing adversarial examples." arXiv preprint arXiv:1412.6572 (2014).
[2] McCloskey, M, Cohen, NJ, Bower, GH. Catastrophic interference in connectionist networks: the sequential learning problem.
[3] Biederman, I. "Recognition-by-components: a theory of human image understanding." Psychological Rev. 94.2 (1987): 115.

Disclosures: A.S. Rios: None. **G.C. Mel:** None. **V. Akopian:** None. **L. Itti:** None. **B.W. Mel:** None.

Poster

580. Vision: Processing of Contrast, Form, and Color

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 580.12/II5

Topic: D.07. Vision

Title: Disentangling feature complexity and pooling region size in metameric texture perception

Authors: *A. V. JAGADEESH, J. L. GARDNER
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Abstract: In peripheral vision, physically different images with similar high-order statistics can be indistinguishable, i.e. metameric. This suggests there are perceptual mechanisms that compute the presence of complex, high-order features and pool these over regions of visual space. Thus, stimuli with similar complex features can appear metameric even if those features are spatially scrambled within pooling regions. If so, both the complexity of features and the pooling region size should determine the degree of metamerism of visual textures. We tested this hypothesis by modifying an algorithm for generating textures using convolutional neural networks (CNNs) to allow control of both the complexity of matched features and the size of pooling regions. To control complexity, we generated stimuli that matched Gram matrices containing the inner product of pairs of filter activations across spatial locations at various CNN layers, since later layers compute more complex, nonlinear features. To control pooling region size, we divided the image into uniform sized square subregions and computed a Gram matrix within each subregion. Starting from a random image, we used gradient descent to generate textures that minimize the squared-error to the Gram matrices of an original image. We generated textures that were matched to 3 different layers of the VGG-19 network (pool1, pool2, pool4), at 4 different pooling region sizes (1.5, 2, 3, 6 degs width) for 20 natural images (6 degs). We conducted a visual search experiment to examine how these manipulations affect perceptual similarity. On each trial, subjects were shown the original image subtending 6 degs at fixation for 500ms. After a 500ms interstimulus interval, 4 images (the original and 3 textures generated to match a particular layer and pooling region size) were presented in each quadrant at an eccentricity of 10 deg for 2 s. During this time, subjects responded with a keypress to indicate which image was the original. Fixation on a centrally presented cross was enforced throughout the trial using eye-tracking. We found that both feature complexity ($F = 346.42$, $p < 0.001$) and pooling region size ($F = 68.59$, $p < 0.001$) had a significant effect on the percent of correct choices. Identification accuracy decreased monotonically with greater feature complexity and smaller pooling region size. These results suggest that both feature complexity and pooling region size contribute to visual metamerism and therefore suggest limits on the complexity of features accessible within regions of visual space in peripheral vision.

Disclosures: A.V. Jagadeesh: None. **J.L. Gardner:** None.

Poster

580. Vision: Processing of Contrast, Form, and Color

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 580.13/II6

Topic: D.07. Vision

Support: NIH Grant EY026753

Title: Shady: A software engine for real-time visual stimulus manipulation

Authors: *J. HILL[1], S. W. J. MOONEY[1], E. RYKLIN[2], G. T. PRUSKY[3]
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Abstract: Shady is a software toolbox that enables precise visual stimulus presentation on mass-produced screens and video cards, without additional specialized hardware. It renders arbitrary stimulus patterns linearly with high dynamic range and high timing precision. It lets you manipulate them in real time while leaving the computer's central processor (CPU) free to perform other time-critical tasks. To do this, it provides a customizable, specialized "shader" program that runs on a graphics processor (GPU) and a high-level wrapper/programmer's interface in the Python programming language. It is available as open-source software: see http://shady.readthedocs.io
With increasing prevalence of multi-modal, integrative and real-time research approaches (such as those that hinge on neuro- and bio-feedback) specialized software components must evolve to avoid dominating users' programming environment, but rather to be good citizens. Shady is designed with this in mind. Its manifesto is:
(1) Precise control of every physical pixel of a display device (to avoid spatial artifacts);
(2) Minimization of "boilerplate" coding: it takes one short line of code to initialize the display and start the engine, one line to create a stimulus, and (often) one line to specify how some parameter of the stimulus should change over time; the housekeeping is done reliably and out of sight;
(3) Good citizenship I: minimization of CPU load. Shady pushes nearly the burden of frame-by-frame pixel-processing (including signal generation, animation, spatial windowing, contrast modulation in time and space, gamma correction, quantization, and dynamic-range enhancement tricks) onto the GPU, leaving the CPU largely free for other tasks; (4) Good citizenship II: compatibility. Shady's Python and C++ code has been confirmed to work on multiple platforms, including recent versions of Windows, macos and Ubuntu Linux. It does not require any proprietary software beyond the base operating system. Both Python 2 and 3 are supported, with graceful tolerance for variation in version-numbering, or even absence, of its few third-party dependencies;

(5) Good citizenship III: Windows-centricity. We bite the bullet and focus on Windows as our primary platform for performance optimization, because that is where support for specialized neuroscience hardware and novel human interaction devices is most prevalent.

Disclosures: S.W.J. Mooney: None. **E. Ryklin:** None. **G.T. Prusky:** None.

Poster

580. Vision: Processing of Contrast, Form, and Color

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 580.14/II7

Topic: D.07. Vision

Support: NIH Grant EY026753

Title: Curveball: A tool for rapid measurement of contrast sensitivity based on smooth eye movements

Authors: *G. T. PRUSKY[1,2], S. W. J. MOONEY[1,2], J. HILL[1,2], M. S. TUZUN[1,2], N. M. ALAM[1], J. B. CARMEL[1,2]
[1]Burke Neurolog. Inst., White Plains, NY; [2]Blythedale Children's Hosp., Valhalla, NY

Abstract: The contrast sensitivity function (CSF) is a highly informative measure of visual system performance and is well correlated with many other measures of visual health and disease. Unlike other measures of spatial visual function, the CSF describes a contour in the two-dimensional space of contrast sensitivity and spatial frequency, and is consequently more challenging to assess than one-dimensional measures such as visual acuity. The time required to measure multiple contrast thresholds with conventional psychophysical staircase procedures is prohibitive in most clinical settings. Here, we describe an efficient new contrast sensitivity measurement tool, 'Curveball', that continuously infers stimulus visibility through smooth eye tracking instead of perceptual report. Curveball moves a band-limited image on a computer screen, measures gaze velocity with an eye tracker and employs an adaptive algorithm to determine the extent to which the eyes move in concert with the image. If a participant smoothly follows the image with their eyes, as most do instinctively, stimulus features are changed in real time to a lower contrast until they can no longer follow, which quantifies the limit of ability. Our findings provide strong evidence that Curveball is a reliable, accurate, and efficient objective measure of contrast sensitivity at working distance. Task repeatability was high, both within the same session (coefficient of repeatability of $\log_{10}$ threshold contrast was 0.275) and across different days (0.227). It was consistent across changes in room illumination suggesting that it is suitable for clinical settings. The procedure produces CSFs that are (a) systematically related to the CSFs obtained from both static and moving stimuli in a conventional staircase task and (b) highly predictive of the difference between corrected and uncorrected eye chart acuity. Curveball

contrast sensitivity estimates change in predictable ways as the user moves closer to the screen, and the algorithm's ability to detect smooth tracking appears to degrade only gradually as viewing distance varies between the optimal and maximum distance allowed by the eye-tracker. This suggests that the participant's distance can be continuously monitored using the eye-tracker and used to compute the true spatial frequencies being measured in each trial when estimating the CSF. The display-mounted eye tracker used here required only a 500ms one-point calibration at the start of the task for our smooth pursuit matching algorithm to perform well. Overall, our findings indicate that Curveball is a promising means of accurately assessing contrast sensitivity in previously neglected populations.

Disclosures: G.T. Prusky: None. **S.W.J. Mooney:** None. **J. Hill:** None. **M.S. Tuzun:** None. **N.M. Alam:** None. **J.B. Carmel:** None.

Poster

581. Multisensory Integration and Cross-Modal Processing

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 581.01/DP07/II8

Topic: D.09. Multisensory Integration

Support: NSF GRFP 0940902
NSF IGERT 1250104

Title: Multiple sensory inputs modulate a short-lived sleep-like state in *C. elegans*

Authors: *D. L. GONZALES[1,2], J. ZHOU[3], J. T. ROBINSON[2,1,3,4]
[1]Applied Physics, [2]Electrical and Computer Engin., [3]Bioengineering, Rice Univ., Houston, TX; [4]Neurosci., Baylor Col. of Med., Houston, TX

Abstract: To survive in complex environments, animals must evaluate information from multiple senses and select an appropriate behavioral response. Indeed, multisensory integration is a major function of the nervous system, but difficulties in simultaneously quantifying both the behavioral and neural state of an animal while controlling multiple sensory stimuli presents challenges in studying this phenomenon. Here, we describe a short-lived, sleep-like state in *C. elegans* that is amenable to whole-brain imaging, quantitative behavioral phenotyping, and microfluidic control of the external environment. When confined in microfluidic chambers for 1 hr, adult animals transition between periods of normal activity and short quiescent bouts hallmarked by a complete cessation of locomotion. The quiescent state, we call μSleep, begins and ends spontaneously, lasts an average of 1-2 min, and meets some of the basic criteria for sleep behavior. In addition, we have discovered that the geometry of the confinement chamber, available fluid volume, fluid flow rate, temperature, and satiation state can all increase and/or decrease the prevalence of quiescent bouts. These results suggest that multiple sensory pathways

converge to drive μSleep. To identify the circuits involved in regulating μSleep, we investigated how loss-of-function mutations in dopaminergic, serotonergic, octopaminergic and stress-related neural circuits affect the quiescent phenotype. Furthermore, we used whole-brain imaging to determine whether the global brain dynamics during μSleep, and other *C. elegans* sleep states, are independent of environmental cues. Together, these results shed light on how the nervous system integrates multiple sensory inputs to control transitions between behavioral states.

Disclosures: D.L. Gonzales: None. **J. Zhou:** None. **J.T. Robinson:** None.

Poster

581. Multisensory Integration and Cross-Modal Processing

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 581.02/II9

Topic: D.09. Multisensory Integration

Title: The effects of early sensory deprivation on multisensory thalamocortical and intracortical connections

Authors: *J. HENSCHKE[1], T. MACHARADZE[2], A. M. OELSCHLEGEL[3], J. M. PAKAN[4], J. GOLDSCHMIDT[5], P. O. KANOLD[6], E. BUDINGER[7]
[1]Otto von Guericke Univ., Magdeburg, Germany; [2]Leibniz Inst. for Neurobio., Magdeburg, Germany; [3]Inst. of Anat., Otto-von-Guericke-University Magdeburg, Magdeburg, Germany; [4]Otto-von-Guericke Univ., Magdeburg, Germany; [5]Leibniz-Institute for Neurobio., Magdeburg, Germany; [6]Biol., Univ. of Maryland, College Park, MD; [7]Leibniz Inst. for Neurobio. Magdeburg, Magdeburg, Germany

Abstract: The nervous system integrates information from multiple senses. This multisensory integration already occurs in primary sensory cortices like A1, S1, and V1 via direct cross-modal thalamocortical and corticocortical connections. In humans, sensory loss that occurs from birth results in functional recruitment of the deprived cortical territory by the spared senses during subsequent development. However, the underlying neural circuit changes accompanying this recruitment are poorly understood. Here, using anatomical tracer injections into three primary sensory cortices (A1, S1, and V1) in a rodent model (Mongolian gerbil), we show that early auditory, somatosensory, or visual deprivation increases the multisensory connections of these areas at the end of the critical period (P28). The anatomical changes encompass lemniscal, non-lemniscal sensory specific, as well as multisensory pathways and are due to axonal reorganization processes but not apoptosis or neurogenesis of projecting neurons. Furthermore, the axonal remodeling is mediated by non-lemniscal thalamic nuclei and the primary areas themselves. Functional single-photon emission computed tomography imaging (SPECT) of regional cerebral blood flow revealed a largely reduced stimulus-evoked activity but a higher functional connectivity specifically between primary areas in deprived compared to non-deprived

animals. Since supragranular pyramidal neurons are the main targets of intracortical and also some thalamocortical connections, we subsequently investigated their morphology in deprived vs. non-deprived animals at P28 using the Golgi-Cox method. Scholl and branch analyses showed that early sensory deprivation leads to a general increase of dendritic branching, i.e., to longer and more widely branched basal and apical dendrites in both the deprived and spared cortical areas. In contrast, the overall number of dendritic spines decreased and accordingly, overall spine density was also decreased. This suggests that the loss of early sensory experience also induces a refinement of intracortical multisensory connections by pruning of dendritic spines at the end of the critical period. Consequently, stimulus-induced activity is decreased but functional connectivity increased.

Disclosures: J. Henschke: None. **T. Macharadze:** None. **A.M. Oelschlegel:** None. **J.M. Pakan:** None. **J. Goldschmidt:** None. **P.O. Kanold:** None. **E. Budinger:** None.

Poster

581. Multisensory Integration and Cross-Modal Processing

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 581.03/II10

Topic: D.09. Multisensory Integration

Support: James S. MacDonnell Fund 220020516 - 0

Title: Early loss of vision leads to enhanced performance on tactilely mediated behaviors in the short-tailed opossum (monodelphis domestica)

Authors: *M. ENGLUND[1], C. IYER[2], S. FARIDJOO[2], L. KRUBITZER[2]
[1]Psychology, Univ. of California Davis, Auburn, CA; [2]Univ. of California Davis, Davis, CA

Abstract: Early loss of vision has been shown to induce massive cross-modal changes in the size, connectivity, and functional organization of cortical areas devoted to processing inputs from both the lost sense, as well as the spared senses. Specifically, neurons in visual cortex respond to auditory and tactile stimuli, and patterns of thalamocortical and corticocoritcal projections are altered. In addition, connections of somatosensory cortex and the neural response properties of S1 are altered in animals with early loss of vision. However, the behavioral correlates of this neural reorganization have yet to be examined. Using two naturalistic sensorimotor tasks in the Brazilian short-tailed opossum *(Monodelphis domestica),* the *ladder rung walking task* and *skilled reaching task,* this study examined how the early loss of vision leads to enhanced performance on behavioral tasks relying on the spared senses. Early-blind opossums significantly outperformed sighted controls in both tasks in the light and dark, committing less errors on average on the variable ladder task, and increased performance in skilled reaching. Neither crossing time nor top performers were found to drive ladder rung walking error. Additionally,

handedness and angle of approach did not contribute to skilled reaching success. Further, we show the reliance of tactiley mediated discrimination in these tasks was predominantly on the whiskers, since whisker trimming resulted in significantly decreased performance. These results suggest that not only does the early loss of vision alter the organization and connections of both the targeted and the spared sensory systems, but that these changes appear to generate adaptive discriminatory behavior mediated by the spared sensory systems.

Disclosures: M. Englund: None. **C. Iyer:** None. **S. Faridjoo:** None. **L. Krubitzer:** None.

Poster

581. Multisensory Integration and Cross-Modal Processing

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 581.04/II11

Topic: D.09. Multisensory Integration

Title: Associative interaction among sensory cortices consolidated by the first and second-order conditionings

Authors: G. TASAKA[1], M. YAMASHITA[2], Y. IDE[4], *E. HIDA[3], T. AIHARA[2]
[1]Grad. Sch. of Engin., [2]Tamagawa Univ., Tokyo, Japan; [3]Tamagawa Univ., Machida 194, Japan; [4]Pharmacol. Evaluation Inst. of Japan (PEIJ), Kanagawa, Japan

Abstract: In our previous study, not only the somatosensory cortex but also the auditory cortex was activated by the electrical foot-shock after a fear conditioning with a foot-shock and a tone-stimulation in guinea pigs. Those activations were simultaneously observed in the recording area covering two sensory cortices by using optical imaging method with a voltage sensitive dye. The result suggests that the associative response was represented by the conditioning with sensory stimuli. However, information representation and consolidation mechanisms of inter-cortical associative interactions for various sensory inputs are still unclear.
In the present study, in order to investigate how various sensory information are consolidated in inter-cortical networks, cortical activations in somatosensory, auditory and visual area were simultaneously measured using the optical imaging method after the first- or second-order conditionings with three sensory stimuli (foot-shock, tone, and light) were performed for guinea pigs.
As a result, we found that not only the main sensory area for an applied stimulus but also the other sensory areas for stimuli used the conditioning came to be activated after conditionings. For example, both somatosensory cortex and visual cortex came to be activated by the foot-shock after the second-order conditioning with three sensory stimuli. In addition, those responses were activated by direct electrical stimulation to the other cortical area. It shows that new connections were consolidated among cortical areas by conditionings. While, it has been reported that projections to sensory areas through amygdala and medial geniculate nucleus

relating cholinergic pathway were facilitated and associative responses was observed in cortical areas. Consequently, our results suggest that existing pathways were facilitated and strengthened by conditionings with sensory stimuli, and additional new information pathways were induced.

Disclosures: G. Tasaka: None. **M. Yamashita:** None. **Y. Ide:** None. **E. Hida:** None. **T. Aihara:** None.

Poster

581. Multisensory Integration and Cross-Modal Processing

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 581.05/II12

Topic: D.09. Multisensory Integration

Support: NIH Grant AA13023

Title: Connectivity of ferret multisensory cortical LRSS area assessed by tracer (BDA) and by rsfMRI methods

Authors: *M. A. MEREDITH[1], E. H. PRICKETT[2], R. P. GULLAPALLI[3], S. TANG[3], A. E. MEDINA[4]
[1]Dept Anat. & Neurobio., Virginia Commonwealth Univ. Sch. Med., Richmond, VA; [2]Anat. and Neurobio., Virginia Commonwealth Univ. Sch. of Med., Richmond, VA; [3]Diagnos. Radiology and Nuclear Med., [4]Pediatrics, Univ. of Maryland, Baltimore, MD

Abstract: The present study examined the connectivity of an auditory-tactile multisensory area in ferret cortex, the lateral rostral suprasylvian sulcal area (LRSS) using two methods that evaluate neural connections: neuronal tract tracing and resting state functional magnetic resonance imaging (rsfMRI). For tract tracing, anesthetized adult ferrets (n=3; 2 females) received pressure injections of tracer (Biotinylated Dextran Amine-BDA, 3kMW) in the LRSS. Following transport, euthanasia and post-fixation processing, BDA labeled neurons were plotted from serial coronal sections of cortex using Neurolucida. Areal locations were determined using an atlas of ferret cortex (Zhou et al., 2016) and revealed that 39% of ipsilateral projections to LRSS originated from somatosensory cortices (S1, S2, S3; PSSC, MRSS), 37% from auditory areas (A1, AAF, ADF/AVF, PPF/PSF, VP) and where 30% of all connections arose from S2 (16%) and A1 (14%). Resting state functional MRI (rsfMRI) was performed on adult ferrets (n=6, male) using a 7T animal MRI system. Regions of interest (ROIs) were defined using the same ferret atlas (Zhou et al., 2016). Correlation coefficients between the time course of LRSS and other ROIs were transformed to a connectivity z-score using Fisher's transformation. Averaged connectivity with somatosensory areas (S1, S2, S3, PSSC, MRSS) was 0.88, while averaged connectivity with auditory areas (A1, AAF, ADF/AVF, PPF, PSF) was 0.41. Areas with highest LRSS average connectivity scores were the somatosensory MRSS (z = 1.37) and

auditory ADF/AVF (z = 0.52). These results show that both tracer injection and rsfMRI methods demonstrate LRSS connectivity with ipsilateral somatosensory and auditory cortical cortices, consistent with the prevalence (68%) of multisensory somatosensory-auditory neurons in the LRSS. However, regions of highest connectivity varied with methodology: that with the highest rsfMRI correlation scores may be accounted for by possible bidirectional connections with the LRSS, which were not sampled by tracer methodology. Ultimately, both sets of findings support the convergence of connections with somatosensory and auditory cortices as a basis for multisensory processing within the LRSS.

Disclosures: M.A. Meredith: None. **E.H. Prickett:** None. **R.P. Gullapalli:** None. **S. Tang:** None. **A.E. Medina:** None.

Poster

581. Multisensory Integration and Cross-Modal Processing

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 581.06/II13

Topic: D.09. Multisensory Integration

Support: Whitehall Foundation

Title: Comparison of visual processing during visual-only and audiovisual contexts in the mouse primary visual cortex

Authors: *J. MCCLURE, JR[1,2], H. KHDOUR[2], P.-O. POLACK[2]
[1]Newark, NJ; [2]Behavioral and Neural Sci., Rutgers Univ., Newark, NJ

Abstract: Classically, multisensory integration was described as occurring in higher-order sensory cortices. This notion was recently challenged in studies demonstrating that the presence of sounds modulates visually evoked activity in the primary visual cortex (V1). However, the net effect of sound modulation on orientation encoding by V1 neurons is still debated. Moreover, the involvement of direct projections from the primary auditory cortex (A1) to V1, during visual processing, remains unclear. The goal of our study is [1] to determine how sound modulation of visually evoked activity in V1 affects orientation encoding and orientation perception, and [2] to test whether sound-induced modulation of visual processing in V1 cannot be attributed, at least in part, to non-specific mechanisms such as an increase in arousal in an audiovisual context.
To address these questions, we performed two-photon calcium imaging in the V1 of mice injected with GCAMP6f, while presenting blocks of audiovisual and visual-only stimuli. At the end of the recording session, we assessed the orientation tuning of the imaged neurons by presenting a series of drifting gratings of 12 evenly spaced orientations. We analyzed the distribution of the preferred orientation of V1 neurons responding to the visual stimulus as well as the orientation encoded by V1 neuronal population activity during the visual stimulus

presentation. We found an improvement of the encoding of visual information during audiovisual blocks compared to visual-only blocks. The improvement of orientation encoding in V1 was due to a potentiation of the response from V1 neurons tuned for the visual stimulus orientation, while the activity of neurons tuned for either the orthogonal orientations or for the opposite direction were decreased. This finding suggested that orientation perception could be facilitated in the audiovisual context, in particular when discriminating visual stimuli that activate overlapping neuronal populations. We tested this hypothesis by training mice to perform a Go/No-Go orientation discrimination task. Once trained to lick for an oriented cue and withhold liking for a cue of orthogonal orientation, the angular distance between the Go and No-Go cues was progressively decreased. We found that at the limit of orientation discriminability, mice performed better during audiovisual blocks than when visual cues were presented in isolation. Finally, we found that during audiovisual blocks, the mouse pupil was more dilated, which suggested that audiovisual cues enhanced arousal and likely participated in the modulation of visual processing in audiovisual contexts.

Disclosures: J. McClure: None. **H. Khdour:** None. **P. Polack:** None.

Poster

581. Multisensory Integration and Cross-Modal Processing

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 581.07/II14

Topic: D.09. Multisensory Integration

Support: NIH 2016 U01 NS

Title: Multisensory processing of external salinity by larval zebrafish

Authors: *K. J. HERRERA, F. ENGERT
MCB, Harvard Univ., Cambridge, MA

Abstract: Avoiding unfavorable environments is a critical task for all organisms. For larval and adult zebrafish, bodies of water with high salinity represent such environments. However, the mechanisms by which information about external salt content is detected and transformed into an appropriate behavioral response are unknown. Here, we first assay the avoidance response to salinity using various concentrations and ion types. Then, we use volumetric imaging with light-sheet microscopy to identify brain regions that can detect external salt concentrations. We identify a range of chemosensory modalities that can encode salt concentrations, including olfaction as well as, surprisingly the lateral line. Currently, we are using chemogentic approaches to determine how these different modalities contribute to the generation of avoidance responses.

Disclosures: K.J. Herrera: None. **F. Engert:** None.

Poster

581. Multisensory Integration and Cross-Modal Processing

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 581.08/II15

Topic: D.09. Multisensory Integration

Support: MOE Singapore
Knut and Alice Wallenberg Foundation

Title: Claustral neurons projecting to the anterior cingulate cortex receive preferential synaptic inputs from higher order and not primary sensory cortical regions

Authors: *Z. CHIA[1,2], G. J. AUGUSTINE[2], G. SILBERBERG[1]
[1]Dept. of Neurosci., Karolinska Institutet, Stockholm, Sweden; [2]Lee Kong Chian Sch. of Med., Nanyang Technological Univ., Singapore, Singapore

Abstract: We previously characterized the intrinsic electrical properties of claustrum neurons that project to the anterior cingulate cortex (ACC). Here we have determined (1) whether these neurons relay information to the ACC from different cortical regions and (2) where inputs are received in the ACC. The ACC of mice were injected with retrograde beads, to identify claustrum neurons projecting to ACC, while anterograde virus expressing channelrhodopsin-2 (ChR2) was injected into various cortical regions to label neurons projecting to the claustrum. Whole-cell patch clamp recordings were made from bead-labelled claustrum neurons and synaptic responses were evoked by photostimulation of ChR2-expressing cortical axon terminals. Photostimulation of terminals from multiple cortical regions evoked monosynaptic EPSPs in claustrum neurons. Cortical areas exciting the claustrum included the contralateral ACC, orbitofrontal cortex and insular cortex. Connection probability from higher cortical areas (such as the contralateral ACC and insular cortex) to the claustrum was 10-fold higher compared to claustral input from sensory areas, including primary somatosensory, visual, and auditory cortices. To characterize the synaptic inputs to the claustrum, the insula-claustrum pathway was probed in more detail. Claustrum neurons receive synaptic input only from the ipsilateral insula. Additionally, these insula projections arise primarily from Layer 5. Dual recordings showed that both claustrum projection neurons and parvalbumin (PV) expressing interneurons receive insula input. PV interneurons received stronger and faster synaptic inputs from insula, suggesting a feedforward inhibitory pathway within the claustrum microcircuitry. Finally, the site of claustrum projections on the ACC was investigated by recording from neurons in different layers of the ACC. We found that claustrum projections send monosynaptic excitatory projections to all layers of the ACC (Layers 1, 2/3, and 5/6). In summary, multicortical integration takes place in the claustrum at a population level, evident by ACC-projecting claustrum neurons receiving input from multiple cortical areas. These ACC-projecting claustrum neurons synapse on all

layers of the ACC. The insula-claustrum-ACC circuit may underlie the “Salience Network”; this is the first evidence of functional intercortical connectivity mediated via the claustrum.

Disclosures: Z. Chia: None. **G.J. Augustine:** None. **G. Silberberg:** None.

Poster

581. Multisensory Integration and Cross-Modal Processing

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 581.09/II16

Topic: D.09. Multisensory Integration

Title: Toward a unified coding of motion: Multisensory integration of moving visual and somatosensory cues in the associative parietal cortex (APC) of the rat

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Abstract: The central nervous system is able to create a stable representation of our own movements in a constantly moving environment. Most frequently, motion information is available concurrently through different sensory channels. Combining multisensory inputs facilitates the elaboration of a motion percept by disambiguating sensory cues, allowing to distinguish object-motion from self-motion. The Middle Temporal area (MT) is dedicated to the processing of visual motion stimuli. For other sensory modalities, however, no specific motion area has been identified. Gathering and processing all sensory information about motion in a unique area could be a parsimonious way to build up a unified percept. Recent investigations suggested a role of MT in multisensory integration of motion. This study aims at characterizing this multisensory integration in the rat’s APC, a potential homolog of MT located at the interface of the primary visual (V1) and somatosensory (S1) cortices. To mimic both exo- and egocentric motions, we presented visual gratings and applied air puffs to the whiskers. To assess whether different sensory inputs reached the APC, we used voltage sensitive dye imaging and found that visual and somatosensory stimuli-evoked activations, respectively in V1 and S1, propagated and converged into that region. To characterize the neuronal responses in this area, we recorded the activity of over 900 single units in the APC and showed that this region contains unimodal (visual or tactile only) neurons (27%). Direction selectivity is a main and defining characteristic of MT neurons, which enables the processing of visual motion. We have been able to comparably reveal this property in APC visual neurons, which fired only to one preferred direction. Remarkably, the somatosensory neurons also showed direction selectivity during whisker displacement, proving APC’s ability to compute motion from several channels. To be considered as a multisensory integration area, the APC must also contain multimodal neurons that extract and combine unimodal motion features. We showed that 61% of the recorded units were multimodal, significantly responding to both sensory stimuli. Visual and somatosensory

direction selectivity was also observed in these bimodal neurons, meeting the requirement for potential multisensory integration of motion. By combining the unisensory stimulations in congruent and incongruent situations, we revealed that the two recorded populations of APC neurons displayed multisensory integrative processes, depending on the stimulation patterns. These results strongly suggest that APC, potential homolog of MT, is a multimodal hub for motion processing.

Disclosures: J. Caron-Guyon: None. **J. Corbo:** None. **Y. Zennou-Azogui:** None. **C. Xerri:** None. **N. Catz:** None.

Poster

581. Multisensory Integration and Cross-Modal Processing

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Program #/Poster #: 581.10/II17

Topic: D.09. Multisensory Integration

Support: Knut and Alice Wallenberg Foundation, project grant KAW2014.0051
Swedish Research Council, project grant 542-2014-2350

Title: Parietal inputs convey multisensory visuo-tactile synaptic inputs to nociceptive-sensitive midcingulate circuits and facilitate their premotor output

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Abstract: Parietal areas are multisensory and their output is conveyed to prefrontal, premotor cortical targets in all mammals studied so far. However, how multisensory synaptic inputs conveyed by identified parieto-frontal streams are processed when the prefrontal target is activated by an adequate stimulus, as well as the functional impact of such projection on the premotor output of the target area, remain to be elucidated at synaptic and microcircuit level. To address these questions, we used the prefrontal projection of the multisensory, visuo-tactile parietal area RL to midcingulate cortex as an approachable model system in the mouse, suitable for microcircuit investigation. Intra and extracellular recordings revealed that association parietal area RL drives subthreshold but also suprathreshold visuo-tactile responses in the target, posterior midcingulate (pMC) cortex, and that the target pMC spot showed multisensory enhancement. pMC revealed to be a pain-sensitive circuit as nociceptive stimulation triggered a long-lasting UP-state-like depolarization that were accompanied by an abrupt switch of the ongoing firing rate (decreased if it was tonically active and increase in case of silence) in a layer-specific pattern. In vivo whole-cell recordings in other visual areas (visual cortex) as well as functional neuroanatomy with c-fos activation, showed that this pattern of pain-driven activation was area-specific. Synaptic multisensory responses increased in amplitude in a subset of neurons

and had a shorter duration during nociceptive stimulation. We next investigated the functional impact of the parietal synaptic inputs onto the premotor output of pMC. Electromyography showed that pMC intracortical microstimulation induced bilateral whisker movements whose amplitude and duration were robustly facilitated by parietal RL activation. pMC-evoked movements had partially a premotor character as the pMC spot innervated by RL was reciprocally connected to a division of M1, whose optogenetic inactivation reduced the amplitude of pMC-driven whisker movement. Taken together, our data show the circuit and synaptic basis behind pMC acting as a sensory-motor hub that triggers faster and stronger visual-tactile motor responses during nociceptive stimulation. Our study reveals and elucidates at the microcircuit and synaptic levels the intrinsic susceptibility of cingulate prefrontal circuits to respond to both nociceptive and parietal inputs, we also provide the first evidence and further discuss the impact on the behavioral level.

Disclosures: S. Papaioannou: None. **E. Malinina:** None. **A. Tripathi:** None. **P. Medini:** None.

Poster

581. Multisensory Integration and Cross-Modal Processing

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Program #/Poster #: 581.11/II18

Topic: D.09. Multisensory Integration

Support: FWO-SB Fellowship to SG
FWO flanders
C1 grant Research Council KU Leuven

Title: Sensory projections towards the mouse posterior parietal cortex

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Abstract: The posterior parietal cortex is a brain region responsible for the processing of spatial information and orientation. This involves for example the spatial ratios between objects and the position of our body to ensure proper movement trajectories or visual attention. To carry out its function, the posterior parietal cortex needs to receive information from the different sensory modalities. In humans and primates this brain region is therefore known as a multisensory area, which is not surprising since it is anatomically situated between the somatosensory, visual and auditory cortical regions. At the behavioral level, the posterior parietal cortex thus contributes to spatial navigation, perceptual decision making as well as multisensory integration. All these features make it highly likely that the posterior parietal cortex is involved in cross-modal cortical recovery processes upon sensory loss. Our research group validated the adult monocular

enucleation mouse model to study this type of plasticity. In this model the deprived visual cortex becomes reactivated by whisker inputs from the spared somatosensory modality. Seven weeks post enucleation, maximal whisker-driven reactivation of the visual cortex is reached (Van Brussel et al., 2011). To create knowledge about the intricate cortical network that drives this recovery from late-onset loss of vision we initiated a connectome study to identify posterior parietal cortex in the mouse in order to validate that this plasticity process indeed relies on the posterior parietal cortex as a hub to transfer information from the somatosensory cortex to the visual cortex. We investigated what cortical subregions may entail the posterior parietal cortex in the mouse and what the specific sensory inputs are from the various sensory cortical areas. We predict that mouse PPC exists of the secondary visual regions RL, A and AM. By using a retrograde tracing approach, where modified Herpes Simplex virus was injected in these regions, we revealed that they indeed have different projection patterns originating from somatosensory, auditory and visual cortex typical for multisensory cortical regions encompassing the posterior parietal cortex. In sum we were able to identify an anatomical substrate that may carry somatosensory inputs to mouse visual cortex upon vision loss.

Disclosures: S.R. Gilissen: None. **K. Buttiens:** None. **K. Farrow:** None. **V. Bonin:** None. **L. Arckens:** None.

Poster

581. Multisensory Integration and Cross-Modal Processing

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 581.12/JJ1

Topic: D.09. Multisensory Integration

Support: NIH R01-EY014882

Title: Cross-modal plasticity of inhibitory thalamic gating in adults

Authors: *D. CHAKRABORTY, J. L. WHITT, H.-K. LEE
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Abstract: Loss of one sensory modality initiates compensatory changes in the spared senses which are generally referred to as cross-modal plasticity (Bavelier and Neville, 2002). We previously reported that loss of vision causes potentiation of thalamocortical (TC) inputs to promote relay of auditory signals in the primary auditory cortex (A1) (Petrus et al., 2014). We hypothesized that TC plasticity may be due to changes in the inhibitory gating of sensory thalamic nuclei. The primary inhibition onto visual thalamus (lateral geniculate nucleus, LGN) and auditory thalamus (medial geniculate body, MGB) comes from a common source, the thalamic reticular nucleus (TRN). To determine if TRN inputs to these primary sensory thalamic nuclei are altered by sensory deprivation, we stereotactically injected double-floxed ChR2 (DIO-

ChR2) into TRN of parvalbumin-Cre or somatostatin-Cre mice to measure the inhibitory synaptic transmission of reticular-thalamic synapses in LGN and MGB. Measurements were done by performing whole-cell recordings in the LGN, MGB, and TRN of three-month-old normal reared mice and mice deprived of vision for 1 week prior to the recordings. We found that visual deprivation differentially alters the inhibitory synaptic strengths at TRN-LGN and TRN-MGB synapses and changes the efficiency of transmission of high-frequency stimulation at TRN-MGB synapses. These findings show that the adult thalamus undergoes experience-dependent plasticity specifically by regulating the inhibitory gating in reticular-thalamic synapses and thus have functional significance for cross-modal sensory adaptation in adults.
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Disclosures: D. Chakraborty: None. **J.L. Whitt:** None. **H. Lee:** 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.; Johns Hopkins University.

Poster

581. Multisensory Integration and Cross-Modal Processing

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 581.13/JJ2

Topic: D.09. Multisensory Integration

Support: Wellcome Trust Studentship 105241/Z/14/Z

Title: Cross-modal gain control in sensory thalamus

Authors: *M. LOHSE, J. C. DAHMEN, V. M. BAJO-LORENZANA, A. J. KING
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Abstract: Considerable attention has been focused on the involvement of cortical areas in multisensory processing. Although visual and somatosensory influences on auditory responses can arise via direct corticocortical pathways, it is likely that some aspects of multisensory cortical processing are inherited from the thalamus. However, we currently know much less about the nature of the multisensory interactions or the circuits involved in sensory thalamus. Using electrophysiology, 2-photon imaging of thalamocortical axons and optogenetics, we investigated multisensory interactions within different divisions of the medial geniculate body (MGB) and the local circuits underlying these effects in mice.
Auditory responses were found to be reliably suppressed (~ 25 % reduction in firing rate across neurons) by somatosensory inputs in both the ventral and dorsal divisions of the MGB. Auditory responses in the more medial structures (medial division of MGB/posterior intralaminar nucleus and suprageniculate nucleus) were facilitated by somatosensory stimulation, with a subgroup of

these medial neurons also being driven by whisker deflection.
Somatosensory suppressive influences were also found in auditory cortex, but absent in the auditory midbrain. In vivo 2-photon calcium imaging of auditory thalamic axons revealed that only the somatosensory inhibitory control of auditory thalamus was relayed to auditory cortex. Optogenetic activation of primary somatosensory cortex (S1) suggests that the pathway from S1 to thalamus is sufficient for mediating this cross-modal gain control. Using a disynaptic circuit tagging approach between sensory thalamic nuclei, we are currently aiming to understand the intra-thalamic circuit contributions to the cross-modal gain control between somatosensory thalamus and auditory thalamus.
Our results suggest that sensory thalamus and surrounding circuits provide an important substrate for cross-modal gain control of auditory inputs.

Disclosures: M. Lohse: None. **J.C. Dahmen:** None. **V.M. Bajo-Lorenzana:** None. **A.J. King:** None.

Poster

581. Multisensory Integration and Cross-Modal Processing

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 581.14/JJ3

Topic: D.05. Olfaction and Taste

Support: NSERC Grant

Title: Effects of congenital blindness on olfactory functions and brain plasticity

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Abstract: Early blindness clearly results in an enhancement of auditory and tactile performances, associated with dramatic cerebral structural and functional changes. On the other hand, human studies showed contradictory results regarding olfactory abilities in early blind individuals. However, recent studies found that olfactory bulb is larger in blind than in sighted humans and visual cortex is activated by olfactory exposure in blinds only. So far, the impact of congenital blindness on olfactory functions and its underlying neurobiological basis remain poorly understood. The present study aims to determine the effects of blindness on olfactory functions and brain plasticity in mouse model of congenital blindness. We used the ZRDBA mouse strain, a unique mouse model, from which half of newborns are sighted and half are anophthalmic. In this study, a series of behavioral tests were performed on 20 anophthalmic and 20 sighted mice to assess their olfactory performances (i.e., odor sensitivity, discrimination, localization, memory tasks). To investigate olfactory processing in the brain, mice were exposed to a 5-minute odor stimulus. After perfusion, the brains were collected, frozen, and cut to

perform serial sections for immunohistochemistry analysis. Preliminary results indicate that anophtalmic mice exhibit higher olfactory abilities in most of behavioral tasks. Ongoing immunohistochemistry analysis will test whether enhanced olfactory performance in blind mice may correlate with structural changes and functional alterations in olfactory processing areas and in the visual cortex. This research brings a better understanding of the impact of visual deprivation on olfactory functions and the underlying neuronal mechanisms.

Disclosures: J. Thibault: None. **E. Luna Amirault:** None. **G. Bronchti:** None. **S. Al ain:** None.

Poster

581. Multisensory Integration and Cross-Modal Processing

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Program #/Poster #: 581.15/JJ4

Topic: D.09. Multisensory Integration

Support: NSERC RGPIN-2014-04947
Google
CIFAR

Title: Biologically plausible deep learning with segregated dendrites and multiplexing

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Abstract: Deep learning refers to learning in networks of neurons with many layers of synaptic connections. While there is growing evidence that the cortex is able to learn complicated tasks using some form of deep learning, it remains unclear how this occurs. This is called the 'credit assignment' problem. In the machine learning world, techniques for achieving deep learning with artificial neural networks (ANNs) have been wildly successful in learning tasks such as image recognition, natural language processing and navigation, in some cases even surpassing human performance. While deep ANNs were originally inspired by the brain, the state-of-the-art learning rules used to train them are wildly biologically implausible for many reasons. As a result, there has been limited impact of AI advances on neuroscience.
The present work helps to unify deep learning principles from AI with what we know about the physiology of the brain in order to help explain how the cortex enables powerful learning abilities. We demonstrate a biologically plausible form of deep learning with ensembles of neurons. Our model makes use of the unique morphological properties of pyramidal cortical neurons (namely, that they have two groups of dendrites that are electrotonically segregated), as well as the theory that ensembles of neurons can communicate two information streams encoded

separately in their *event rates* and *burst probabilities* (called multiplexing). Our model also makes use of inhibitory interneurons to shape activity at apical dendrites in a way that ensures proper credit assignment. The combination of inhibitory input, multiplexing and segregated dendrites may enable cortical circuits to perform credit assignment through many layers of synaptic connections without needing separate phases of learning.
Our model demonstrates that biologically plausible deep learning can be accomplished using segregated dendrites and multiplexing of bottom-up and top-down signals, therefore providing a unique interpretation for the morphology of cortical neurons. It also generates several experimental predictions about the types of signals that are communicated in bottom-up and top-down information streams, and how top-down feedback at apical dendrites shapes plasticity in the neocortex. This work is important for helping to further our understanding of how deep learning can be implemented by biological networks of neurons to enable the powerful learning capabilities of the brain.

Disclosures: J. Guerguiev: None. **T. Mesnard:** None. **B.A. Richards:** None.

Poster

581. Multisensory Integration and Cross-Modal Processing

Location: SDCC Halls B-H

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Program #/Poster #: 581.16/JJ5

Topic: D.09. Multisensory Integration

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NSERC Discovery Grant (02417)
CIFAR Learning in Machines and Brains Program

Title: Can machine learning models account for predictive coding-like features observed in sensory cortex?

Authors: *C. J. GILLON, J. GUERGUIEV, B. A. RICHARDS
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Abstract: Sensory processing in the brain relies on a hierarchy of regions which respond to increasingly complex stimulus features. Computational neuroscientists have postulated that this structure implements a predictive coding system, whereby each region sends predictions of the inputs it expects to lower-order regions, allowing the lower-order regions to efficiently propagate only prediction errors, i.e. unexpected or novel features of stimuli. This hypothesis has multiple lines of support: (1) predictive coding models trained on natural images develop similar receptive fields to those seen in visual cortex areas like V1, (2) top-down predictive signals and (3) prediction error signals have been found in sensory cortex. However, although these findings are consistent with predictive coding, there are other forms of hierarchical statistical model used

in machine learning that might also show these properties, but which are rarely considered by neuroscientists. Here, we investigate three such models that are effective at solving visual tasks: the bidirectional Helmholtz machine, variational autoencoder, and generative adversarial network. If V1-like receptive fields, and prediction and error signals also emerge in some or all of these models, this would suggest that the predictive coding model should be considered just one possible explanation for existing neuroscience data. We then show preliminary two-photon recording data showing population dynamics in mouse visual cortex in anticipation of expected stimuli (2) and in response to unexpected stimuli (3). By comparing these dynamics to predictions drawn from the models, we can begin to establish which, if any, the brain is most likely implementing during sensory processing.

Disclosures: C.J. Gillon: None. **J. Guerguiev:** None. **B.A. Richards:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 582.01/JJ6

Topic: D.09. Multisensory Integration

Support: NIH R01 Grant 5R01EY025978-02

Title: Neural correlates of sound symbolic crossmodal correspondences

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Abstract: Humans share consistent associations, known as crossmodal correspondences (CCs), between seemingly unrelated features in different sensory modalities. While one of the fundamental properties of language is the assumed arbitrariness between sound and meaning, sound symbolism is a notable exception that has been studied empirically using CCs between auditory pseudowords (e.g. ‘loh-moh’ and ‘kee-kay’) and visual shapes (e.g. rounded or pointed). The characteristics of the neural signals that underpin sound symbolic CCs are not well understood. Here, we report the results of a functional magnetic resonance imaging (fMRI) study that examined blood oxygenation level dependent (BOLD) responses to auditory pseudowords and visual shapes. Participants also provided post-scan perceptual ratings of roundedness and pointedness for a range of auditory nonwords and visual shapes. Representational dissimilarity matrices (RDMs) for the perceptual ratings of the auditory and visual stimuli were significantly correlated ($r = 0.93$, $p<0.0001$), indicating a close relationship between ratings in the two modalities. During fMRI scanning, participants attended to pairs of audio-visual stimuli and responded that the pairs either matched (congruent pairs e.g. ‘kee-kay’ and pointed shape or ‘loh-

moh' and rounded shape) or did not match (incongruent pairs e.g. 'kee-kay' and rounded shape or 'loh-moh' and pointed shape). Behaviorally, participants were faster to respond to the congruent pairs than the incongruent pairs, indicating their sensitivity to sound symbolic CCs. For a univariate contrast comparing BOLD activity during the perception of congruent pairings to that during the perception of incongruent pairings, the BOLD signal was stronger for the congruent condition in areas important for multisensory integration, such as the right posterior superior temporal sulcus, as well as areas in auditory and visual cortex, and in areas important for attention, such as the right inferior frontal gyrus. This research provides insights into the fundamental nature of sound symbolic CCs and how they might evoke specific interpretations of physical meaning in natural language at the perceptual and neural levels.

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Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

Location: SDCC Halls B-H

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Program #/Poster #: 582.02/JJ7

Topic: D.09. Multisensory Integration

Support: Cluster of Excellence DFG 1077 ''Hearing4all''

Title: Age-related hearing loss impacts functional connectivity at rest

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Abstract: Previous research investigating cortical plasticity in age-related hearing loss provides evidence for cross-modal reorganization in the auditory cortex, additional recruitment of the frontal lobe and increased coupling of visual and auditory cortex for matching audio-visual input. These changes already occur after mild to moderate impairment and not only after severe hearing loss. By using functional magnetic resonance imaging (fMRI), we here investigated the influence of mild to moderate hearing-impairment on functional connectivity during resting state. Nineteen hearing-impaired subjects with a mean age of 63.5 ± 5.3 years and nineteen normal-hearing participants with a mean age of 63.2 ± 5 years participated in the study. The group of hearing-impaired subjects showed a uniformly varying degree of mild to moderate and symmetrical age-related hearing loss. The measurement included a resting state MRI, as well as assessment of listening effort and the McGurk illusion outside the MRI. In this study, our main aim was to relate high-frequency hearing loss to changes in network dynamics during resting state. Additionally, the relation of listening effort and McGurk illusion with resting state connectivity was investigated. Regions of interest included visual and auditory cortex as well as the default

mode network, salience network, dorsal attention network and frontoparietal network. Behaviorally, the hearing-impaired group showed a significantly higher listening effort and significantly stronger McGurk illusion. At the neural level, between-group comparisons showed an increased positive correlation in the salience network for hearing-impaired participants as well as an increased negative correlation in the dorsal attention network in normal-hearing participants. The multiple regression with listening effort demonstrated significant correlations between listening effort and negative connectivity in default mode, dorsal attention and salience networks. Further, listening effort was related to a negative connectivity between auditory cortex and frontal lobe. McGurk illusions were correlated to an increased positive connectivity within the default mode network. We here provide evidence of resting state connectivity changes due to age-related hearing loss. Hearing loss affects both the salience and dorsal attention networks, whereas listening effort is related to changes in default mode, dorsal attention and salience networks as well as to changed auditory cortex connectivity. These results suggest that already mild to moderate hearing impairment leads to disruption of network connectivity during rest.

Disclosures: S. Rosemann: None. **C.M. Thiel:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 582.03/JJ8

Topic: D.09. Multisensory Integration

Title: Effects of ownership sense of the virtual body induced by the full body illusion on the sound localization

Authors: *C. TOI, A. ISHIGUCHI
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Abstract: One of critical factors of the body ownership sense, or the body-related sense of self (bodily self-consciousness: BSC) is the multisensory integration. If only this integration is completed, we could gain the sense of body ownership to even fake bodies. This effect is shown by the various experiments of full-body (out-of-body) illusion (Ehrsson, 2007), and suggests that the sense of body ownership can be easily deceived. In the typical full-body (out-of-body) illusion experiment, participants sit on the chair wearing the Virtual Reality Head-Mounted Display (VR-HMD). They see their own backs through VR-HMD on which the video image of their own bodies captured by the camera 2 m behind them is projected in real-time. In this situation, while they see a rod approaching the camera, they are touched by another rod with simultaneous position and timing. This makes them start to feel like they are sitting behind themselves. In our current experiment, applying this method to the sound localization task, we tested whether participants' ability of sound localization was changed while they felt the illusion

of the sense of virtual body ownership. We hypothesized that their sense of sound localization would be deceived by the illusion and the degree of the impairment of the ability of sound localization depends on the extent of the change of the sense of their own bodies' positions. Our results partly confirmed this hypothesis, and it suggests that although our sense of body ownership or BSC is derived from visual-tactile integration in this experiment, it could affect even auditory perception, which is not manipulated directly in the experiment.

Disclosures: C. Toi: None. **A. Ishiguchi:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

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Program #/Poster #: 582.04/KK1

Topic: D.09. Multisensory Integration

Support: SFI 15/CDA/3316

Title: Indexing multisensory integration of natural speech using canonical correlation

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Abstract: Speech is most commonly perceived as multisensory. Indeed, integrating auditory and visual information from a talker's face is known to benefit speech comprehension. However, the neural mechanisms underlying this integrative process are not well understood, especially in the context of natural, continuous speech. Recent work relating EEG to the acoustic speech envelope has shown enhanced neural tracking of congruent audiovisual (AV) speech relative to unisensory (A+V) speech (Crosse et al., 2015), especially under challenging listening conditions (Crosse et al., 2016). This approach of relating EEG activity to the acoustic envelope however, is limited in its ability to deal with more complex representations of the speech signal. This is unfortunate given recent work demonstrating the ability to index auditory speech encoding at different hierarchical levels using EEG (Di Liberto et al., 2015). In order to overcome this limitation we have used canonical correlation analysis (CCA) to relate a multivariate representation of a speech stimulus to the multivariate EEG response. Specifically, CCA applies a linear transformation to both the stimulus and response with the goal of optimizing the correlation between the two. This allows us to examine integration effects at different hierarchical levels

using spectrotemporal and phonetic-feature representations of speech.
Our results show that when we represent the speech in terms of its spectrotemporal information there is a significant multisensory integration effect for speech in noise - suggesting that seeing the speakers face restores tracking of the spectrotemporal information in the speech signal. When we represent the speech in terms of its phonetic content however, we find a significant multisensory effect both for speech in quiet and speech in noise. Thus it appears that having access to the visual articulations of the speaker benefits phonetic encoding of the speech signal above what the acoustic information alone can provide. Further analyses will seek to isolate the unique contributions of spectrotemporal and phonetic processing to the EEG signal. The overarching goal is to provide a framework for testing hypotheses about how the temporal dynamics and articulatory information from a speaker's face help us to understand speech in challenging listening conditions.

Disclosures: A.E. O'Sullivan: None. **M.J. Crosse:** None. **G.M. Di Liberto:** None. **J. Majeski:** None. **A. de Cheveigne:** None. **E.C. Lalor:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 582.05/KK2

Topic: D.09. Multisensory Integration

Support: JSPS KAKENHI 16K10980

Title: Enhanced functional connectivity correlated with weight-loss at pre-match period in professional boxers

Authors: *Y. OGINO[1], H. KAWAMICHI[2], D. TAKIZAWA[4], S. K. SUGAWARA[3], Y. H. HAMANO[5], M. FUKUNAGA[5], Y. WATANABE[6], K. TOYODA[7], O. ABE[6], N. SADATO[8], S. SAITO[1], S. FURUI[7]
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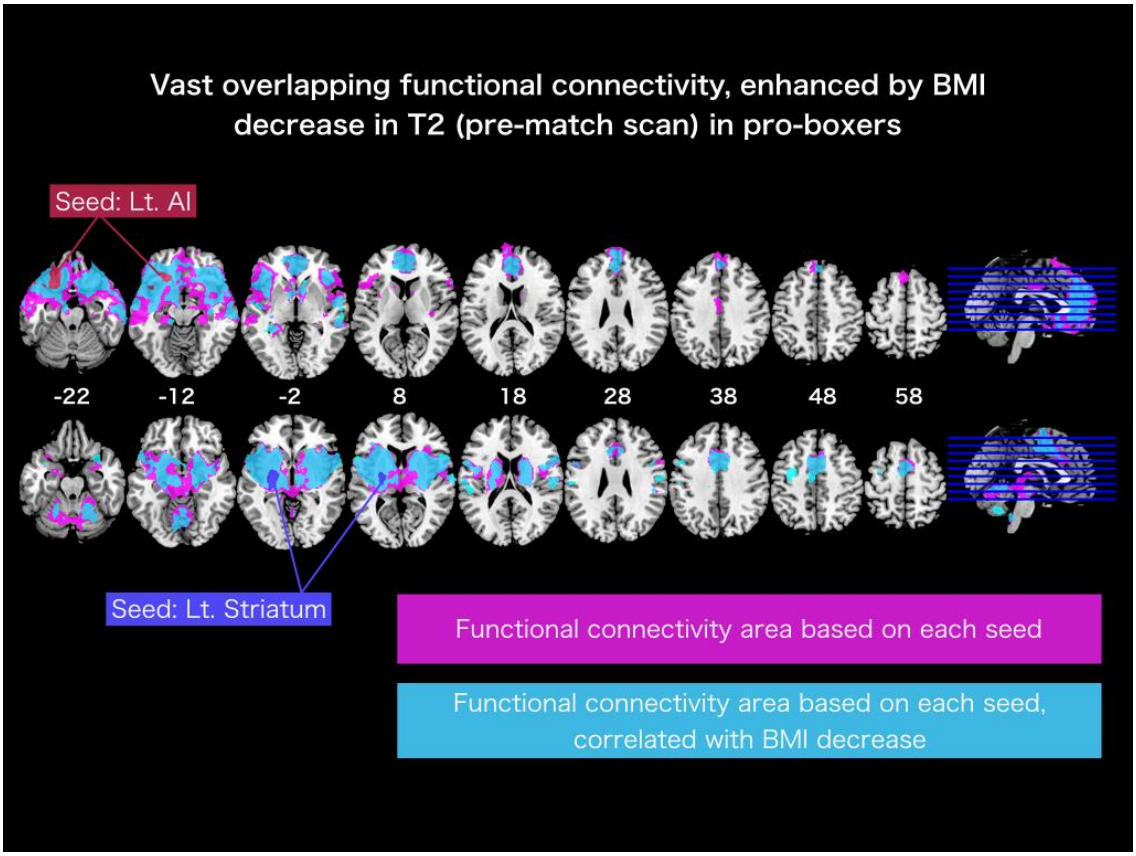
Abstract: Over months prior to the weigh-in (24h before the match), professional boxers (Pro-boxers) typically keep training and reduce their body mass (BM) empirically to gain a strength/size advantage over opponents. Few studies have investigated sport elite’s neural plasticity, and none have explored the impact of weight-making effect on neural structure and functional connectivity (FC). To address this issue, using voxel-based morphometry (VBM),

resting-state functional magnetic resonance imaging (rs-fMRI), we included twenty-one male licensed Pro-boxers (26.7 ± 4.0 years) in the time point of one-month before match (T1, mean BM index [BMI]: 21.9 ± 4.0) in comparison to age-sex-BMI matched Controls (27.2 ± 3.8 years, BMI: 21.4 ± 1.6). Then we longitudinally followed the Pro-boxers at the time point of within-one week before the match (T2, BMI: 20.6 ± 1.1) and one-month after the match (T3, BMI: 22.3 ± 1.4). In the time point of T1, Pro-Boxers presented significant higher gray matter density compared to controls, in left anterior insula (AI) ($p<0.001$, cluster level FWE [family wise error] corrected) and the left caudate ($p<0.016$), generally representing sensory integration and motor control respectively. In rs-fMRI analysis seeding these two clusters (left AI and caudate), significantly higher FC were found between left AI and left hippocampus ($p<0.033$), and between left caudate and bilateral insula/post-central gyrus ($p<0.001$) in Pro-boxers. In the time point of T2, the FC seeding the AI and caudate clusters had extended to the network including middle cingulate cortex/orbital gyrus ($p<0.001$) and dorsal anterior cingulate cortex ($p<0.001$) respectively, which is overlapped with the FC area correlated with BMI decrease in Pro-boxers ($p<0.001$). No significant FC were found in T3. These findings suggest the structural and functional plasticity in Pro-boxers and its widely distributed FC were enhanced by BMI decrease, implying the significance of weight-making in pre-match period.



Disclosures: Y. Ogino: None. **H. Kawamichi:** None. **D. Takizawa:** None. **S.K. Sugawara:** None. **Y.H. Hamano:** None. **M. Fukunaga:** None. **Y. Watanabe:** None. **K. Toyoda:** None. **O. Abe:** None. **N. Sadato:** None. **S. Saito:** None. **S. Furui:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 582.06/KK3

Topic: D.09. Multisensory Integration

Title: Multisensory processing in the elderly

Authors: *B. M. SEXTON, H. BLOCK
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Abstract: With age, degradation of sensory systems leads to poor motor control which may result in earlier placement in a long-term care facility. It is well documented that elderly adults have reduced proprioception and sensorimotor function. However, the effect of age on multisensory processing is unknown. When executing a motor command with the hand, we typically have information from multiple senses: vision from the retina, and proprioception from the muscles and joints. Integrating multiple sensory systems helps reduce variability to provide a more reliable goal directed movement. Here we ask whether elderly adults integrate vision and proprioception in a way that minimizes variance as young adults do, and whether elderly subjects compensate for an imposed visuo-proprioceptive mismatch as young adults do. Five elderly right handed participants with no history of neurological disease or injuries and normal or corrected to normal vision participated. Subjects sat at a custom 2D virtual reality apparatus with a touchscreen. Subjects were instructed to match their right index finger (indicator finger) to visual (V), proprioceptive (P), or combined (VP) targets, with no direct vision of either hand. The V target was a 1 cm white square that appeared in the plane of the touchscreen, the P target was the subject's left index finger (target finger) placed on a tactile marker beneath the reaching surface, and VP targets were a combination of the two. After a veridical baseline block, a misalignment was gradually imposed by shifting the V component forward without the subject's awareness. At the end of the misalignment block, the V component was displaced 70 mm from the P component. Compared to a group of 72 young adults, elderly subjects tended to have higher variance and bias in matching V and P targets. Weight of vision versus proprioception was similar for young and elderly subjects, with both relying slightly more on proprioception. Visual and proprioceptive realignment were similar for both young and elderly subjects in the misalignment block, suggesting elderly subjects are able to realign as much as young subjects. Intact multisensory processing in the elderly needs to be explored as a means of mitigating degradation in individual sensory systems.

Disclosures: B.M. Sexton: None. **H. Block:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 582.07/KK4

Topic: D.09. Multisensory Integration

Support: Vetenskapsradet
European Research Council

Title: The perceptual illusions of dual body ownership and dual self-location

Authors: *A. GUTERSTAM[1,2], J. SZCZOTKA[2], D. LARSSON[2], H. EHRSSON[3]
[1]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [3]Dept. of Neurosci., [2]Karolinska Institutet, Stockholm, Sweden

Abstract: The feeling of being a unitary physical entity-that is, owning one body located in one given location in the external space-is a fundamental subjective experience. Previous research has shown that is it possible to elicit a perceptual illusion of owning two copies of the same limb (e.g. two right arms). However, it remains unclear whether the coherent feeling of owning a full-body may be duplicated in the same manner, and, if so, how it relates to the sense of self-location. To this end, we adapted a full-body illusion (Petkova et al 2008) in which ownership of a mannequin's body is induced through correlated visuo-tactile stimulation. Specifically, participants wearing head-mounted displays were presented two full-bodies lying in parallel being touched by an object while receiving correlated tactile stimulation (Fig1A). In a series of five experiments (n=138; 83 females; 27±7yrs), we systematically manipulated the visuo-tactile congruence and visual perspective (first- vs third-person; 1PP vs 3PP) and quantified the senses of ownership and self-location using questionnaire ratings and threat-evoked skin conductance responses (SCR). The presentation of two bodies viewed from the 1PP and receiving synchronous visuo-tactile stimulation was associated with higher ratings of dual body-ownership questionnaire items ($p<0.001$; Fig1B) and increased threat-evoked SCR ($p<0.05$; Fig1C), suggesting that the two bodies were owned simultaneously. We failed to find support for the hypothesis that splitting the visual field in two and placing each of the bodies in different spatial environments would lead to an illusion of dual self-location, as evident from the subjective ratings on a self-location task ($p>0.05$). However, a strong sense of dual self-location and dual body ownership was induced when the visual perspective repeatedly 'jumped' between two bodies' 1PPs and a common 3PP (Fig1D). In summary, these findings suggest that congruent, ambiguous visuo-tactile stimulation of two bodies can elicit the illusion of owning two separate full-bodies and being in two locations at once.

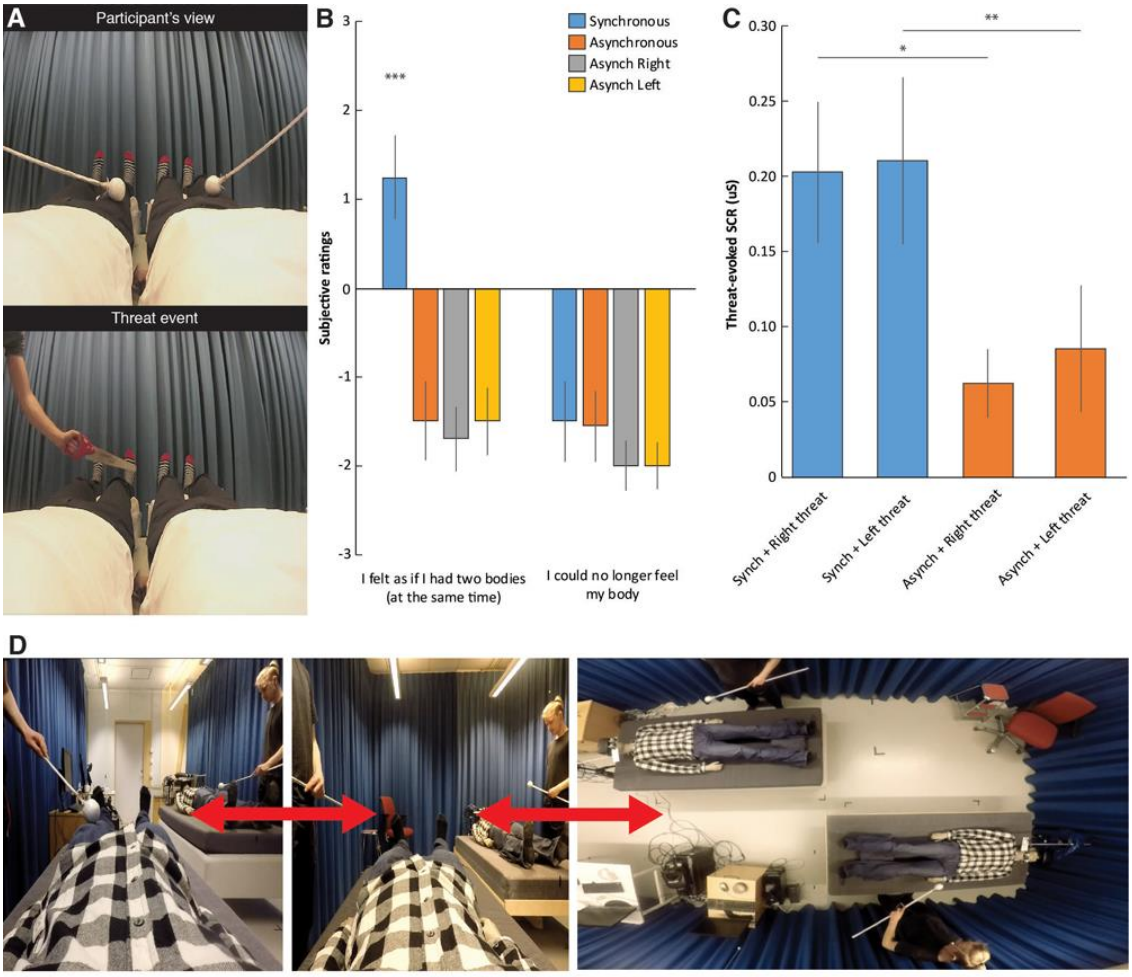

Figure 1. A. Participant's view of visuo-tactile stimulation and threat event. **B.** Questionnaire results. **C.** Threat-evoked SCR. **D.** Visul stimuli in experiment #5, in which the visual perspective changed (here indicated by the red arrows) every 18 s between two 1PPs and one 3PP. *p<0.05. **p<0.01. ***p<0.001.

Disclosures: A. Guterstam: None. **J. Szczotka:** None. **D. Larsson:** None. **H. Ehrsson:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 582.08/KK5

Topic: D.09. Multisensory Integration

Support: NIH R01NS06395
NIH U01NS098976
NIH R25NS070694

Title: Rapid modulation of activity in auditory cortex by visual speech information revealed by electrocorticography

Authors: P. J. KARAS[1], *B. METZGER[1], J. F. MAGNOTTI[1], Z. WANG[2], D. YOSHOR[1], M. S. BEAUCHAMP[1]
[1]Dept. of Neurosurg., Baylor Col. of Med., Houston, TX; [2]Dept. of Statistics, Rice Univ., Houston, TX

Abstract: Speech perception is inherently multisensory: observers make use of visual information from the talker's mouth as well as auditory information from the talker's voice. The neural interchange between auditory and visual cortex that allows this is poorly understood. In the present study, we recorded activity from electrodes implanted in epilepsy patients. Patients were presented with auditory-only (A), visual-only (V), and audiovisual (AV) speech consisting of two different types of words: words in which visual mouth movements preceded the onset of auditory speech (visual-leading; *e.g.* 'drive'); and words in which auditory speech preceded the visual mouth movements (auditory-leading; *e.g.* 'known'). We recorded from 111 electrodes implanted on the superior temporal gyrus across 8 patients. Average high-frequency gamma broadband activity (75-150 Hz) in the window from 0 ms to 500 ms after auditory stimulus onset was used as a measure of local neuronal response. From these 111 electrodes, we selected only electrodes that showed significant activity in any of the experimental conditions ($F > 25$, $R2 > 0.18$, $p < 10^{-15}$), resulting in 58 electrodes. The mean percent signal change response to A and AV speech across all words was 121% (+/- 11% standard error) and 120% (+/- 11%), respectively. To compare how the signal changed between A-only and AV words, we first calculated the AV reduction for each word type in each electrode by taking the mean difference between A trials and AV trials. We then compared the AV reduction for visual-leading (mean A-AV = 8% +/- 7%) and auditory-leading words (mean A-AV = -7% +/- 4%). On average, visual-leading words showed a larger difference between A and AV conditions, $t(57) = 2.74$, $p = 0.008$). Across electrodes, 40 of 58 were consistent with the overall finding that visual-leading words have a greater AV reduction than auditory-leading words. We explain this observation with reference to theories of predictive coding in sensory processing. Under this framework, the visual component of AV speech provides a prediction of the incoming auditory component, but only if the visual information precedes auditory speech (visual-leading words). The additional visual information reduces the computational burden on auditory cortex, leading to more efficient word identification and reduced neural activity.

Disclosures: P.J. Karas: None. **B. Metzger:** None. **J.F. Magnotti:** None. **Z. Wang:** None. **D. Yoshor:** None. **M.S. Beauchamp:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 582.09/KK6

Topic: D.09. Multisensory Integration

Support: VCU Presidential Research Quest Fund

Title: Children with idiopathic toe walking showed differences in areas of tactile and vestibular processing

Authors: *V. W. CHU, J. LEE, B. CHAN
Occup. Therapy, Virginia Commonwealth Univ., Richmond, VA

Abstract: Idiopathic toe walking (ITW) is characterized by the absence of heel contact during gait, without a medical cause for the toe walking. Long term consequences of persistent toe walking include shortened Achilles tendon and ankle equinus. A link between ITW and sensory processing dysfunction has been suggested, but to date, there is limited research examining this relationship. Areas of sensory processing that potentially relate to ITW include: sensory seeking, tactile defensiveness, poor proprioception, vestibular dysfunction and difficulties with sensory modulation. Identifying the specific sensory function that underpin ITW will be invaluable to the development of specific diagnoses and treatments for this gait abnormality. We recruited children with ITW between the ages of 4 to 12 years from VCUHS and local clinics. Age-matched typically walking participants were recruited from the local community. Children completed a set of activities to assess their sensory processing related to walking. Balance-related proprioceptive, vestibular and visual processing was assessed with the Sensory Organization Test (SOT) and perturbation tests using the Neurocom SMART Balance Master®. Vibration perception threshold was measured with a vibrometer and tactile threshold was measured with the Semmes Weinstein filaments. Proprioceptive processing was assessed by testing ankle position sense and ankle force perception without visual feedback. Sensory modulation response to stimuli was examined by measuring skin conductance in response to tactile and vestibular stimuli. Preliminary results showed that children with ITW have difficulty with balance tasks that involved ankle perturbations, and reduced use of effective ankle strategies for balancing. They also have increased latency and larger response magnitude to balance perturbation. Children with ITW also showed abnormally high skin conductance response indicating potential difficulty with sensory modulation. Children with ITW also showed either extremely high or low tactile detection thresholds compared to typically developing children. Children with ITW did not show significant deficits in proprioception discrimination tasks. Further testing in more children with ITW will allow us to examine subtypes of sensory differences in ITW. This research will significantly advance our understanding of ITW by

providing a framework to detect and analyze the underlying sensory differences in children with ITW. Our research strives to better understand the causes of ITW, so that we can develop effective treatments to guide earlier intervention to prevent long-term consequences of persistent toe walking.

Disclosures: V.W. Chu: None. **J. Lee:** None. **B. Chan:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 582.10/KK7

Topic: D.09. Multisensory Integration

Title: Evaluating the effects of english second language status on fixations and first-pass skip rates during a continuous reading task

Authors: *C. Y. DELGADO[1], T. A. DOTY[3], D. L. LARRANAGA[4], D. A. DEL CID[5], C. MCGINNIS[2]
[1]California State Univ. Northridge, Tarzana, CA; [2]California State Univ. Northridge, Northridge, CA; [3]Cal State Northridge, Northridge, CA; [4]Psychology, VISN Lab. at California State University, Northridg, Altadena, CA; [5]Psychology, Vision Sci. Information Lab. @ CSUN, Sherman Oaks, CA

Abstract: Previous literature has indicated that bilinguals are more likely to make errors while reading function words aloud than content words (i.e. word class) in mixed language texts (Gollan, Schotter, Gomez, Murillo, & Rayner, 2014). More specifically, bilinguals are more likely to make intrusion errors (e.g saying *pero* instead of *but*) when fixating in a non-target word in a mixed-language text (Gollan et al., 2014). Studies have shown that eye movements are ahead of the voice, indicating that overt and covert attention are not aligned. This implies an increased vulnerability to intrusions when reading mixed-language text. (Gollan et al., 2014.) There has been a significant difference in overt attention in both fixation and first-pass skip reading (i.e. skipping words) on word length and word class (Chamberland, Saint-Aubin, & Légere, 2013). Previous work implied mechanisms of language control inhibit the dominant language when switching between two languages to reduce intrusions (Gollan et al, 2014). Conflicting studies used fMRI to demonstrate language inhibition is not possible. The bilingual brain cannot inhibit and avoid language conflicts, because both left caudate and anterior cingulate cortex have been observed to be active in the neural networks of language selection of both languages (Abutalebi et al., 2007; van Heuven et al., 2008).
The present work aims to evaluate a possible interaction with English second language (ESL) individuals on word length, word class and gaze durations through fixations and first-pass skip rates of words. Twenty-three participants' gaze and ocular movements were recorded using an

EyeLink 1000 Plus (SR-Research) eye-tracker to investigate this possible interaction. Results confirm previous findings that there are significant differences in both fixation and first-pass skip rates based on word length and word class. However, no significant effects were observed based on ESL status of the reader.

Disclosures: C.Y. Delgado: None. **T.A. Doty:** None. **D.L. Larranaga:** None. **D.A. Del Cid:** None. **C. McGinnis:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 582.11/KK8

Topic: D.09. Multisensory Integration

Title: Brain regions involved in sound-to-meaning mapping and its relationship to phoneme perception

Authors: *S. ITAGAKI, S. MURAI, K. I. KOBAYASI
Doshisha Univ., Kyotanabe, Kyoto, Japan

Abstract: In the association between meanings and sounds (i.e., phonemes), a phenomenon termed “sound symbolism” has been confirmed by several studies. This is the idea that a sound makes a certain of impression (e.g., phoneme “p” is associated with small impression) and it could serve as a psychological basis for the word-meaning association. The purpose of this study is to clarify the involvement of a phoneme-perception-related brain region in the sound symbolism. We conducted two experiments. In the Experiment I, we focused on sound symbolism in visual size. Subjects were all Japanese native speakers, and they did not have knowledge about sound symbolism and this experiment. They were required to answer visual size difference between a standard and a target stimulus. Visual stimuli had 1 type of standard and 2 types of target. Target size was either smaller or larger than the standard. Sound stimuli were voice sounds (/bo/, /bi/, /po/ and /pi/), noise and click sound (control). Voice sounds were assumed to have impression of “larger” or “smaller”, according to previous researches. The subject performed the task under functional magnetic resonance imaging (fMRI) scanning. We defined the congruent and incongruent conditions as follows and analyzed the data accordingly. It is congruent condition when the impression from the visual stimulus is consistent with the impression of the sound stimulus. It is incongruent condition when the relationship between stimuli was reversed. As a result, reaction times in incongruent condition were longer than congruent condition suggesting that sound symbolism was observed between visual size and syllables under fMRI, and right superior temporal gyrus was more activated in congruent condition. This region has been reported to relate to vowel perception by previous studies. In the Experiment II, we investigated the brain region related to phoneme perception. Subjects were all

Japanese native speakers and most of them participated in the Experiment I as well. They were required to discriminate phoneme. Sound stimuli were voice sounds (/bo/, /bi/, /po/, /pi/, /a/, /i/ and /o/) and noise. In Experiment II, right superior temporal gyrus and left and right fusiform gyrus were activated when subjects discriminate phoneme. We will discuss relationship between the activation area for sound symbolism and for phoneme perception in individual level.

Disclosures: S. Itagaki: None. **S. Murai:** None. **K.I. Kobayasi:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 582.12/KK9

Topic: D.09. Multisensory Integration

Support: University of Tuebingen, Fortuene grant 2292-0-0 and 2454-0-0
DFG RO 5587/1-1

Title: The pre- and post-stimulus dynamics of the brain's multisensory causal inferences

Authors: *T. ROHE[1], A.-C. EHLIS[1,2], U. NOPPENEY[3]
[1]Dept. of Psychiatry and Psychotherapy, [2]LEAD Grad. Sch. & Res. Network, Univ. of Tuebingen, Tuebingen, Germany; [3]Computat. Neurosci. and Cognitive Robotics Ctr., Univ. of Birmingham, Birmingham, United Kingdom

Abstract: Humans integrate stimuli from multiple modalities to obtain more reliable multisensory representations of their environment. However, given the multitude of multisensory stimuli in any environment, the brain needs to integrate stimuli only if they arouse from a common cause, but has to segregate stimuli from independent causes (Koerding et al., 2007). Humans infer a common cause if they perceive a small temporal, spatial and structural disparity between multisensory stimuli. Current fMRI studies (Rohe & Noppeney, 2015, 2016, 2018) demonstrate that the brain implements such causal inference processes along a cortical hierarchy. However, the temporal dynamics of the brain's causal inference processes remain unknown. In the current EEG study, human participants (N = 23) were presented with one to four synchronous flashes and beeps and they counted either the number of flashes or the number of beeps. As predicted by the Bayesian Causal Inference (BCI) model, participants behaviorally integrated the stimuli if a small numeric disparity (≤ 1) between the number of flashes and beeps suggested a common source, while the stimuli were segregated for a large disparity. Representational similarity analysis demonstrated that the geometry of participants' audiovisual numeric representations was predicted by the BCI model's internal estimates of stimulus number and their likely causal structure. 300 ms up to 100 ms before stimulus onset, the power of alpha and gamma EEG oscillations as well as alpha phase modulated participants' perceptual prior of the

stimuli's likely causal structure. Starting from 150 ms after stimulus onset, a multivariate pattern analysis on EEG recordings decoded the number of presented stimuli with high accuracy. The decoding approach demonstrated that the brain first represented the stimuli's numeric disparity and computed the stimuli's likely causal structure. Next, the brain integrated the stimuli in case of a small numeric disparity, but segregated the stimuli in case of a large disparity. Overall, the brain's neuronal dynamics before and after stimulus onset reflect the hierarchical organization of multisensory causal inference across the cortices.

Disclosures: T. Rohe: None. **A. Ehlis:** None. **U. Noppeney:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 582.13/KK10

Topic: D.09. Multisensory Integration

Support: BBSRC (BB/M007847/1)

Title: Use of click-based echolocation may preserve retinotopic-like representation of space in calcarine cortex in early-blind people

Authors: *L. J. NORMAN, L. THALER
Durham Univ., Durham, United Kingdom

Abstract: In sighted people, calcarine cortex (CC) in each hemisphere contains a neural mapping of the contralateral visual field, where more peripheral points in visual space are represented more anteriorly. CC in early-blind people is known to process input from non-visual modalities - e.g. auditory (1) or tactile (2) - and there is some evidence from resting state functional connectivity that the visual cortex of congenitally blind people is functionally connected in a way that resembles retinotopic organisation (3). It is not known, however, whether this organisation can be used for spatial representations of sensory input - a condition that may be essential for successful visual rehabilitation following vision loss (e.g. visual prostheses). Therefore, here we tested if basic principles of retinotopic organisation (contralaterality and eccentricity mapping) are used to map acoustic space in CC of early-blind people, who either had a history of using click-based echolocation (n=5) or not (n=5), as well as in sighted blindfolded controls (n=5). Echolocation is the ability to perceive objects through sound echoes, and is associated with processing in CC (4). During fMRI, each participant listened to individualized binaural recordings of clicks and click echoes reflected from objects located at one of eight positions along the horizontal meridian, or to binaural recordings of source sounds from the same positions. We then used cross-correlation to map representations of these echo- and source- positions in CC. In three out of the five echolocators, we found evidence

of contralateral mapping of acoustic space for both echo- and source- positions. Furthermore, in two of these three echolocators there was evidence of preserved eccentricity mapping, and this was more prominent for echo- than source-acoustic positions. There was no evidence of contralaterality or eccentricity mapping in blind people who did not use echolocation, or in sighted blindfolded people. This suggests that the results found in the early-blind echolocators were driven specifically by expertise in using echolocation, and not by vision loss or by the ability to form mental imagery, for example. Overall, this result provides evidence that the use of click-based echolocation by early-blind individuals may allow some retinotopic-like representation of space to be preserved in CC, and this may have implications for successful visual rehabilitation in individuals with early vision loss (e.g. visual prostheses). 1 Collignon et al (2011). PNAS. 108, 4435-4440 2 Cheung et al. (2009). Current Biology, 19, 596-601 3 Striem-Amit et al (2015). Brain, 138, 1679-1695 4 Thaler et al. (2011). *PLoS ONE*, 6(5): e20162

Disclosures: L.J. Norman: None. **L. Thaler:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 582.14/KK11

Topic: D.09. Multisensory Integration

Support: SNF Grant PZ00P3_167836/1
H2020 Grant UE8-BRAINCOM-732032

Title: Neural correlates of cross-modal influences in top-down processing of visual speech

Authors: *R. THÉZÉ[1], A.-L. GIRAUD[1], P. MEGEVAND[2,1]
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Abstract: Audiovisual speech processing results from a mixture of bottom-up sensory information and top-down predictions, and it is hypothesized that the integration of information from each modality across cortical areas involves cortical oscillations. The aim of this study was to probe the interplay between bottom-up and top-down influences on audiovisual speech processing by dissociating them. We developed a cross-modal “pop-out” task where the same speech stimulus is presented thrice: first (V1), in visual modality only (i.e. no sound), second (AV), with the corresponding sound information and, third (V2), again in the visual modality only. We built a set of 60 stimuli (i.e. sentence long video samples from known movies with a character speaking to the camera) and asked participants (n = 10) to rate the intelligibility on each presentation. On average, the videos were rated as not intelligible on the first presentation, and highly intelligible on the second. On the third presentation they were rated as significantly

more intelligible than the first presentation but less than the second. In other words, a given visual speech stimulus became more intelligible if it immediately followed presentation of the corresponding audiovisual speech stimulus. Two patients with epilepsy agreed to perform the experimental task while we recorded their brain activity with intracranial subdural EEG electrodes while they undertook the task. We identified a subset of electrodes located for the most part in the right superior temporal cortex and precentral gyrus in which high-frequency activity correlated with speech envelope during presentation of audiovisual stimuli (AV). Importantly, we found one electrode in the motor cortex where high-frequency activity did not respond to pure visual stimuli on the first presentation (V1) but tracked speech envelope during the second presentation of visual stimuli (V2), thus mirroring the behavior of subjective intelligibility ratings.These findings support the idea that top-down predictions on the contents of visual speech influence its perception, a process that takes place outside of the auditory speech areas.

Disclosures: R. Thézé: None. **A. Giraud:** None. **P. Megevand:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 582.15/KK12

Topic: D.09. Multisensory Integration

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European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 720270 (HBP SGA1)

Title: Multisensory data-driven modeling of fMRI responses across primate species

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Abstract: Multi-sensory processing has been studied in both human and non-human primates (Driver and Noesselt, 2008). Recent comparative efforts focused on functional correspondences of sensory brain networks based on comparisons of fMRI timeseries across species (Mantini et al., 2012; Mantini et al., 2013). Yet, comparative multi-sensory processing remains largely unexplored territory. Here, we aimed to activate large portions of monkey and human cortex using multisensory stimulation. Specifically, we collected fMRI responses from both species while identically-ordered sequences of visual, auditory and tactile stimuli where randomly presented in an event-related manner to the awake and fixating subjects. We first calculated

voxel-by-voxel inter-subject correlations within each species to identify voxels showing reliable correlations (Hasson et al., 2004). These voxels were extracted to establish ROIs responding reliably to our multimodal stimuli. Next, these ROIs were used for computing intra-species correlation matrices. We then used a hierarchical clustering analysis, which segregated three groups of ROIs corresponding to the three sensory systems. Then, we averaged ROI responses within each cluster to obtain three independent timecourses (visual, audio, tactile) separately for each species. To account for differences in HRF between species, we convolved these sensory components with a canonical HRF from the other species. Using the resulting sensory signals, we modelled voxel responses throughout the brain across species. Thus, the sensory-driven functional responses from one species are used to model the responses in the other species. This enabled us to identify regions across species involved in sensory processing for the three modalities. Most interestingly, we identified overlap of the three sensory maps based on the inter-species modeling. Evidence for trimodal processing was found in inferior-frontal (IFJ, IFS), somatosensory (1, 3, 4), insular, posterior STS and even early visual (V1) regions of the human cortex. In the monkey, we found trimodal overlap based on intra-species modeling in ventral prefrontal (46v, 12m), insular, posterior STS (Tpt, pTPO), somatosensory (3a-b), and the MT-complex. Virtually no trimodal overlap, however, was detected using the inter-species modelling in monkey, which may suggest a higher capacity for cross-modality integration in human. Overall, we present a purely data-driven approach, whereby the fMRI responses in one primate species (and not the stimulus design per se) are used to reveal large-scale multi-sensory driven brain regions in other species.

Disclosures: M. Armendariz: None. **D. Mantini:** None. **W. Vanduffel:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 582.16/LL1

Topic: D.09. Multisensory Integration

Support: NRF-2017M3C7A1047225

Title: The effect of head direction on gait trajectory in human

Authors: *H. JOO[1], S. KIM[2,4], J.-K. RYU[3], K. LEE[2,5]
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Abstract: Background
There have been numerous research findings on neural structures that process spatial orientation such as place cell, grid cell, head direction cell, and etc. These structures, however, are located in the subcortical region which made human-based research has been explored to a limited extent. The purpose of the study was to examine the influence of head direction on straight locomotion task in behavior level in human. As many researches revealed that head direction cells exist in different animal species, this study will base its idea that human also has direction cells and plays an important role in motor control behavior.
Method
To isolate the head direction effect, two conditions were investigated: 0-degree and 30-degree rightward rotation in a horizontal plane. Since vision is the dominant sense in the human sensory system, subjects were examined with and without the eye mask. Eleven subjects(5 female) participated and were asked to walk in a straight line ten times in each condition (four-conditions in total) from the same starting point. To calculate walking orbits, participants wore a cap type motion tracker. A Factorial ANOVA was conducted to compare the main effects of type of head direction and visual sense availability as well as the interaction between the type of head direction and vision on the angular difference at the five-meter point from the baseline. Baseline was derived from the averaging walking path in the 0-degree head direction with vision.
Result
The main effect for vision type yielded an F ratio of $F(1, 109)=30.211$, $p<.001$, indicating a significance difference between using vision condition($M=-2.587$, $SD=.071$) and mask condition($M=-3.878$, $SD=.226$). The main effect for head direction type yielded an F ratio of $F(1, 109)=9.651$, $p<.01$, indicating that the effect for head direction was significant, 0-degree($M=-2.917$, $SD=.163$) and 30-degree($M=-3.549$, $SD=.151$). The interaction effect was significant, $F(1,109)=11.539$, $p<.01$. There was no significant sex difference.
Conclusion
These findings suggest that vision exerts a crucial role in modulating heading direction even though the head's direction doesn't match with the body's direction to walk. The head direction factor also modulates subjects' path to drift in the direction of head rotation as compared to frontward head direction. To further understand the role of head direction, possible input signals to head direction cells such as the vestibular system, proprioception, etc. should be systematically controlled.

Disclosures: H. Joo: None. **S. Kim:** None. **J. Ryu:** None. **K. Lee:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

Location: SDCC Halls B-H

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Program #/Poster #: 582.17/LL2

Topic: D.09. Multisensory Integration

Support: NRF-2017M3C7A1047225

Title: Anisotropic decline of ownership illusion intensity in spatial mismatch condition: A guideline to modulating pain signal

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Abstract: Ownership illusion modulates pain perception by raising pain threshold. Ownership illusion is a phenomenon, which feels ownership of object that do not belong to one's own body. The illusion is induced when the object is similar to one's own body. Similarity can arise from location, movement, texture and visual properties like shape and color. When inducing ownership illusion, synchronous tactile-visual stimulation is usually used. Pain modulation of ownership illusion is still available in virtual reality.
To control the intensity of ownership illusion precisely, quantification of relationship between ownership illusion intensity and spatial mismatch was preceded. Participants were instructed to put on HMD and see the left or right hand made up by using UNITY. The virtual hand and participant's real hand was collocated as much as possible. Then virtual and real hand were stimulated by using Geomagic Touch. Stimulation was given synchronously but location was different. There were 8 conditions of spatial mismatch, which differs in distance and direction. Intensity of ownership illusion was measured by a modified questionnaire originally presented by Botvinick and Cohen(Botvinick and Cohen, 1998).
As a result, ownership illusion intensity declined as size of spatial mismatch increased. Same tendency was discovered in several papers(Samad M, Chung AJ, Shams L, 2015). Meanwhile, the ownership illusion intensity found to be more robust to vertical spatial mismatch than horizontal spatial mismatch.
This anisotropy can be explained by dermatomal distribution. According to dermatomal distribution of hands, vertically different tactile signal can share same afferent fibers while the horizontal cannot. This anatomical difference could be the cause of anisotropy of ownership illusion intensity by influencing multisensory integration. This phenomenon should be considered when studying an analgesic effect of ownership illusion.

Congruent	Horizontal mismatch 01	Horizontal mismatch 02	Vertical mismatch 01	Vertical mismatch 02
Right hand	2cm			
Left hand	3cm			

Disclosures: M. Seo: None. **S. Kim:** None. **J. Ryu:** None. **K. Lee:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

Location: SDCC Halls B-H

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Program #/Poster #: 582.18/LL3

Topic: D.09. Multisensory Integration

Support: Creative Endeavors Scholarship

Title: Grapheme-color synesthetes and inhibitory differences during an anti-saccade task

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Abstract: Research into synesthesia can inform many fields, including cognitive neuroscience, language, emotion, imagery, and attention (Kadosh & Henik, 2007). Grapheme-color synesthetes consistently report the same color being associated with a given letter. One of the primary theories of why this occurs involves an inability to inhibit feedback between neurological areas (Grossenbacher & Lovelace, 2001). The present study examined whether this lack of inhibition extends to other domains of functioning by means of an antisaccade task. Three grapheme-color synesthetes, along with two age- and gender-matched controls for each, were recruited and asked to complete an antisaccade task in the lab. Results indicate a significant difference between synesthetes and non-synesthetes in the latencies for their pro- and antisaccades. These data indicate partial support for the disinhibition-feedback theory of synesthesia as inhibited processing extends to domains other than grapheme processing.

Disclosures: D.L. Larranaga: None. **R. Esquenazi:** None. **M.F. Awad:** None. **S.A. Drew:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

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Program #/Poster #: 582.19/LL4

Topic: D.09. Multisensory Integration

Support: FINEP 01·12·0514·00
CNPq 573966/2008-7

Title: The peripersonal space representation in paraplegic patients depends on the level of lower-limb residual neurological functions

Authors: *S. SHOKUR[1], F. ASNIS[1], S. ALMEIDA[1,2], M. A. NICOLELIS[3,1,4]
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Abstract: The peripersonal space (PPS) is a multisensory representation of the space immediately surrounding the body encoded in frontoparietal cortical areas. PPS representation is essential in the sensory guidance to generate suitable motor acts and allowing us to interact with objects (Ladavas 2015). Numerous studies have shown that PPS is plastic and changes, for example, when a subject uses an external tool (Canzeroni 2013, Serino 2007) or shrinks for amputee patients around their stump.
Here we study the PPS representation for patients with sensory-motor deficiency due to spinal cord injury (SCI). We recorded the lower-limb PPS representation for a group of 13 paraplegic patients, throughout 16 months of a training protocol developed by our laboratory (Donati 2016), which has previously demonstrated to induce partial neurological recovery in SCI patients.
To measure the PPS limit, we used an audio-tactile discrimination paradigm (Canzeroni 2012). We used the ASIA evaluation (golden standard for neurological assessment, Ditunno 1994) to assess patients' sensory-motor functions.
First, we found that the PPS limit for SCI patients was significantly shorter than for healthy subject (ttest, $P<0.05$). Second, among the SCI patients, we found a significant correlation between patients' lower-limb PPS limit with both their sensory score ($R= 0.69$, $P=0.008$, $N=34$) and their motor score ($R=0.62$, $P = 0.02$). These results suggest that the low-level neurological recovery, which was induced by our training protocol, triggers a reorganization of the patients' body perception and as a result expands their PPS.
Ref:
Làdavas, Elisabetta, et al. "Neuropsychological evidence of an integrated visuotactile representation of peripersonal space in humans." Journal of Cognitive Neuroscience 10.5 (1998): 581-589.
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Ditunno, J. F., et al. "The international standards booklet for neurological and functional classification of spinal cord injury." Spinal Cord 32.2 (1994): 70.

Disclosures: S. Shokur: None. **F. Asnis:** None. **S. Almeida:** None. **M.A. Nicolelis:** None.

Poster

582. Multisensory Integration: Cross-Modal Processing in Humans II

Location: SDCC Halls B-H

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Program #/Poster #: 582.20/LL5

Topic: D.09. Multisensory Integration

Support: NIH Grant EY023268
FNRS Grant FC7159
NIH Grant P20 GM103650

Title: Directional visual motion is represented in the auditory and association cortices of early deaf individuals

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Abstract: Individuals who are deaf since early life may show enhanced performance at some visual tasks, including discrimination of directional motion. The neural substrates of such behavioral enhancements remain difficult to identify in humans, although neural plasticity has been shown for early deaf people in the auditory and association cortices, including the primary auditory cortex (PAC) and superior temporal sulcus (STS) region, respectively. Here, we investigated whether neural responses in auditory and association cortices of early deaf individuals are reorganized to be sensitive to directional visual motion. To capture direction-selective responses, we recorded functional magnetic resonance imaging (fMRI) responses frequency-tagged to the 0.1 Hz presentation of central directional (100% coherent random dot) motion persisting for 2 s contrasted with non-directional (0% coherent) motion for 8 s. We found direction-selective responses in the STS region in both deaf and hearing participants, but the extent of activation in the right STS region was over seven times larger for deaf participants. Minimal but significant direction-selective responses were also found in the PAC of deaf participants, both at the group level and in five out of six individual deaf participants. In response to stimuli presented separately in the right and left visual fields, the pattern of activation across the right and left hemispheres was similar in both the PAC and STS region of deaf participants, and could support a right visual field advantage reported previously in behavioral studies. Taken together, these results show that the reorganized auditory cortices of early deaf individuals are sensitive to directional motion. More speculatively, these response suggest that auditory and association regions can be remapped to support enhanced visual performance.

Disclosures: T.L. Retter: None. **M.A. Webster:** None. **F. Jiang:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.01/LL6

Topic: E.02. Cerebellum

Title: TMEM240: A novel cerebellar synaptic protein

Authors: *M. L. HOMA, A. LOYENS, D. MAZUR, V. HUIN, L. BUÉE, B. SABLONNIÈRE
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Abstract: Introduction. Dominantly inherited spinocerebellar ataxias (SCAs) are heteregeneous neurodegenerative diseases characterized by cerebellar impairment. To date, 38 different loci and 27 genes have been described for the SCAs. Recently, missense mutations and a stop mutation in transmembrane protein 240 (TMEM240) have been reported in spinocerebellar ataxia 21 (SCA21) among 11 french families. SCA21 stands out by its association with severe cognitive impairment and early age onset. Nowadays, TMEM240 function is still unknown.
Objectives. 1. Establish a general brain mapping of TMEM240 expression in mice 2. Determine TMEM240 specific expression in mice cerebellum 3. Define TMEM240 cellular and subcellular expression.
Methods. Immunohistochemistry analyses are performed on mice brain tissues. To establish cellular and subcellular localization, we realized immunohistochemistry on mice brain sections. Immunostaining is studied by confocal microscopy. TMEM240 synaptic expression is analyzed by electron microscopy.
Results. Immunostaining shows that TMEM240 is mainly expressed in cerebellum and especially in the uvular lobe (IX) and nodulus lobe (X). At a cellular level, TMEM240 is localized in neurons from cerebellar cortex : molecular layer, cerebellar glomeruli in the granular layer and in the soma and dendritic arborization of Purkinje cells. TMEM240 is located among synapses between Purkinje cell and granular cells, and co-localized with synaptic markers as validated by confocal microscopy.
Conclusion. TMEM240 protein is expressed in neurons from cerebellar cortex. TMEM240 expression is mainly observed in synapses. TMEM240 could have a synaptic function in cerebellar cortex neuronal network.
Perspectives. SCA21 model in zebrafish, identification of TMEM240 partners.

Disclosures: M.L. Homa: A. Employment/Salary (full or part-time):; University Lille. **A. Loyens:** None. **D. Mazur:** None. **V. Huin:** None. **L. Buée:** None. **B. Sablonnière:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.02/LL7

Topic: E.02. Cerebellum

Support: HHMI
NIH
NSF

Title: Cortex-cerebellum dynamics in the execution and learning of a motor task

Authors: *M. J. WAGNER, T. H. KIM, J. KADMON, N. D. NGUYEN, S. GANGULI, M. J. SCHNITZER, L. LUO
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Abstract: The neocortex and cerebellum have expanded together during mammalian evolution, and the cortex-pons-cerebellum pathway is among the densest long-range connections in the brain. However, little is known about cortico-cerebellar information transmission. Here we show, by simultaneous two-photon Ca^{2+} imaging in premotor layer 5 pyramidal and cerebellar granule cells during a motor planning task, that cortical representations of behavior are communicated to granule cells with high fidelity. Moreover, granule cells represented events more reliably than did their correlated cortical partners. Transiently silencing basal pontine neurons indicated that coherent activity requires cortico-cerebellar transmission. Chronic cortex-cerebellum imaging over weeks of learning revealed that cortex and cerebellar task representations emerged in parallel. Cortico-cerebellar and intra-cortical correlations also rose substantially with learning. These findings support a circuit model in which pons amplifies coherent layer 5 dynamics that emerge with learning before relaying signals to granule cells. Thus, the cerebellum receives detailed representations of cortical dynamics that are substantially enhanced by learning, which likely facilitates cerebellar participation in cortical computation.

Disclosures: M.J. Wagner: None. **T.H. Kim:** None. **J. Kadmon:** None. **N.D. Nguyen:** None. **S. Ganguli:** None. **M.J. Schnitzer:** None. **L. Luo:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

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Program #/Poster #: 583.03/LL8

Topic: E.02. Cerebellum

Support: NIH Grant R37-NS39395
NIH Grant F32-NS106720

Title: The role of the medial cerebellum in modulating synaptic responses in the ventrolateral periaqueductal gray

Authors: *C. E. VAAGA, I. M. RAMAN
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Abstract: Stimulation of the ventrolateral periaqueductal gray (vlPAG) can elicit freezing, an innate defensive behavior necessary to avoid predation. Other brain regions provide input to the PAG and are therefore likely involved in processing innately threatening stimuli. For example, lesions of the cerebellar vermis selectively impair innate freezing, and cerebellar projections from the medial cerebellar nucleus (mCbN) to the vlPAG have been reported (although primarily attributed to oculomotor function). These results suggest that the cerebellum may modulate vlPAG circuitry via a direct, monosynaptic projection; however, the synaptic contribution of the mCbN to vlPAG circuitry has not been tested. Here, we examined the functional connectivity of cerebellar afferents to vlPAG circuitry using anatomical and slice electrophysiological techniques in mice (both sexes, p17-p56). Stereotaxic injections of CTb-GFP into the vlPAG labeled neurons in the mCbN. Neurons in the mCbN had high spontaneous firing rates (121.5±8.7 Hz), 25-50 Hz higher than in the neighboring interpositus nucleus. To selectively target glutamatergic vlPAG neurons involved in freezing behaviors we used a Chx10-cre mouse, which labels a subset of glutamatergic neurons in the vlPAG thought to drive freezing. Anterograde injections of AAV-DJ-ChR2-eYFP into the mCbN labeled axon terminals in close proximity to Chx10+ neurons in the vlPAG. Chx10+ neurons had small diameters (7-8 μm) and high input resistances (584.1±47.3 MΩ). In current clamp recordings, 70% of Chx10+ neurons were regular firing (5.8±1.2 Hz), whereas the remaining 30% showed prominent bursting. To assay the functional connectivity between the mCbN and the vlPAG, we injected a ChR2-expressing viral vector into the mCbN. Optical stimulation of cerebellar afferents in the vlPAG resulted in glutamatergic EPSCs (4.2 to 110 pA; mean 33.7 pA) in 6 of 32 cells unlabeled small cells, confirming that direct, though small, excitatory cerebello-PAG connections exist. To test whether the cerebellum exerts effects on the PAG through other means, we tested the effects of cerebellar activity on IPSCs. Electrically evoked IPSCs were 322.2±90.3 pA in control conditions. Interestingly, high frequency stimulation (25 Hz) of cerebellar afferents potentiated

electrically evoked IPSCs in 5 of 8 cells by 162.5±8.2%. Because disinhibition is thought to be a primary means of activating freezing responses in the vlPAG, these results suggest that the cerebellum may be well positioned to modulate freezing responses through both direct and modulatory effects on vlPAG circuitry.

Disclosures: C.E. Vaaga: None. **I.M. Raman:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

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R21 EY-020631
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P30 EY-019007

Title: Midlateral cerebellar purkinje neurons participate in visuomotor associative learning

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Abstract: The cerebellum has been primarily considered to have roles in motor learning and coordination. However, Recent clinical, anatomical and electrophysiological evidence suggest that the cerebellum has a role in cognition as well as in motor control. There is a growing consensus that visuomotor associative learning requires a network that includes the cerebellum, the prefrontal cortex, and the basal ganglia. To test this hypothesis at the level of single neuron activity, we trained Rhesus monkeys to associate well-learned left- and right hand movements with arbitrary visual symbols. We first studied the activity of midlateral cerebellar Purkinje cell simple spikes while the monkeys performed the visuomotor association task using familiar, overtrained symbols. 83% of neurons increased their activity during the hand-movement, and 17 % of neurons had no movement-related activity. We then changed the symbols to non vebalizable (by humans) fractal stimuli that the monkeys had never seen. The monkeys had to figurev out, by trial and error, which symbol was associated with which hand, which took them 20-60 trials. The kinematics of the movements did not differ before and after the symbol switch. After the symbol switch, both types of Purkinje neurons showed a global change in firing

activity, and both types of Purkinje neurons began to report the prior trial's outcome: simple spike activity differed between prior correct and prior wrong trials; but only in a particular epoch of the trial for each neuron. Across the population, the epochs tiled the whole trial period. The neurons reported the trial outcome independent of changes in reaction time, hand movement kinematics or laterality, visual symbol novelty and reward expectation. This activity was not merely a report of reward: the neurons did not signal when the monkey failed to receive a reward while performing the overtrained task. Our results suggests that that cerebellum's unique structure for learning is suited for a purely cognitive learning context as well as a motor learning context.

Disclosures: N. Sendhilnathan: None. **M.E. Goldberg:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

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Program #/Poster #: 583.05/LL10

Topic: E.02. Cerebellum

Support: NIH Grant NS-13742

Title: Computations in the cerebellar flocculus - Divide and conquer

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Abstract: In the rabbit's cerebellar flocculus both Type 1 and Type 2 mossy fiber responses to horizontal vestibular rotations are present in about equal numbers. Type 3 is present, but to a lesser degree. (Type 1 responses have activity increases for rotations to the recording (ipsilateral) side and/or decreases for rotations to the contralateral side. Type 2 responses have the oppositely directed activity changes, and Type 3 responses have activity increases for both rotation directions.) Curiously, the simple spike responses of horizontal Purkinje cells are virtually only Type 2. To address how this asymmetry may occur, we recorded from various neuron classes in the anesthetized rabbit's flocculus (mossy fibers, granule cells, basket/stellate cells, Purkinje cells, unipolar brush cells (UBCs), and Golgi cells). The rabbits were sinusoidally rotated in the light. Neurons were classified using our previous algorithm (Ruigrok et al., 2011). Of the recorded interneuron classes, UBCs and Golgi cells do not synapse directly on Purkinje cells, but rather their influence is embedded in the activity of those granule cells with which they synapse. We, thus, simplified the computations into two parts: those occurring through inner paths involving cells that do not synapse on Purkinje cells and those occurring through outer paths involving cells that do synapse on Purkinje cells. Granule cells rarely had significant background

activity, so their Type 1 or Type 2 responses consisted largely of only increased activity. Since the excitatory activity of specific granule cells combined with the inhibitory activity of specific molecular layer interneurons (basket/stellate cells) generates Purkinje cell activity, the Type 2 Purkinje cell activity may be most simply explained as dominance of Type 1 molecular layer interneuron activity in combination with Type 2 excitatory granule cell activity. Similarly, with a different subset of granule cells and molecular layer interneurons, Purkinje cell activity could be unmodulated, accounting for the near absence of Type 1 Purkinje cells. Another kind of asymmetry consisting of Purkinje cell responses to only ipsilateral rotations or to only contralateral rotations was unexpectedly present. A way for that behavior to occur can now be understood as a consequence of using only Type 2 granule cell activity or only Type 1 molecular layer interneuron activity. This study suggests that a general way to comprehend cerebellar computations may be, as described above for the flocculus, to divide interneuron activity into two anatomically based inner and outer paths and to focus on the diversity of granule cells and molecular layer interneurons.

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Poster

583. Cerebellum: Cortex and Nuclei I

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Program #/Poster #: 583.06/LL11

Topic: E.02. Cerebellum

Support: NIH (F31-NS095476)
NIH (R37-NS39395)
NIH (R01-NS067299)

Title: Synaptic responses and spiking of cerebellar output neurons in larval zebrafish during fictive swimming

Authors: *T. HARMON[1], D. L. MCLEAN[2], I. M. RAMAN[1]
[1]Neurobio., [2]Northwestern Univ., Evanston, IL

Abstract: Cerebellar output neurons receive excitatory drive related to motor commands, which is integrated with inhibition from Purkinje cells (Pkj). To test how these inputs interact during movements in larval zebrafish, we made whole-cell recordings from olig2+ eurydendroid neurons (ENs, homologous to glutamatergic cerebellar nuclear cells) while monitoring spontaneous and evoked fictive swimming with ventral root (VR) recording. Voltage clamp recordings revealed basal synaptic input in the form of parallel fiber EPSCs and Pkj IPSCs. During swimming, EPSCs and IPSCs both increased in rate and summated. Responses to

spontaneous and sensory-evoked swimming were different, however. For spontaneous swimming, EPSCs preceded the VR response (-25 ± 11 ms) and arrived before IPSCs (-9.2 ± 1 ms). For evoked swimming, both responses lagged the onset of the VR response (EPSC: 6.3 ± 3 ms, IPSC: 27 ± 8 ms). The amount of synaptic drive also differed, with evoked swimming associated with greater charge transfer (EPSC: 3.3 ± 0.6 nC, IPSC: 11.5 ± 2.3 nC) than spontaneous swimming (EPSC: 1.0 ± 0.3 nC, IPSC: 4.7 ± 0.9 nC). Current clamp recordings revealed that firing rates increased to comparable levels during spontaneous and evoked swimming. However, the onset of spiking closely matched the onset of spontaneous swimming and lagged evoked swimming (lag, 1 ± 8 ms, vs. 27 ± 8 ms). A subset of EN cells showed activity related to the motor burst cycle. Also, lateral ENs received greater and earlier Pkj input than medial cells. To further characterize Pkj inhibition of ENs, we analyzed spontaneous IPSCs and IPSCs recorded while optogenetically suppressing Pkj simple but not complex spikes. While the mean control amplitude of IPSCs was 26 ± 2 pA, the distribution was skewed toward larger values (40-60 pA). With simple spikes suppressed, mean amplitudes were similar (23 ± 2 pA), indicating that large IPSCs were not exclusively from presynaptic complex spikes. To estimate the Pkj-EN convergence, we compared the IPSC rates to rates of Pkj spiking. Basal IPSCs occurred at 13 ± 2 IPSCs/s, about twice the mean Pkj firing rate (~7 spk/s). Simple spike suppression led to a rate of 1.4 ± 0.3 IPSCs/s, ~5 times the complex spike rate (~0.3 spk/s). Thus, the Pkj-EN convergence ratio is likely between 2 and 5. These results demonstrate that cerebellar output neurons can receive distinct patterns of synaptic input, and that their responses differ according to whether swimming is sensory-evoked or spontaneous.

Disclosures: T. Harmon: None. **D.L. McLean:** None. **I.M. Raman:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.07/LL12

Topic: E.02. Cerebellum

Support: REP of Seattle Children's Research Institute

Title: Diversity of cellular morphology and physiology of Purkinje cells in the adult zebrafish cerebellum

Authors: *V. Z. HAN
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Abstract: This study was designed to explore the functional circuitry of the zebrafish cerebellum, with a focus on its Purkinje cells, using whole-cell patch recordings in slice preparations. Following physiological and pharmacological characterizations, the recorded cells

were labeled for morphological identification. It was found that the zebrafish Purkinje cells are surprisingly diverse .Based on their physiology and morphology, they can be classified into at least three subtypes: a *narrow spike cell* (Type 1), which fires only narrow Na^+ spikes (<2 ms in duration), and has a single primary dendrite with an arbor restricted to the distal molecular layer; a *broad spike cell* (Type II), which fires broad Ca^{2+} spikes (5-7 ms in duration) and has a primary dendrite with limited branching in the inner molecular layer and then further radiates throughout the molecular layer ; and a *very broad spike cell* (Type III), which fires very broad Ca^{2+} spikes (≥ 10 ms in duration) and has a dense proximal dendritic arbor that is either restricted to the inner molecular layer (Type IIIa), or radiates throughout the entire molecular layer (Type IIIb). The graded paired-pulse facilitation of these Purkinje cells' responses to parallel fiber activations are largely similar to those reported in mammals. However, two types of CF responses were observed: one a simple waveform and the other a complex-like one, with both all-or-none and paired-pulse depressed. The labeled axon terminals of these Purkinje cells end locally, as reported for other teleosts. The present study, for the first time, provides evidence that zebrafish Purkinje cells are remarkable diverse in their physiology and morphology, suggesting that the corresponding functional circuitry and information processing differ from what has been well-established in the mammalian cerebellum.

Disclosures: V.Z. Han: None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.08/DP08/LL13

Topic: E.02. Cerebellum

Support: Wellcome Trust Grant 535790

Title: Heterogeneous mossy fiber activity patterns and their implications for sensorimotor encoding in the cerebellar cortex

Authors: *H. ROS, S. SADEH, N. CAYCO-GAJIC, R. SILVER
Neuroscience, Physiol. and Pharmacol., Univ. Col. London, London, United Kingdom

Abstract: The brain gathers information about the body and the surrounding world, enabling it to build internal representations and to plan and execute movement. The cerebellum is thought to predict the sensory consequences of movements and coordinate movement by learning sensorimotor relationships. Cerebellar mossy fiber (MF) inputs convey a wide range of sensory and motor related information that is integrated by granule cells. But little is known about how populations of MFs encode sensory and motor signals locally within the input layer. To address this we used adeno-associated viruses to express the genetically encoded calcium indicator

GCaMP6f in distinct precerebellar nuclei, implanted a chronic window over Crus I/II and vermis of the cerebellar cortex and performed two-photon (2P) imaging of MFs in awake behaving mice. Since MF synaptic rosettes are sparsely distributed, we used high speed 3D 2P Acousto-Optic Lens (AOL) microscopy to record their activity within a 250 x 250 x 250 μm imaging volume. We observed a wide range of activity patterns across MFs, with individual MFs exhibiting either an increase or a decrease in activity with locomotion. Surprisingly, positively and negatively modulated MFs were often observed within the same local region (i.e. 10 - 100 μm), suggesting that individual GCs could be innervated by functionally opposed inputs that cancel out. Examining the spatio-temporal patterns of MF population activity and relating this to behaviour will allow us to identify how information from specific pathways is encoded.

Disclosures: H. Ros: None. **S. Sadeh:** None. **N. Cayco-Gajic:** None. **R. Silver:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.09/LL14

Topic: E.02. Cerebellum

Support: NIH Grant R37 NS39395
NIH Grant T32 MH067564

Title: Sensorimotor processing in the cerebellar corticonuclear circuit amplifies reflexive whisking via well-timed spiking

Authors: *S. BROWN, I. M. RAMAN
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Abstract: To test how cerebellar crus I/II Purkinje cells and their targets in the lateral cerebellar nuclei (CbN) integrate sensory and motor-related inputs and contribute to reflexive movements, we recorded extracellularly from awake, head-fixed mice during non-contact whisking. Air puffs to the whisker pad elicited changes in population instantaneous firing rates of Purkinje simple spikes, which matched and slightly preceded (~15 ms) the change in whisker position (~1 Hz/1 degree protraction) over the first few hundred milliseconds after the puff. Purkinje spike rates changed similarly whether the location of the air puff was on the ipsilateral or the contralateral side, suggesting little role for these responses in fine sensory discrimination. Responses also remained relatively unaffected when ipsilateral sensory feedback was removed by lidocaine. The later portion of the response was reduced by optogenetically inhibiting the reticular nuclei but not motor cortex, consistent with a motor-command-related signal. Optogenetically silencing cerebellar output suppressed movements by about 30%. CbN cell responses during puff-evoked whisking did not match whisker kinematics but showed only a brief elevation of firing rates in

the first 50 ms following the puff. Examination of spike timing during puff-evoked whisks demonstrated that both Purkinje and CbN cells generated well-timed spikes in sequential 2-4 ms windows at response onset, such that they alternately elevated their firing rates just before protraction. In contrast, with spontaneous whisks, which were smaller than puff-evoked whisks, although Purkinje cell spiking matched whisker kinematics, CbN cells were slightly inhibited (by ~5 spikes/sec), and well-timed spikes were absent from both Purkinje and CbN cells. Thus, sensory input can facilitate millisecond-scale well-timed spiking in Purkinje and CbN cells and permit cerebellar amplification of reflexive whisker movements.

Disclosures: S. Brown: None. **I.M. Raman:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.10/MM1

Topic: E.02. Cerebellum

Support: NSERC

Title: Lugaro cells in the avian cerebellum (?)

Authors: *D. R. WYLIE[1], I. CRACIUN[1], C. G. GUTIERREZ-IBANEZ[1], A. S. M. CHAN[1], H. LUKSCH[2]
[1]Neurosci. and Mental Hlth. Inst., Univ. of Alberta, Edmonton, AB, Canada; [2]Tech. Univ. of Munich, Freising, Germany

Abstract: Lugaro cells are inhibitory interneurons that reside in the upper granular layer of the cerebellar cortex, just below or within the Purkinje cell layer. They are characterized by: 1) a fusiform cell body oriented in the parasagittal plane; 2) two pairs of dendrites emanating from opposite ends of the cell body; 3) innervation from Purkinje cell axon collaterals; and 4) an axon that projects into the molecular layer and travels parallel to granular cell parallel fibers. Lugaro cells have previously been described in mammals, but not others vertebrate classes, save one report in a teleost fish. Here we propose the existence of Lugaro cells in the avian cerebellum based on the morphological characteristics and connectivity described above. Immunohistochemical staining for the calcium binding protein secretagogin, using an antigen retrieval protocol, revealed Lugaro-like cells in the pigeon cerebellum (see Figure). These cells exhibited fusiform somata and horizontally projecting dendrites when viewed in the parasagittal plane. We also observed long axons projecting deep into the molecular layer and turning sharply to travel alongside parallel fibers in the coronal plane. Immunohistochemisty to other molecular markers was explored, including calretinin, calbindin, and glutamic acid decarboxylase (GAD). While mammalian Lugaro cells are known to express calretinin, the secretagogin-labelled cells

in the pigeon did not. Additionally, secretagogin was not expressed in rat Lugaro cells. GAD was expressed in the pigeon secretagogin-labelled cells, confirming their inhibitory function. Calbindin labelling revealed Purkinje cell terminals surrounding the secretagogin-expressing cells (see Figure). Our results suggest that Lugaro cells are more wide spread among vertebrates than previously thought and may be a characteristic of the cerebellum of all vertebrates.

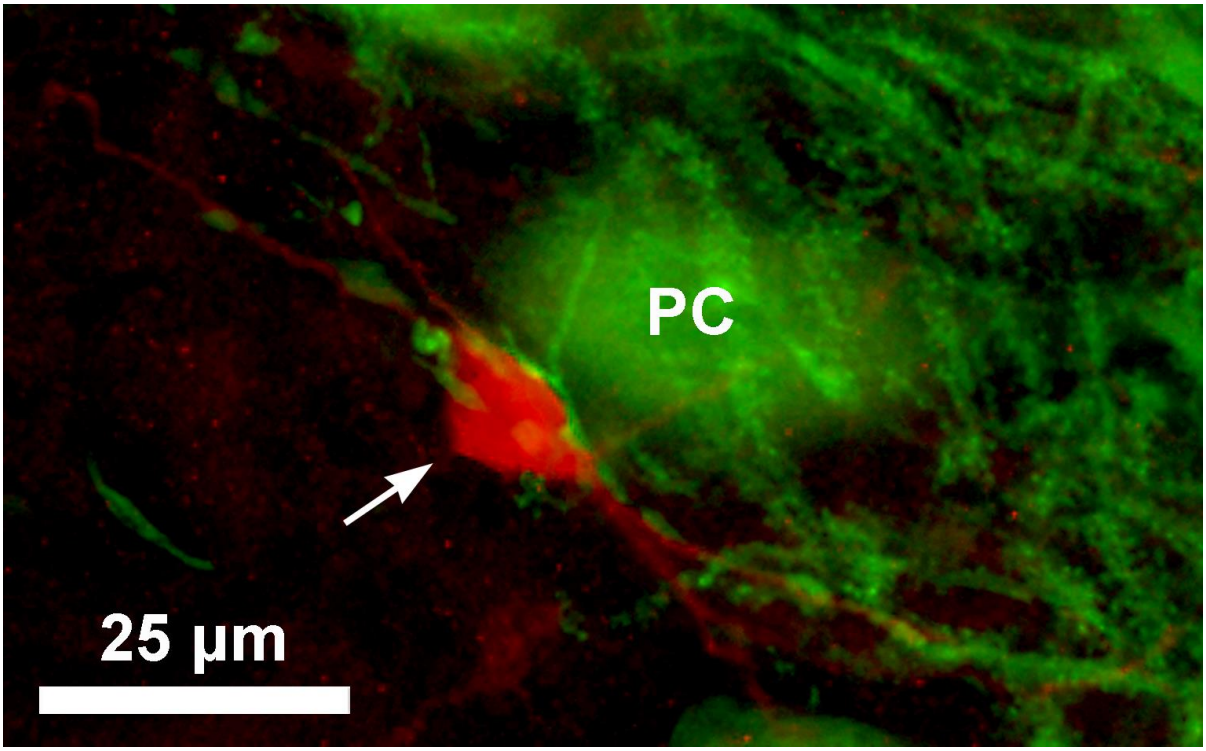

Figure: Secretagogin immunochemistry (red) reveals Lugaro-like cells (arrow) in the granular cell layer. Note the contacts from Calbindin +ve (green) varicosities, presumably from Purkinje cell (PC) collaterals.

Disclosures: D.R. Wylie: None. **I. Craciun:** None. **C.G. Gutierrez-Ibanez:** None. **A.S.M. Chan:** None. **H. Luksch:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.11/MM2

Topic: E.02. Cerebellum

Support: CONACYT (BALF 576171)
CUERPO ACADEMICO DE NEUROQUIMICA (UVCA304)

CUERPO ACADEMICO DE NEUROCIENCIAS (UVCA28)

Title: Activation of purkinje cells of the cerebellum during the appetitive andconsummatory phase of sexual behavior in the wistar male rat

Authors: *B. A. LARA[1], G. J. SANCHEZ[1], D. HERRERA[2], F. ROJAS[2], G. A. CORIA-AVILA[2], J. MANZO[2], R. TOLEDO-CARDENAS[2]
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Abstract: The cerebellum is a structure that has been associated with multiple functions, such as motor skills, learning processes, and sexual behavior, among others. The correlation that exists between cerebellum and sexual behavior has been described by different authors; in experiments performed in rats, it has been shown that there is an activation in the granular layer of the cerebellum during the execution of the behavior. However, the participation of Purkinje cells (Pk) is still unclear. Two behavioral paradigms were used to evaluate the participation of Pk cells during sexual behavior: 1) non-contact stimulation in presence of a receptive female (appetitive phase), and 2) the execution of one, two, three, and four ejaculations in the same session (consummatory phase). Sexually experienced adult male rats (300-350g) were used. For the treatment of cerebellar tissue, the immunohistochemical technique was performed using the c-Fos protein as a marker of cellular activation, and Calbindin (Cb) as a specific marker of Pk cells. Only the cells that showed colocalization were counted. The analysis of Pk cells was made in the apical and basal area of the 10 lobes of the cerebellar vermis. Results showed a significant increase in the number of Pk c-Fos Cb-ir cells during the appetitive phase of sexual behavior (receptive female) compared to the control group (non-receptive female). Regarding the consummatory phase, a significant increase in the number of Pk c-Fos Cb-ir cells was observed in the one ejaculation group compared to the control group. However, in the second and third ejaculation groups a decreased number of Pk c-Fos-Cb-ir cells was observed, that was significant only in the third ejaculation. However, in the fourth ejaculation, the immunoreactivity increased again, until reach the levels by the control group. The activation of the apical region of the lobes was significantly higher compared to the basal region. Based on the results, it is suggested that the activation of Pk cells in the appetitive phase, could indicate the preparation of the system to execute further patterns of sexual behavior when achieving the consummatory phase. The increase during the first ejaculation is maintained, suggesting an optimization in the activity of the cells of Pk so that the behavior can continue. Then, this activation decreases as the behavior is repeated in the next ejaculations of the same session. For the fourth ejaculation, the activation Pk cells increases again, suggesting that is a preparation to start another ejaculatory series.

Disclosures: B.A. Lara: None. **G.J. Sanchez:** None. **D. Herrera:** None. **F. Rojas:** None. **G.A. Coria-Avila:** None. **J. Manzo:** None. **R. Toledo-Cardenas:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.12/MM3

Topic: E.02. Cerebellum

Support: R01MH112143
R01NS089664
R01NS100874

Title: Cerebellar involvement in controlling the intrinsic variability of the respiratory rhythm in mice

Authors: Y. LIU[1], S. QI[1], R. V. SILLITOE[2], *D. H. HECK[1]
[1]Anat. & Neurobio., Univ. of Tennessee, Memphis, TN; [2]Pathology and Immunol., Baylor Col. of Med., Houston, TX

Abstract: The cerebellum has strong reciprocal connections with the brain stem in both rodents and primates [1, 2]. Investigations into the functional significance of these projections have linked the cerebellum to various brain stem controlled functions such as blood pressure regulation, cardiovascular and respiratory activity. However, those studies were mostly conducted in anesthetized or decerebrate conditions and thus did not provide information about what aspects of these brain-stem controlled behaviors the cerebellum controls in an awake behaving animal. Activity of neurons in the medial cerebellar nucleus has been shown to represent orofacial behaviors controlled by brain-stem pattern generators, including respiration, and neurons from the medial cerebellar nucleus project to brain-stem areas that contain the respiratory pattern generating circuits [3]. These projections provide a potential route for cerebellar modulation of respiratory activity. Here we asked what aspects of respiratory behavior the cerebellum might control by measuring respiratory behavior after genetically targeting the output of Purkinje cells. We used the Cre/LoxP approach to selectively block Purkinje cell GABAergic neurotransmission, thereby functionally disconnecting the cerebellar cortex from the cerebellar nuclei [4]. Respiratory behavior was monitored for 30 min by placing mice in a plethysmograph. Peak pressure changes related to inspiration were marked and the inspiration times were used for further analysis of different aspects of the respiratory rhythm, such as mean interval duration, coefficient of variation (CV), and intrinsic variability (CV2). The CV2 is represents the standard deviation of two adjacent inter-inspiration intervals. Our results show that loss of cerebellar Purkinje cell neurotransmission did not affect the average respiratory frequency or the CV of the respiratory rhythm. However, the mutant mice did show a significant decrease in CV2 of the respiratory rhythm ($p < 0.001$, t-test). A reduced CV2 in mutant mice signifies reduced local interval variability, indicating that influence from the intact cerebellum somehow

increases variability. This is consistent with the assumption that the intact cerebellum is involved in fine temporal control of the respiratory rhythm, which might be related to a proposed cerebellar involvement in the coordination of respiratory with other orofacial behaviors [3,5]. 1. Päällysaho et al., Neurosci. Res. 1991; 12: 217; 2. Asanuma et al., Brain Res 1983; 286(3): 299; 3. Lu et al., Front Neural Circuits 2013; 7:56; 4. White et al., J Neurosci 2014; 34(24): 8231; 5. Bryant et al., Eur J Neurosci 2010; 32(1): 41-52.

Disclosures: Y. Liu: None. **S. Qi:** None. **R.V. Sillitoe:** None. **D.H. Heck:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.13/MM4

Topic: E.02. Cerebellum

Support: National Natural Science Foundation of China (31321091, 91332203 and 31490591)

Title: The cellular mechanism of Prrt2-associated paroxysmal dystonia

Authors: *B. LU[1], Z.-Q. XIONG[2]
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Abstract: Paroxysmal dyskinesia is a brain disorder characterized by sudden attacks of involuntary movements. Paroxysmal kinesigenic dyskinesia (PKD), a subtype of paroxysmal dyskinesia, was caused by loss-of-function mutations in PRRT2 gene. In our previous study, genetically engineered animal models of PKD has been generated. However, how does the dysfunction of PRRT2 contribute to stimulus-triggered dystonia is still not fully understood. In this study, we found dystonia was closely accompanied with hyperexcitability in cerebellum of Prrt2-mutant mice. By monitoring the activity of population neurons with electrophysiological recording, we found that optical stimulation induced more extended excitability in cerebellum of Prrt2-mutant mice compared to which in wild type mice. The carbamazepine, an effective medicine for preventing PKD attack in clinical, reduced cerebellar excitability and alleviated dystonia attack in animal model of PKD. Together, our findings provided persuasive evidence for the hypothesis that cerebellar hyperexcitability might be an underlying neuropathological mechanism in Prrt2-associated paroxysmal dyskinesia.

Disclosures: B. Lu: None. **Z. Xiong:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.14/MM5

Topic: E.02. Cerebellum

Support: JSPS KAKENHI 16K070025

Title: Cerebellar modules in the olivo-cortico-nuclear loop labeled by pcdh10 expression in the adult mouse

Authors: G. A. SARPONG[1], S. VIBULYASECK[1], Y. LUO[1], M. S. BISWAS[1], H. FUJITA[2], S. HIRANO[3], *I. SUGIHARA[1]
[1]Neurophysiol., Tokyo Med. & Dent. Univ., Tokyo, Japan; [2]Otolaryngology-Head and Neck Surgery, Johns Hopkins Univ., Baltimore, MD; [3]Lab. Cell Biol., Kansai Med. Univ., Hirakata City, Japan

Abstract: Topographic connection between corresponding compartments of the cerebellar cortex, cerebellar nuclei and inferior olive forms parallel cerebellar modules, which are essential for the cerebellar function. Compared to the striped cortical compartments which are labeled by molecular markers such as aldolase C or zebrin II, the supposedly-corresponding nuclear of olivary compartmentalization has not been much clarified. Some members of the cadherin family of cell adhesion molecules are expressed in subdivisions of the cerebellar cortex, cerebellar nuclei and inferior olive, implying their involvement in topographic axonal connection. We previously clarified expression of *pcdh10*, which encodes protocadherin 10 protein, in embryonic Purkinje cell subsets by using *pcdh10-lacZ* knock-in mice. Here, we focused on the expression pattern of *pcdh10* in the adult mouse to compare it with aldolase C stripes, the standard marker of molecular compartments of the cerebellar cortex. In the cerebellar cortex *pcdh10* was strongly expressed in (1) aldolase C positive vermal stripes a+//2+ in lobules VI-VII, and (2) paravermal narrow stripes c+, d+, 4b+, 5a+ in crus I and neighboring lobules and (3) paravermal stripes 4+//5+ across all lobules from lobule III to paraflocculus, areas less involved in somatomotor function. In the cerebellar nuclei, *pcdh10* was enriched in the caudal part of the medial and posterior interposed nuclei which project less to the medulla or to the red nucleus than to other metencephalic, mesencephalic and diencephalic areas. In the inferior olive, *pcdh10* was enriched in the rostral and medioventrocaudal parts of the medial accessory olive which have connection with the mesencephalic areas rather than the spinal cord. Axonal labeling experiments confirmed that the three cortical *pcdh10*-positive areas were topographically connected to the nuclear and olivary *pcdh10*-positive areas. This showed that these *pcdh10*-positive areas coincide with modular structures in the olivo-cortico-nuclear loop. We speculate that these modules are

functionally involved in various non-somatomotor functions through their afferent and efferent connections.

Disclosures: G.A. Sarpong: None. **S. Vibulyaseck:** None. **Y. Luo:** None. **M.S. Biswas:** None. **H. Fujita:** None. **S. Hirano:** None. **I. Sugihara:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.15/MM6

Topic: E.02. Cerebellum

Support: ERC-2014-STG 640093

Title: Spatial and temporal locomotor learning in mouse cerebellum

Authors: D. M. DARMOHRAY, J. R. JACOBS, *M. R. CAREY
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Abstract: Stable and efficient locomotion requires precise coordination of movements across the body. Learned changes in locomotor patterns can be induced by exposure to a split-belt treadmill that imposes different speeds on the limbs on each side of body. We developed a transparent split-belt treadmill for mice that provides quantitative readouts of locomotor behavior in order to study the neural circuit mechanisms underlying this form of motor learning. Here we show that mice adapt to split-belt walking in a way that is remarkably similar to humans. Like human learning, mouse locomotor adaptation is specific to measures of interlimb coordination, has spatial and temporal components that adapt at different rates, and is highly context-specific. Further, split-belt adaptation in mice is dependent on an intact cerebellum, but insensitive to large lesions of cerebral cortex. To begin to narrow down the potential sites of plasticity underlying locomotor adaptation, we targeted inhibitory DREADDs to Purkinje cells projecting to each of the three distinct deep cerebellar nuclei. Using this chemogenetic approach, we identified a subregion of the cerebellum that is necessary for this form of locomotor learning. Consistent with predictions from our interlimb coordination analyses, this region shows differential lateralization for spatial and temporal aspects of locomotor adaptation. These findings provide a starting point for a circuit-level model for how movements of four independent limbs are coordinated and maintained during locomotion.

Disclosures: D.M. Darmohray: None. **J.R. Jacobs:** None. **M.R. Carey:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

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Program #/Poster #: 583.16/MM7

Topic: E.02. Cerebellum

Support: CONACyT (LVC) 575913
CONACyT (JRGP) 595412
BECA-SNI-MGRL, CA-28-Neurociencias

Title: Changes in the pattern of multiunit activity in cerebellum after electrolytic lesion of the ventrolateral striatum

Authors: *L. VASQUEZ CELAYA[1], J. R. GUTIÉRREZ PÉREZ[1], M. G. ROCHA[2], C. GONZÁLEZ[2], P. CARRILLO[3], G. A. CORIA ÁVILA[4], J. MANZO DENES[4], M. MIQUEL[5], L. I. GARCIA[4]
[1]Doctorado en Investigaciones Cerebrales, UV, Xalapa, Mexico; [2]Facultad de Bioanálisis, Univ. Veracruzana, Xalapa, Mexico; [3]Inst. de Neutoetología, Univ. Veracruzana, XALAPA, Mexico; [4]Ctr. de Investigaciones Cerebrales, Xalapa, Mexico; [5]Jaume I Univ., Castellon, Spain

Abstract: Studies in patients with Parkinson's disease (PD) have shown that cerebellar function is modified by an alteration in basal ganglia. This suggests an anatomical and functional relationship and would imply a possible compensatory mechanism for the dysfunction of the cortico-basal circuits. These observations support the idea that both structures function as an integrated system. In the present study, we altered the ventrolateral striatum (VLS) of the basal ganglia with an electrolytic lesion in male Wistar rats (250 and 300 gr). Multiunit activity (MUA) recordings were made in cerebellum. Rats were divided into three groups, a control, a sham which also an electrode descended in the VLS and the lesion group to which an electrolyte lesion was performed (3.5 mV / 30 s) in the VLS. In each of these three groups, three subgroups were formed (Sim b, Crus II lobes, and dentate nucleus) according to the structure where MUA was registered. Thus, all animals were independent between groups and structures. The aim was to analyze and determine the effect of the electrolytic lesion of the VLS on the MUA of the granular neurons of the cerebellum. In all the groups the basal activity and the maximum amplitude reached during the mandibular tremor caused by the lesion were analyzed. The results show differences in the Crus II lobe and in the dentate nucleus during the recording of basal activity. In both the sham and the lesion group decreased the amplitude compare to the control group. The mandibular tremor was observed in the lesion group as expected, surprisingly the rats of the Sham group also showed the behavioral pattern of mandibular tremor and bursts during AMU registration. Our results confirm a role of the cerebellum in the alteration of the

ventrolateral striatum. Further studies are needed in the sham group as this could be proposed as an acute model of parkinsonism in the rat.

Disclosures: L. Vasquez celaya: None. **J.R. Gutiérrez Pérez:** None. **M.G. Rocha:** None. **C. González:** None. **P. Carrillo:** None. **G.A. Coria Ávila:** None. **J. Manzo Denes:** None. **M. Miquel:** None. **L.I. Garcia:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.17/MM8

Topic: E.02. Cerebellum

Support: CIHR FRN-143320
NIH NIDCD grants R01-DC002390

Title: The activity of Purkinje cells in the vestibular cerebellum during active versus passive rotational head movements

Authors: *O. ZOBEIRI, K. E. CULLEN
Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: The cerebellum, a structure that is well-conserved across vertebrates, is thought to contain a forward model that predicts the sensory consequences of self-generated movement. Comparing this prediction with the actual sensory feedback, allows the brain to distinguish sensory inputs that are the consequence of active self-generated versus passive externally-generated movements (i.e., sensory reafference versus exafference). Previous studies by our group have shown that neurons in the vestibular nuclei as well as most medial of the deep cerebellar nuclei - the rostral fastigial nucleus (rFN) - are significantly more sensitive to passively-applied than actively generated vestibular stimulation. However, the neural mechanism underlying the cancellation of vestibular reafference is unknown. Accordingly, here, to investigate the neuronal basis of vestibular reafferent suppression, we recorded from Purkinje cells in the Nodulus and Uvula of the vestibular cerebellum. Single unit extracellular recording were made in rhesus monkeys during comparable active and passive head rotational movements, and Purkinje cell simple spikes were detected via a semi-automated clustering algorithm. Our analysis of responses during passive motion first revealed robust simple spike responses to head motion that were either bidirectional or unidirectional. Next, comparison of neuronal responses during passive and active head rotations, demonstrated that simple spike responses were markedly attenuated (~60%) across our population of Purkinje cells during active versus passive rotations. We hypothesized that these neurons might sum a neck motor-derived (e.g., efference copy) signal with the vestibular input to cancel vestibular reafference. To address this possibility,

we measured neuronal responses while monkeys attempted to make gaze shifts between two targets but their heads were restrained. In this condition, monkeys produced large neck torques, signifying the generation of motor commands comparable to those generated during active head movements. Consistent with our hypothesis, we found that cells that were sensitive to rotational head velocity, also responded when the monkey generated a motor command to move the head as measured by a torque sensor. Thus, taken together, these results provide new insights into the computations preformed by Purkinje cells in Nodulus/Uvula that underlie the cancellation of vestibular reafference.

Disclosures: O. Zobeiri: None. **K.E. Cullen:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.18/MM9

Topic: E.02. Cerebellum

Support: Wellcome trust

Title: M1 and cerebellar responses to spatial and temporal perturbations during visuomotor tracking

Authors: *W. XU, A. JACKSON
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Abstract: The cerebellum is essential for controlling coordinated movements. Internal forward models in the cerebellum are thought to enable smooth and accurate movements in the face of slow and noisy feedback signals by predicting the expected outcome of movements and signalling discrepancies with actual consequences. We explored the neural implementation of such internal models by simultaneously recording from the primary motor cortex and the contralateral cerebellar cortex in a macaque performing a visually guided isometric wrist torque tracking task. 52 putative cerebellar Purkinje cells and 37 M1 neurons formed the dataset. Neurons were recorded using custom-made flexible tungsten wire electrodes designed to enable long-term stable single-unit recordings without the need for head fixation. The monkey had to follow a target that moved from a central torque-neutral position to the periphery and then returned to the central position. We found that whereas M1 neuronal firing largely represented the force produced (monophasic response profile with peak firing rates during the increase in wrist torque), cerebellar Purkinje cell simple spike firing better represented the magnitude of the disparity between target and current states (having a peak in firing both when the target moved to the periphery and when it returned to the torque-neutral home position). Moreover, the initial peak in M1 firing preceded that of cerebellar firing by around 20ms - compatible with the

cerebellum receiving an efference copy of the motor command from M1. We also found that both M1 and cerebellar neurons responded similarly (with an increase in firing) to sudden perturbations that required an abrupt increase in wrist torque irrespective of whether the cursor was abruptly moved away from the target or the target was abruptly moved away from the cursor. This reinforces the notion that cerebellar neurons encode the disparity between current state and desired state rather than the state of either the cursor or target position separately. In a second experiment, we introduced time delays (200 to 600ms) between the wrist torque and the cursor position on screen. This introduced delay-specific changes to both M1 and cerebellar firing rate profiles. In particular, elevated firing of Purkinje cells occurred at a time consistent with the maximal discrepancy between expected and actual visual feedback. These results are consistent with the cerebellum acting as a forward model representing that compares expected and actual movement outcomes whilst taking into account delays in sensory feedback.

Disclosures: W. Xu: None. **A. Jackson:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.19/MM10

Topic: E.02. Cerebellum

Support: KAKENHI 17H03543
KAKENHI 17H06313

Title: Organization of the functional inputs from the sensorimotor cortex to the cerebellum revealed by transcranial optogenetic mapping

Authors: *M. CHOO[1,3], R. HIRA[4], M. MATSUZAKI[2,4], K. IKEZOE[3], G. J. AUGUSTINE[5], M. KANO[1], K. KITAMURA[1,3]
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Abstract: The cerebellum receives information from the neocortex, which is crucial for execution and learning of skilled movements. Such information is thought to be particularly important for the operation of cerebellar internal models. Although the structure and function of the cerebro-cerebellar communication loop have been extensively studied in various species from rodents to primates, details of functional connectivity between the two brain structures are still largely unknown.
To comprehensively examine the functional inputs from the sensorimotor cortex to the

cerebellum, we performed cell-attached recordings from single Purkinje cells in various cerebellar lobules (lobule VI to VIII in the vermis, and lobule simplex, crus I and II, paramedian lobule, and copula pyramids in the hemisphere) while layer 5 (L5) pyramidal neurons located at various areas of the cerebral cortex were optogenetically stimulated in Thy1-ChR2-EYFP mice. Photostimulation of L5 pyramidal neurons reliably evoked simple and complex spikes in Purkinje cells, which represent mossy and climbing fiber inputs to the cerebellar cortex, respectively. For each recorded Purkinje cell, changes in simple spikes and complex spikes were plotted separately along the location of photostimulation in order to obtain a map of mossy and climbing fiber inputs from the sensorimotor cortex to single Purkinje cells.
Consistent with previous studies, inputs from the cerebral cortex to the cerebellum via mossy and climbing fibers are largely overlapped. However, the simple and complex spike maps show different patterns depending on the lobules such that each lobule receives spatiotemporally distinct inputs from the motor, sensory and association areas of the cerebral cortex. On average, inputs from the primary areas (S1 and M1) are mainly contralateral whereas those from the higher-order area (M2) are bilateral. However, the laterality is variable at single cell level even in the same lobule, and dependent on the medio-lateral position, i. e., zones where PCs are located. These results suggest that different aspects of information transferred from these cortical areas are integrated in specific cerebellar lobules and zones to form output patterns of simple and complex spikes, which may contribute to accurate coordination of movements.

Disclosures: M. Choo: None. **R. Hira:** None. **M. Matsuzaki:** None. **K. Ikezoe:** None. **G.J. Augustine:** None. **M. Kano:** None. **K. Kitamura:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.20/MM11

Topic: E.02. Cerebellum

Support: NIH Grant EY11027
NIH Grant NS095232

Title: The cerebellar vermis modulates activity in the prefrontal cortex

Authors: *H. FUJITA, T. KODAMA, S. DU LAC
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Abstract: Cerebellar involvement in cognitive functions and mental disorders such as autism and schizophrenia is increasingly evident from clinical and imaging studies. In particular, lesions in the posterior part of the cerebellar vermis lead to impaired executive function (planning, set-shifting, abstract reasoning, verbal fluency, working memory), which have been historically

attributed to the medial prefrontal cortex (mPFC). Inspired by reports of anatomical and functional connectivity between the vermis and prefrontal cortex (Kyuho and Kawaguchi, 1985; Steriade, 1995; Kelly and Strick, 2003; Watson et al., 2014), we examined neuronal activities in the mPFC in response to optogenetic stimulation of the posterior vermis in awake behaving mice. We found that brief (10s of msec) inhibition of Purkinje cells in vermal lobule VII can robustly excite or inhibit mPFC neurons with minimal latency of ~25 ms. Despite the polysynaptic nature of the circuits linking the posterior vermis and mPFC, response onset latency to the cerebellar stimulation was well preserved across trials, indicating temporal precision of cerebellar control over the mPFC. To anatomically investigate neural substrates mediating cerebellar activity to the mPFC, we made double tracer injection into the mPFC and the fastigial nucleus, the main vermal output nucleus to retrogradely label mPFC-projecting neurons and to anterogradely label fastigial axons. We found major overlap of signals from the two tracers in the VM and MD thalamic nuclei and sparser overlap in the brainstem reticular formation. These results suggest that brief disinhibition of fastigial neurons can robustly modulate activity in the mPFC in heterogeneous ways to subserve distinct aspects of executive function.

Disclosures: H. Fujita: None. **T. Kodama:** None. **S. du Lac:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.21/MM12

Topic: E.02. Cerebellum

Support: NIH NS099577
NSF CBET-1631912

Title: Cerebellar granule cells acquire a predictive neural signal in the go-no go associative learning task

Authors: *M. MA[1], G. FUTIA[2], B. OZBAY[3], E. GIBSON[5], D. RESTREPO[4]
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Abstract: Previous studies have shown that cerebellar granule cells carry a predictive signal for motor action and the expectation of reward. However how these cerebellar input neurons respond in the associative learning in self-initiated go no-go odorant discrimination task has not been investigated in awake behaving animals.
To answer this question, we selectively expressed the Ca2+ sensor GCaMP6s in granule cells of cerebellum to enable the neuron activity detection. We performed head-fixed two-photon

imaging to perform large ensemble recordings of Ca2+ during behavioral training. The water-deprived mouse was trained with two odors to perform the go no-go task and neural activity of 100-200 granule cells was recorded simultaneously with two-photon imaging. To explore the possible roles of granule cells in associative learning, we collected data from mice learning to discriminate two odorants (forward training session) and we switched the rewarded odorant (reverse session). We also recorded licking and monitored movements though video. Information content of the neural responses was evaluated through perceptron analysis. Initially, a large fraction of granule cells responded similarly to both the rewarded (S+) and the unrewarded odorant (S-). Ca2+ increased following odorant delivery and decreased before the end of odor application and delivery of reinforcement. As learning progressed, the majority of recorded granule cells developed differential responses to S+ and S- trials. In proficient S+ trials, Ca2+ increased before and reached the peak after odor onset, and was maintained several seconds after delivery of reward. In S- trials, the neurons responded similarly before odor onset, but the response reached peak faster than S+ trials. Perceptron analysis of the activity of all neurons in the field classified the odorants correctly shortly after odorant onset. We then performed reverse training with the same animal and interestingly we found that the granule cells maintain the responses to odor values for the forward training in the first few trials, and the reversed task perceptron analysis differentiated between the odorants only after delivery of the reward. After the animals learned to discriminate the odorants in the reversed session the response to the rewarded and unrewarded odorants was re-established and perceptron analysis was able to discriminate between odorants shortly after odor onset.
Currently we are in the process of analyzing the relationship of the Ca2+ responses to motor actions and ultimately we want to figure out how the information stored at cerebellar granule cells contributes to behavioral responses.

Disclosures: M. Ma: None. **G. Futia:** None. **B. Ozbay:** None. **E. Gibson:** None. **D. Restrepo:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.22/MM13

Topic: E.02. Cerebellum

Title: Occupancy of sigma-1 receptors- A mass spectrophotometry based assay

Authors: *J. B. THENTU[1], K. BANDARU[2], G. BHYRAPUNENI[2], R. DYAVARASHETTY[2], A. MOHAMMED[3], R. ALETI[3], N. PADALA[3], D. AJJALA[3], R. NIROGI[3]
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Abstract: The use of liquid chromatography coupled with mass spectrometry (LC-MS/MS) is advantageous in in-vivo receptor occupancy assays at pre-clinical drug developmental stages. Relatively, its application is beneficial in terms of high throughput, data reproducibility, sensitivity, and sample processing. In this perspective, we have evaluated the use of FTC-146 as a non-radiolabelled tracer to determine the sigma-1 receptor occupancy of test drugs in mice brain. Further, the brain and plasma exposures of test drug were determined at their corresponding occupancies. In this occupancy method, primary study conditions like sacrification time after intravenous administration of tracer, dose of tracer, and specific brain regions were optimized during initial evaluation. Mice were pre-treated orally with SA4503, fluspidine, haloperidol, and donepezil followed by tracer treatment. In this occupancy assay, SA4503 was used as positive control for the derivation of relative occupancies. There was a dose-dependent decrease in brain regional FTC-146 binding in pre-treated mice. From the occupancy curves of SA4503, fluspidine, haloperidol, and donepezil the ED_{50} values in specific brain regions were observed to be in the range of 0.74-1.45, 0.09-0.11, 0.11-0.12, and 0.07-0.09 mg/kg, respectively. Brain regional distribution and binding inhibition upon pre-treatment were comparable to data reported with labeled [18F]FTC-146. Brain and plasma exposures of test compound were determined and correlated with corresponding sigma-1 occupancy from the same experiment. Their corresponding brain EC_{50} values are 74.3-132.5, 3.4-3.7, 122.5-139.5, and 8.8-11.0 ng/g and plasma EC_{50} values are 34.3-53.7, 0.08-0.10, 7.8-9.5, and 0.6-0.7ng/mL. With this mass spectrometry based assay, a wide category of drugs can be screened for sigma-1 receptor engagement along with their correlation to exposures can be derived which will aid in selecting suitable clinical doses.

Disclosures: J.B. Thentu: A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **K. Bandaru:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **G. Bhyrapuneni:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Dyavarashetty:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **A. Mohammed:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Aleti:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **N. Padala:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **D. Ajjala:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Nirogi:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.23/MM14

Topic: E.02. Cerebellum

Support: This work is supported by a GRF grant from the Research Grant Council of the Hong Kong Special Administrative Region Government (CityU 11100015)

Title: Alternation of cortical synaptic plasticity delays peripheral nervous system regeneration

Authors: *L. H. WEERASINGHE ARACHCHIGE[1], K. K. SINGH[1], G. KUMAR[1], P. ASTHANA[1], W. Y. TAM[1], K. M. KWAN[2], C. H. E. MA[1,3]
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Abstract: Axonal regrowth after lesion to the central nervous system (CNS) usually fails because of the limited capacity of CNS regeneration. In contrast, peripheral nervous system (PNS) axons readily regenerate after injury to form functional synapses with target muscle within a critical time period of 35 days in mice. Our previous work showed that motor function recovery was observed only if regenerating axons arrived at the target muscle within the critical time period. There is no doubt that damages in the PNS alter somatosensory cortex activities; however, the study on cortical reorganization is very limited. Cerebellum functions as a movement coordination centre for voluntary motor control. Purkinje cells are the major output neuron for fine-tuning motor activity in the cerebellar cortex. Here, we showed that motor functional recovery was delayed in a conditional knockout mouse with ablation of a transcription factor specifically in mature PCs after sciatic nerve crush. We established a mouse model to study critical period by performing repeated sciatic nerve crushes to prevent regenerating axons from reaching target muscle at specific period of time. Our preliminary data demonstrated that critical period was shortened significantly in mutant mice in terms of motor function recovery assessed by animal behavioral tests, electrophysiology and histology studies. Mutant mice exhibited reduction of toe spreading reflex, along with a decrease in evoked compound muscle action potential and neuromuscular junction formation in target muscle. We believe that current study will provide new insight into the development of neuroprosthetics and neurorehabilitation strategies for treating traumatic PNS injuries.

Disclosures: L.H. Weerasinghe arachchige: None. **K.K. Singh:** None. **G. Kumar:** None. **P. Asthana:** None. **W.Y. Tam:** None. **K.M. Kwan:** None. **C.H.E. Ma:** None.

Poster

583. Cerebellum: Cortex and Nuclei I

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 583.24/NN1

Topic: E.02. Cerebellum

Support: NMRC/CBRG/0075/2014

Title: Control of skilled reach through modulation of specific forelimb muscles by the IntA nucleus of the cerebellum

Authors: *A. R. THANAWALLA[1], A. I. CHEN[2]
[1]Sch. of Biol. Sci., [2]Nanyang Technological Univ., Singapore, Singapore

Abstract: The final output of the cerebellum, the deep cerebellar nuclei (DCN) have the ability to influence movement through connections with the spinal cord and motor cortex. The recent identification of a mouse line which permits recombination in specific neurons of the DCN provides an opportunity to manipulate these neurons and explore their functional relevance. To investigate the influence of glutamatergic projection neurons of the IntA nucleus on the muscular system, we performed EMG recordings in mice that express ChR2 in a subpopulation of IntA neurons, *Ucn3*::Cre recombined neurons. Through optogenetic manipulation of these neurons during a skilled reaching behavioral paradigm, we found that IntA^{Ucn3}neurons have the ability to change the EMG profile of selective forelimb muscles. Furthermore, we explored the temporal nature of these effects and found that IntA^{Ucn3}neurons exert their influence within a specific time window during the reaching movement. Our results show that IntA^{Ucn3}neurons control discrete movement through specific forelimb muscles in the context of skilled reaching. These results provide additional evidence supporting the functional specificity of neuronal subpopulations in the DCN.

Disclosures: A.R. Thanawalla: None. **A.I. Chen:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 584.01/NN2

Topic: E.02. Cerebellum

Support: Korea Institute of Science and Technology Institutional Program No. 2E27850
National Research Foundation of Korea Grant No. 2016008165

Title: Novel substructure in input layer of cerebellar cortex depending on projection of granule cell

Authors: *T. KIM, Y. YAMAMOTO, K. TANAKA-YAMAMOTO
KIST, Seoul, Korea, Republic of

Abstract: The input layer of cerebellar cortex processes various inputs through the synaptic connections between mossy fibers (MFs) and granule cells (GCs), and relays the refined inputs to Purkinje cell via GC axons, parallel fibers (PF). Thus the MF-GC organization is one of essential factors to fully understand cerebellar network. However due to the massive number of

cells and synapses within small volume of the input layer, the studies have been restricted to local unit network, and global organization have been assumed to be simply the repetition of the units. Furthermore, because no attempts have been performed to classify GCs, variability in basic properties of GCs or synaptic connections was assumed to be originated from simple randomness. On the other hand, our recent development of labeling technique enabled the clear dissection of a group of GCs which stretch their PFs at similar distances from Purkinje cell layer. We applied this technique to label two separate groups of GCs with different fluorescent molecules, and analyzed acquired images of the input layer by custom-built program. We then calculated the ratios of two groups of GC dendrites connecting to individual MF terminals, and tested how the ratios are different according to the distance of two PF bundles. To interpret analyzed results, computational network models of three possible distribution patterns of MF-GC connections were generated. The comparison of experimental results with model results revealed the tendency of biased chance to make MF-GC synapses for the same groups of GCs distinguished by our labeling technique. In addition, network model that could mimic experimental results was utilized to simulate activity transfer from MFs to PFs through the MF-GC connections. The simulation suggested that the organized network formation rather than random formation would have advantage to convey spatially distinct patterned input at molecular layer. Based on the results from the model, in this presentation, we claim that network organization of input layer by GCs and MF terminals needs to be considered to have GC's projection dependent substructure unlike the assumptions so far.

Disclosures: T. Kim: None. **Y. Yamamoto:** None. **K. Tanaka-Yamamoto:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

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Program #/Poster #: 584.02/NN3

Topic: E.02. Cerebellum

Support: Korea Institute of Science and Technology Institutional Program (Project No., 2E27850)
National Research Foundation of Korea (NRF) grant funded by the Korean Ministry of Education, Science and Technology (NRF grant No., 2016008165)

Title: Role of the organized formation of parallel fibers during the cerebellar development

Authors: *H.-Y. PARK[1,2], T. KIM[1], Y. YAMAMOTO[1], K. TANAKA-YAMAMOTO[1,2]
[1]KIST, Seoul, Korea, Republic of; [2]Div. of Bio-Medical Sci. and Technol., Korea Univ. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: Neural activity is generally believed to play an essential role in the network formation. During the postnatal development of cerebellar cortex, granule cells migrate and they form their axons, parallel fibers (PFs). Coincidentally, Purkinje cells (PCs) extend their dendrites to the molecular layer (ML) where PFs are being made. Considering that PFs act as a presynaptic input to PCs through temporally and spatially overlapped development, it raises a hypothesis that the PF activity would affect the development of cerebellar network. However, it is not yet clear whether the activity of the sequentially formed PFs functions on the cerebellar development. To address this question, we utilized a new method using the adeno-associated virus (AAV) that allows us to express molecules in only a bundle of developing PFs, instead of all PFs. We injected this AAV expressing tetanus toxin (TeTx) in a lobule 4/5 of mouse cerebellar cortex and investigated the effects of blockade of neurotransmitter release from a bundle of PFs on the development of the cerebellar network. The blockade led to the reduction of PC viability, represented by reduced intensity of calbindin staining, the reduction of PC dendrite complexity, and the decrease in the molecular layer thickness. The abnormal PC properties were accompanied by the motor dysfunctions. We also found a partial increase in the density of molecular interneurons, which are developed around the time when PC dendrites and PFs are extending. In addition, innervation of another inputs onto PCs, climbing fibers (CFs), was impaired, as expected from the studies showing that PF inputs are required for normal CF innervation. Thus, the inputs from sequentially organized PFs seem to be necessary for the network development and functions of the cerebellum. We further found that severity of impairments was varied depending on the location and timing of PF bundles expressing TeTx, suggesting that there would be critical periods of PF inputs for the cerebellar development. Interestingly, the abnormal cerebellar networks and motor dysfunctions were observed even after TeTx was no longer expressed in adulthood, indicating that the impairment during the development is irreversible. Based on these results, we conclude that the spatially and temporally organized formation of PFs and their inputs are crucial for the cerebellar development.

Disclosures: H. Park: None. **T. Kim:** None. **Y. Yamamoto:** None. **K. Tanaka-Yamamoto:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 584.03/NN4

Topic: E.02. Cerebellum

Support: the Korea Institute of Science and Technology Institutional Program (Project No., 2E27850)
the National Research Foundation of Korea (NRF) grant funded by the Korean Ministry of Education, Science and Technology (NRF grant No., 2016008165)

Title: Projection-dependent labeling of cerebellar granule cells

Authors: *Y. YAMAMOTO, T. KIM, K. TANAKA-YAMAMOTO
KIST, Seoul, Korea, Republic of

Abstract: Cerebellar granule cells (GCs) compose approximately half of all neurons in brain, and have unique morphological features. Although the morphology of individual cerebellar GCs have been long known, the network structure of GCs associated with their projection areas through parallel fibers (PFs) have been overlooked, owing to the very large number of GCs that prevent their systematic analysis. One way to address this issue is to dissect specific groups of GCs according to the location of their PFs. Our newly developed adeno-associated viral (AAV) vector triggered sufficient molecular expression in a portion of GCs, which are postmitotic but immature at the time of viral injection. This property of the AAV enabled us to label only a particular group of PFs and their GC somas by the stereotaxic injection at a certain time during postnatal development. The systematic injection at different developmental time points resulted in the labeling of different bundles of PFs, and confirmed that earlier-born GCs have PFs in the deeper layer, whereas later-born GCs have PFs in the more superficial layer. Thus, we established a technique that is capable of achieving projection-dependent dissection of cerebellar GCs. By using this technique, we attempted to examine the three dimensional spatial relationship between the PF locations in the molecular layer and the distribution of their GC somas in the GC layer. When a group of GCs was labeled by the AAV expressing GFP, GFP-positive GC somas were not clustered but were dispersed throughout the GC layer of sagittal slices. It has been demonstrated that Purkinje cells expressing some molecules, such as zebrin, or the innervation patterns of climbing fibers, as well as mossy fibers are localized in certain transverse zones. However, transverse slices did not show any zonal stripes of the GFP-positive GCs in the GC layer, whereas they have the GFP-positive PF bundles in the molecular layer. These results indicate that GC somas are randomly distributed in any axis regardless of their PF projections. Our technique is further applicable for the broad range of investigation, such as comparing structural or functional properties of GCs by performing double or triple labeling of different groups of GCs, or testing the effects of activity modulation in a group of GCs and PFs by expressing modulatory molecules in these GCs.

Disclosures: Y. Yamamoto: None. **T. Kim:** None. **K. Tanaka-Yamamoto:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

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Program #/Poster #: 584.04/NN5

Topic: E.02. Cerebellum

Support: NIH Grant 1R01MH093727

NIH Grant 1F31NS103427-01

Title: Transient stimulation of the inhibitory cerebello-olivary pathway generates a negative prediction error and causes extinction of conditioned eyelid responses

Authors: *O. A. KIM, J. F. MEDINA
Dept. of Neurosci., Baylor Col. of Med., Houston, TX

Abstract: To suppress a previously learned behavior, the brain is thought to generate negative prediction error signals (NPE) that trigger extinction learning. Although NPE signals are recorded in many brain areas, it is not known if they cause extinction. Here, we use an optogenetic approach to generate an artificial NPE signal in the inferior olive (IO) and to examine whether it causes extinction of a cerebellar-driven behavior. Mice with an implanted optical fiber in the IO were trained to blink in response to a tone that was repeatedly paired with an ocular airpuff. After learning, photostimulation during the airpuff caused gradual extinction of learned blinks in mice expressing channelrhodopsin in inhibitory cerebello-olivary synapses, but not in control mice expressing EYFP. Furthermore, photostimulation immediately before or after the airpuff did not cause extinction. Our results reveal an effective mechanism for generating NPE signals and triggering extinction of previously learned cerebellar-driven behaviors.

Disclosures: O.A. Kim: None. **J.F. Medina:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 584.05/NN6

Topic: E.02. Cerebellum

Support: NIH 1R01MH093727

Title: Plasticity of ponto-cerebellar circuits generates predictive responses in climbing fibers

Authors: *S. OHMAE, J. F. MEDINA
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Abstract: During eyeblink conditioning climbing fibers learn to fire in response to the conditioned stimulus (CS, e.g. a tone or LED-flash), thus providing the cerebellar cortex with a predictive signal that anticipates the impending delivery of an aversive airpuff to the eye. The sites of plasticity and the underlying neural circuits responsible for generating this Predictive-CS response are unknown. Here we performed two experiments to examine the role of the ponto-cerebellar pathway: (1) To test whether Pons activity is sufficient to drive the Predictive-CS response of climbing fibers, we trained mice in an eyeblink conditioning task that used direct

photostimulation of the Pons as the CS (Pons CS). We found that climbing fibers fire robustly in response to the Pons CS after learning, but not before learning. This finding demonstrates that climbing fibers can learn to generate a predictive-CS response via plasticity in the Pons or in areas downstream. (2) To test whether areas of the cerebellum that are downstream of the Pons may be involved, we stimulated the cerebellar Interpositus nucleus of naïve mice while recording climbing fiber responses in the eyeblink area of cerebellar cortex. We found that climbing fiber responses could be reliably elicited by stimulating a small eyeblink-controlling zone of the cerebellar interpositus, but much less by stimulating other neighboring sites (e.g. jaw-controlling zone). This finding reveals a hardwired excitatory connection that links functionally-related areas of the deep cerebellar nucleus and the source of climbing fibers in the inferior olive. Altogether, our results suggest a model in which the cerebellum drives predictive responses in climbing fibers, by increasing the strength of the CS-related inputs that it receives from the Pons during learning.

Disclosures: S. Ohmae: None. **J.F. Medina:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

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Program #/Poster #: 584.06/DP09/NN7

Topic: E.02. Cerebellum

Support: NIH Grant MH093727
NIH Grant NS104836

Title: Predictive control of a motor synergy by the cerebellum

Authors: *S. A. HEINEY[1], J. F. MEDINA[2]
[1]Iowa Neurosci. Inst., Univ. of Iowa, Iowa City, IA; [2]Dept. of Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Aversive stimuli elicit defensive reflexes that require activation of neural circuits specialized for coordinating the movement of multiple body segments. Here, we show that when the aversive stimulus can be predicted, mice learn to make the same defensive motor synergy preemptively. Furthermore, we identify a small area in the rostral anterior interpositus nucleus of the cerebellum that is both sufficient and necessary for performing the entire defensive motor synergy. Neurons recorded within this critical region are activated predictively in anticipation of the aversive stimulus, and display firing rate modulations that are correlated with the vigor of the synergistic movement on a trial-by-trial basis. The same neurons have sensorimotor receptive fields with mixed selectivity for multiple body segments, providing a neural substrate for the synergy. Our results suggest that some regions of the cerebellum may be organized in

ethologically-relevant “action maps” whose neurons can be selectively engaged via predictive mechanisms to reduce dimensionality and simplify motor control.

Disclosures: S.A. Heiney: None. **J.F. Medina:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 584.07/NN8

Topic: E.02. Cerebellum

Support: NIH 1R01MH093727

Title: Cerebellar participation in a cognitive timing task in mice

Authors: *G. J. WOJACZYNSKI, J. F. MEDINA
Dept. of Neurosci., Baylor Col. of Med., Houston, TX

Abstract: A growing body of evidence from human studies suggests that the cerebellum plays a significant role in cognitive function. Achieving a full mechanistic understanding of the cerebellar contribution to cognition would be greatly facilitated by developing cerebellar-dependent cognitive tasks for rodents, which offer a wealth of methodological advantages. Here, we introduce a novel subsecond timing task for mice, based on cognitive “omitted oddball” detection tasks previously developed for humans and non-human primates. We used lesions to identify a region of the anterior interpositus nucleus of the cerebellum that is necessary for performing the task. Furthermore, we found that neurons within this critical region of the cerebellum fire strongly in correct trials when the mouse successfully reports the omitted oddball stimulus, and are less active in unsuccessful trials. Collectively, our results establish a cerebellar-dependent cognitive task for mice, paving the way for future research into the underlying neural mechanisms.

Disclosures: G.J. Wojaczynski: None. **J.F. Medina:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 584.08/NN9

Topic: E.02. Cerebellum

Support: ERG-Stg

Title: Regional differences in the development of the cerebellar cortex

Authors: *M. SCHONEWILLE[1], G. C. BEEKHOF[2], C. OSORIO[2], F. BLOT[2], J. J. WHITE[2]
[1]Erasmus MC, Rotterdam, Netherlands; [2]Neurosci., Erasmus MC Rotterdam, Rotterdam, Netherlands

Abstract: The cerebellar Purkinje cell is one of the largest neurons in the mammalian brain. In mice, as in most other mammals, the inputs to the Purkinje cell as well as its massive dendritic tree are largely configured postnatally. Coincidentally, cerebellar lesions have been link to deficits in cognitive and emotional abilities and cerebellar injury in early life forms a major risk factor for development of autism spectrum disorder. Here we describe physiological, morphological and immunohistochemical changes that occur during Purkinje cell development in normal mice. Our data indicate the presence of early regional variation in the activity of Purkinje cells during development. Deeper analysis of these pre-symptomatic changes will provide the framework needed to study the spatial and temporal profile of pathogenesis and thereby facilitate early identification of disorders and the testing of potential treatments.

Disclosures: M. Schonewille: None. **G.C. Beekhof:** None. **C. Osorio:** None. **F. Blot:** None. **J.J. White:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 584.09/NN10

Topic: E.02. Cerebellum

Support: 311237

Title: The zona incerta modulation of precerebellar nuclei

Authors: *R. BHUVANASUNDARAM, S. WASHBURN, J. E. KRZYSPIAK, K. KHODAKHAH
Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: The zona incerta (ZI) is one of the least-studied regions of the brain, despite its robust projections throughout the brain. Recently a number of studies have associated a few potential functions with ZI. One study showed that stimulation of GABAergic neurons in ZI drives rapid binge eating in mice. Another study identified a GABAergic sub-population of neurons in the

ventral ZI that promote sleep. Additionally, caudal ZI has been shown to be a potential target for deep brain stimulation (DBS) for treatment of some forms of tremor. Anatomical findings show that ZI sends projections to pontine nuclei (PN) and inferior olive (IO), two of the major precerebllar nuclei that in turn provide the main inputs to the cerebellum known as the mossy (mf) and climbing fibers (cf). In this study, we examined the functional properties of ZI inputs to PN and IO. We performed *in vivo* extracellular single unit recordings in PN in awake head-fixed mice, and optogenetically activated the ZI-PN pathway. Optogenetic stimulation of the ZI axons in the PN elicited rapid responses in more than 75% of recorded cells with a short latency of 2-3 milliseconds. Using trains of stimuli, we found the ZI-PN pathway remained robust at 20 Hz. Next, we used *in vitro* electrophysiology to study synaptic transmission at ZI-IO. Our data suggest that optogenetic activation of channelrhodopsin expressing ZI axonal fibers in IO evoking large synaptic response. Together, our data suggest that ZI sends functional projections to neurons in the pontine nuclei and inferior olive. Future studies will explain the function of these pathways.

Disclosures: R. Bhuvanasundaram: None. **S. Washburn:** None. **J.E. Krzyspiak:** None. **K. Khodakhah:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 584.10/NN11

Topic: E.02. Cerebellum

Support: NIH R01 NS096289

Title: Serotonin regulates tonic inhibition at the input stage of cerebellar processing

Authors: *E. FLEMING, C. HULL
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Abstract: The cerebellum plays a key role in motor learning, in particular by harnessing diverse sensorimotor inputs to form learned associations that refine the dynamics of movement. Recent work indicates that the cerebellum can also form contextual associations in order drive appropriate motor output and learning according to behavioral state (Courtemanche et al., 2009, Kimpo et al., 2014, Brooks et al., 2015, Lawrenson et al., 2016). While such context-dependent representations of sensory input are evident in the granule cell layer (Ozden et al., 2012), it is unknown what circuit mechanisms could modulate granule cell layer synaptic integration in a manner consistent with changes in brain state or behavioral context.
Anatomical evidence suggests that the cerebellum receives significant serotonergic (5-HT) projections from the raphe and reticular nuclei (Bishop & Ho, 1985) that are particularly dense in

the granule cell layer (Takeuchi et al., 1982). Here, we use an acute brain slice preparation from young adult rats to demonstrate that 5-HT depolarizes granule cell layer inhibitory interneurons called Golgi cells through activation of the 5-HT2A receptor without directly affecting either granule cells or mossy fibers. As a result, 5-HT acts to significantly increase spontaneous inhibition onto both granule cells and Golgi cells. While 5-HT produces a net depolarization of Golgi cells that elevates their firing, it does not significantly alter the probability or timing of evoked Golgi cell inhibition onto granule cells. Thus, the increased spontaneous inhibition paired with normal feed-forward evoked inhibition acts to reduce mossy fiber driven spike probability in granule cells without degrading spike timing. Hence, these data provide a circuit mechanism by which 5-HT can regulate the gain of input/output transformations in the granule cell layer by adjusting signal-to-noise ratios in a manner consistent with enhancing pattern separation. Such changes in network integration could underlie the types of context-dependent gating of sensorimotor input that have been observed in the cerebellum in vivo.

Disclosures: E. Fleming: None. **C. Hull:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 584.11/NN12

Topic: E.02. Cerebellum

Support: Beckman Young Investigator Award

Title: Drd1 receptor activation in cerebellar cortex increases granular cell layer activity

Authors: *J. CANTON-JOSH, Y. KOZOROVITSKIY
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Abstract: The cerebellum is influenced by a wide range of neuromodulatory circuits. These circuits are likely to mediate significant effects on activity and plasticity within the cerebellum, thereby modulating motor control. Using transgenic Drd1a-Cre mouse line (Dopamine receptor type 1) and fluorescent in situ hybridization, we have discovered and quantified evidence for mRNA expression of Drd1a receptors within the granule layer of the cerebellar cortex. Since Drd1 receptors are Gs-coupled and have been shown in the striatum and mPFC to lead to increases in cellular excitability, we hypothesized that Drd1Rs could have similar effects in cerebellar neurons. Using ex vivo electrophysiological recordings we have found that selective pharmacological activation of Drd1Rs leads to an increase in tonic firing rates ($n=13$, $p<0.05$) and increases in NMDAR ($n=7$, $p<0.05$) mediated excitatory inputs. These changes are blocked by the addition of Drd1a antagonists ($n=13$, $p=0.5$). Our current studies are examining the

presynaptic sources of dopamine in the cerebellum using optogenetic circuit dissection techniques.

Disclosures: J. Canton-Josh: None. **Y. Kozorovitskiy:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 584.12/NN13

Topic: E.02. Cerebellum

Support: NIH Grant NS084996

Title: Monosynaptic tracing within the cerebellum reveals distinct Purkinje innervation patterns of diverse cell types within the nuclei

Authors: S. M. LEWIS, D. G. HECK, *A. L. PERSON
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Abstract: The cerebellar nuclei sit at the interface between the learning machinery of the cerebellar Purkinje neurons and the rest of the brain. Purkinje cells project to the nuclei with broad parasagittal topography, but the organization of convergent Purkinje cells at the single cell level has, to our knowledge, never been tested, despite having important computational implications. To identify the organization of inputs to premotor output neurons, Ntsr1-Cre (n = 15) or Vglut2-Cre (n=2) mice of either sex were unilaterally injected into the interposed nucleus (IN) with AAV-DIO-mCherry-TVA and AAV-DIO-H2B-GFP-oG to express an engineered EnvA receptor (TVA) and optimized glycoprotein (oG) in Cre-expressing cells. After 6 weeks, the same IN was injected with EnvA-G-deleted-rabies-eGFP, with mice sacrificed one week later and processed for histology. To identify presynaptic partners to inhibitory neurons, we used the same procedure with Gad1-Cre mice (n=8). Our injection parameters limited uptake of rabies helper viruses, restricting monosynaptic rabies jumps to afferents of very few starter neurons. Using this method, we have identified distinct and highly reproducible Purkinje convergence patterns that differed distinctly between premotor output neurons and neighboring inhibitory neurons. Purkinje neurons presynaptic to premotor output neurons in IN occupied extremely circumscribed parasagittal stripes between one and three neurons wide that appeared consistently in disparate zones in posterior Lobule 8, the border between Lobule 6 and Crus I, and the paraflocculus, with counts totaling an average of 110 Purkinje neurons (+/- 70 s.d.; 9.4 +/- 4.1 neurons per stripe; n = 8). In contrast, Purkinje neurons presynaptic to Gad1-Cre IN neurons were much more numerous (average 3010 +/- 2002 s.d.; n = 3) and occupied a broad parasagittal band spanning Lobules simplex, 4/5 and Crus I. Together these data suggest distinct convergence

patterns of Purkinje neurons onto different postsynaptic cell types in the nuclei which could support diverse computations.

Disclosures: S.M. Lewis: None. **D.G. Heck:** None. **A.L. Person:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 584.13/NN14

Topic: E.02. Cerebellum

Title: Spontaneous activity change of Purkinje cell under direct current stimulation

Authors: *T. YANG[1], M. KC[1], L. LAM[1], H. LU[2]
[1]Philadelphia Col. of Osteo. Med. - Geo, Atlanta, GA; [2]PCOM - Georgia Campus, Suwanee, GA

Abstract: Transcranial direct current stimulation (tDCS) is a noninvasive technique that has been used to potentially correct cerebellar dysfunctions such as ataxia. To understand the mechanism of tDCS to the cerebellar Purkinje cells (PCs), whole cell patch clamp was used to record from these cells. Direct current stimulation (DCS, 200 μA) was delivered at different electrode polarities to mimic the tDCS. The focus of this study is to measure the spontaneous activity of PCs under DCS. Student's t-test was used to study the frequency changes from DCS. The spontaneous activity of PCs every 60 seconds was used to study the effects of DCS. There was no significant increase in firing rate under positive DCS ($p = 0.19$, $n = 7$) and no significant changes in firing rate under negative DCS ($p = 0.82$, $n = 4$). We also tested the firing rate changes with external current injection (+0.2 nA) under DCS, no significant changes were observed with negative stimulation ($p = 0.82$, $n = 13$) or with positive stimulation ($p = 0.07$, $n = 11$). To test our hypothesis that the dendritic tree of individual PC oriented in each folium determines the final output change caused by DCS, more cases will be needed to study the effects with the consideration of this orientation.

Disclosures: T. Yang: None. **M. Kc:** None. **L. Lam:** None. **H. Lu:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 584.14/NN15

Topic: E.02. Cerebellum

Support: NIH Grant F31 NS096887

Title: Anatomical analysis of afferents to the red nucleus in mice

Authors: *C. S. BEITZEL[1], B. D. HOUCK[2], A. L. PERSON[3]
[1]Neurosci., Univ. of Colorado Denver Sch. of Med., Aurora, CO; [2]Hendrix Col., Conway, AR; [3]Physiol. and Biophysics, Univ. of Colorado Sch. of Med., Aurora, CO

Abstract: The red nucleus (RN) is a major target of cerebellar premotor output neurons and forms a rapid route for cerebellum to influence movement through direct projections to spinal motor pools. Increasing evidence indicates that motor cortex, cerebellum, and basal ganglia work together in service of motor control. The red nucleus is innervated by all three of these motor regions and various brainstem nuclei, setting up the RN as a potential hub of integration linking multiple motor systems. Defining rules for interaction between these systems with in the RN, however, requires identifying afferent overlap and sources of inhibition to the RN which are poorly characterized in any species. We explored the anatomically overlap between the motor cortex and the cerebellum, two major inputs to the red nucleus. We injected anterograde viral tracers expressing either GFP or RFP to the cerebellar interposed nucleus and sensorimotor cortex in wild type mice (n=3). We found limited overlap of terminal fields from the cerebellum and sensorimotor cortex restricted to the most rostral extent of magnocellular RN. Sensorimotor cortex preferentially innervated neighboring parvocellular RN and pararubral areas, which were devoid of cerebellar afferents. To better understand if these areas project into RNm, we examined the sources of inputs to the RN, with particular attention to potential sources of inhibition. Using biotinylated dextran amine injections into either wild type mice or GlyT2.eGFP mice (n=4, n=2, respectively) we identified multiple areas that innervate the RN, including known sources such as the cerebellar nuclei, sensorimotor cortices, and substantia nigra-pars reticulata. We also found retrograde label in multiple brainstem reticular nuclei. Interestingly, we identified two sources of glycinergic inhibition to the RN from pontine reticular nuclei (PNo,PNC, RtTg) and gigantocellular reticular nucleus (Gi). Sparse anterograde label from sensorimotor cortex was seen in some of these areas, including Gi, PNo, as well as mesencephalic reticular nuclei, providing a potential conduit for feed-forward inhibition through known and putative inhibitory afferents to the RN. Together these data support RN as a point of integration between multiple upstream motor centers.

Disclosures: C.S. Beitzel: None. **B.D. Houck:** None. **A.L. Person:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

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Topic: E.02. Cerebellum

Support: NIH Grant F31NS09075-04
NIH Grant R01NS050808

Title: Mechanisms underlying stress-induced motor attacks in a mouse model of episodic ataxia type 2

Authors: *H. D. SNELL, A. VITENZON, E. TARA, K. KHODAKHAH
Albert Einstein Col. of Med., Bronx, NY

Abstract: Episodic ataxia type 2 (EA2) is a channelopathy that arises from mutations in the *CACNA1A* gene encoding for the α1 pore forming subunit of P/Q-type voltage-gated calcium channels. Patients with this disorder exhibit motor attacks in the form of ataxia and dyskinesia, which are brought about by physical or emotional stress, or consumption of caffeine or alcohol. We used a well-established mouse model of EA2, *tottering*, to explore the mechanisms by which stressors trigger attacks. Previously, our lab has shown that a decrease in SK channel activity in Purkinje cells is the underlying mechanism for the baseline ataxia, but the mechanism of the attacks, remained unknown. Because cerebellar Purkinje cells (PCs) are required for the expression of attacks in *tottering* mice, we recorded their activity in awake head-restrained mice when they had attacks. We found that PCs exhibited high frequency burst firing during attacks independent of the stressor used. This finding suggested that the triggers might share a common mechanism to induce attacks. Given that stress is the most ubiquitous trigger among channelopathies, we explored its mechanism of action. Previous work in *tottering* mice showed α1 adrenergic receptors play a role in stress-induced attacks, thus we examined whether the noradrenergic system is required for these attacks. We found that pharmacologically activating α1 adrenergic receptors in the cerebellum was sufficient to induce attacks. We also found that activation of α1 adrenergic receptors in the cerebellum was required for stress-induced attacks. To delineate the mechanism by which stress induces erratic activity of PCs we recorded PC activity in acutely prepared slices. Consistent with our *in vivo* data, we found that bath application of norepinephrine (NE) increased PCs irregularity, and this effect was mediated by activation of α1 adrenergic receptors.
It was previously shown that NE down regulates SK channel activity through activation of α1 adrenergic receptors, and a casein kinase II (CK2) dependent phosphorylation mechanism. We found that knock down of CK2 in the cerebellum of *tottering* mice using shRNAs prevented stress-induced attacks, and prevented burst firing of *tottering* PCs *in vivo*. Consistent with our *in vivo* results, we found that pharmacologically blocking CK2 with 4,5,6,7-Tetrabromobenzotriazole (TBB)
prevented NE induced irregularity in PCs, in slice recordings. Overall, these data suggest the adrenergic pathway may be a potential therapeutic target for patients with EA2.

Disclosures: H.D. Snell: None. **A. Vitenzon:** None. **E. Tara:** None. **K. Khodakhah:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 584.16/OO1

Topic: E.02. Cerebellum

Title: Intensity dependent effects of cathodal tDCS on cerebellum using an *in vivo* approach

Authors: J. SELZMAN, V. YARABARLA, A. JAMSHAD, C. PICOU, *H. LU
PCOM - Georgia Campus, Suwanee, GA

Abstract: This research aims to examine the physiological activity in the primary motor cortex as the result of transcranial direct current stimulation (tDCS) on the cerebellar cortex. Cerebellar ataxia affects a significant amount of the population and impairs one's quality of life. This form of ataxia can manifest as a result of trauma from stroke, autoimmune attacks, or other various CNS diseases, and tDCS has been shown to have promising results as a form of potential therapeutic treatment. Using normal animal models can give insight into the mechanisms that underlie tDCS therapy and can be used as a precursor for further research involving diseased animal models. Sprague-Dawley rats (n=10) were used to isolate Purkinje cells (n=14) from the cerebellar cortex as well as to record local field potentials in both the cerebellar and cerebral cortices. Cathodal stimulation intensity was set at 100 μA and 200 μA. The mean frequency of firing rate was analyzed to determine the output of individual Purkinje cells. Some Purkinje cells (n=10) had a decrease in overall firing rate following the stimulation whereas other Purkinje cells (n=4) exhibited an increase in firing rate from the same stimulation. A one tailed t-test (p=0.23) indicated there was no significant difference in firing rates based on intensities. A power spectrum analysis was conducted to study the changes in cerebellar cortical activity, and results from this analysis showed an increase in amplitude at approximately 5-10 Hz in (n=7) cells. Other cells (n=2) were observed to have a change in higher frequency around 80 Hz. The remaining cells (n=5) exhibited no significant changes in amplitude. Overall, cathodal tDCS was shown to cause a decrease in the firing rate of Purkinje cells. Power spectrum analysis revealed an increase in amplitude of low frequency activity of local field potential under cathodal stimulation in some of the cells. Further, cross correlation and coherence analyses demonstrate that the activity changes of the motor and cerebellar cortices are interrelated. The correlation level decreases with tDCS. The analysis based on current cases indicates that the correlation level is more depressed at an intensity of 200 μA. All of these analyses suggested that the cerebellar tDCS altered activity in the primary motor cortex due to a change in cerebellar output. Future analysis should focus on comparisons between cathodal and anodal direct-current stimulation, as well as alternating-current stimulation, to determine the most effective form of treatment.

Disclosures: J. Selzman: None. **V. Yarabarla:** None. **A. Jamshad:** None. **C. Picou:** None. **H. Lu:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

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Program #/Poster #: 584.17/OO2

Topic: E.02. Cerebellum

Support: NIH Grant F31NS105406
NIH Grant R01NS050808

Title: Serotonergic modulation of cerebellar circuitry

Authors: *K. PALARZ, K. KHODAKHAH
Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Ataxia (uncoordinated movement) is a debilitating disorder that interferes with patients' ability to perform activities of daily living. Ataxia is often caused by dysfunction of the cerebellum, a brain area involved in motor coordination and maintenance of balance. There are few therapies available for treatment of ataxia, and the ones used, such as serotonergic agents, have limited efficacy often only in a subset of patients. Thus, there is a real need for new and improved therapeutic approaches for the management and treatment of ataxia.
A major cause of cerebellar dysfunction is abnormal Purkinje cell (PC) activity. PCs, the sole output of the cerebellar cortex, are intrinsically active cells that integrate synaptic input from over 150,000 parallel fiber (PF) synapses and one climbing fiber (CF). Various experiments in vivo and in vitro have suggested opposing effects of serotonin in the cerebellum. The mechanisms by which serotonin causes these opposing effects are not understood. Nevertheless, because serotonergic drugs are promising for the treatment of ataxia, it is important to delineate the mechanism by which they modulate cerebellar function.
Serotonergic drugs that have been most efficacious in lessening motor dysfunction were chosen to target the 5-HT1A receptor. However, these drugs can also activate 5-HT7 receptors. Because 5-HT1A and 5-HT7 receptors typically have opposing effects on firing and synaptic transmission, it is plausible that the limited efficacy of serotonergic drugs used to treat ataxia is due to activation of multiple receptors that elicit opposing effects on cerebellar function. In the cerebellar cortex, 5-HT1A and 5-HT7 receptors are only found on PCs and granule cells, and in turn PFs. Thus, PC firing and PF synaptic transmission are likely targets of the serotonergic drugs used to treat ataxia.
As a first step to elucidate the mechanism of serotonergic modulation in the cerebellum, we examined the effect of serotonergic receptor agonists on the firing rate of PCs. We performed extracellular recordings of PCs in acute sagittal cerebellar slices. Serotonergic receptor agonists

were then bath applied at concentrations that preserve selectivity. Our data indicates that 5-HT1A, 5-HT7, and 5-HT2A receptor agonists have no effect on the firing rate of PCs in vitro. Therefore, the beneficial effects of serotonin observed in patients might have occurred via modulation of PF-PC synaptic transmission. Future efforts will delineate the effect of selective 5-HT1A and 5-HT7 receptor activation on PF synaptic transmission and plasticity.

Disclosures: K. Palarz: None. **K. Khodakhah:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 584.18/OO3

Topic: E.02. Cerebellum

Support: NIH R01 NS092623
NIH F32 NS103216

Title: The cerebellar representation of learning in smooth pursuit eye movements across hundreds of trials

Authors: *N. J. HALL[1], Y. YANG[2], S. G. LISBERGER[1]
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Abstract: We studied the motor learning related changes in neuronal firing of neurons in the cerebellum as monkeys performed smooth pursuit eye movements during hundreds of repetitions of a direction-change task. In each learning trial, a target moved in an initial pursuit direction as the animal followed it with his eyes. After a short, fixed interval of 250 ms, the moving target suddenly, but predictably, changed direction. Over multiple presentations of the learning trials, behavioral learning was measured as a modification of eye velocity just before the target changes direction. As reported by others, we found that neurons in the cerebellum express large changes in firing rate that appear over a few or tens of trials as monkeys perform the direction learning task. However, longer recordings over blocks of hundreds of learning trials revealed that the learned changes are not stable in all neurons. In some neurons, learning changes decreased in amplitude or disappeared entirely as behavioral learning evolved over trials. Our results suggest that motor learning is a dynamic process and might be consolidated outside the cerebellum or only in specific subsets of cerebellar neurons.

Disclosures: N.J. Hall: None. **Y. Yang:** None. **S.G. Lisberger:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

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Topic: E.02. Cerebellum

Support: New Jersey Brain Injury Research Fellowship
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Nancy Lurie Marks Family Foundation
National Science Foundation Graduate Research Fellowship DGE-1148900

Title: Regulation of flexible learning, social interaction, and whole-brain cellular activity by lobule VI of posterior vermis

Authors: *J. VERPEUT[1,2], T. PISANO[1,3], M. KISLIN[1], L. WILLMORE[1], L. TAO[1], D. PACUKU[1], T. D. PEREIRA[1], A. M. BADURA[4], S. S.-H. WANG[1,2]
[1]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [2]Mol. Biol. Princeton Univ., Princeton, NJ; [3]Rutgers-Robert Wood Johnson Med. Sch., New Brunswick, NJ; [4]Netherlands Inst. For Neurosci., Amsterdam Zuidoost, Netherlands

Abstract: The posterior cerebellar vermis is a likely substrate for cognitive and social function: adults with damage to this region show deficits in affect and executive function, and perinatal injury leads to a 36-fold increase in the risk of autism spectrum disorder. We mapped vermal influence over brainwide distal targets, and quantified behavioral consequences of adult and developmental postnatal disruption. To identify distal targets, we performed anterograde transsynaptic tracing using herpes simplex virus (HSV)-H129 injections into lobule VI. After 80 h incubation, we observed GFP-expression in intralaminar thalamic nuclei as well as anterior cingulate, prelimbic, and orbitofrontal cortex. Next, to identify functional targets of lobule VI, we used the light-sensitive proton pump ArchT to inhibit Purkinje cells (PCs; AAV-CAG-FLEX-ArchT-GFP into L7-Cre +/- mice). The resulting brain-wide disinhibition pattern was visualized using quantitative c-Fos mapping and light-sheet microscopy. Compared with L7-Cre -/- littermates receiving the same injection and light stimulation, we found more c-Fos positive neurons in nucleus accumbens (5.3-fold increase; $p<0.05$, Mann-Whitney U, two-tailed), intralaminar nuclei of the thalamus (6.0-fold increase, $p<0.05$), and anterior cingulate cortex (4.1-fold increase, $p<0.05$).
To characterize behavioral consequences of lobule VI perturbation, we reversibly inhibited molecular layer interneurons (MLIs) by expressing hM4Di DREADD (Designer Receptor

Exclusively Activated by Designer Drugs) using AAV-hSyn-hm4Di-mCherry. The DREADD agonist CNO evoked decreases in the rate and modulation of PC simple-spike firing in vivo. To identify consequences of lobule VI disruption during development, we delivered CNO from postnatal days (PND) 30 to 56. Two weeks after CNO, mice (PND >70) showed deficits in reversal learning, social behavior, and novelty-exploration. CNO also acutely impaired reversal learning and novelty-exploration (A. Badura et al., Soc. Neurosci. Abstr. 2017). To test CNO's effects on developing neocortical circuitry, pyramidal neuron dendritic spine morphology of medial prefrontal cortex demonstrated increased mature mushroom spines (26.8%) compared to littermate controls (18.6%; $p<0.05$, t-test, two-tailed). In summary, lobule VI has both developmental and acute effects on flexible behavior, and provides activity sufficient to influence neocortical dendritic refinement. We are now applying automated pose-tracking (see L. Willmore et al. abstract, this meeting) to identify behavioral consequences of lobule VI disruption in freely-behaving mice.

Disclosures: J. Verpeut: None. **T. Pisano:** None. **M. Kislin:** None. **L. Willmore:** None. **L. Tao:** None. **D. Pacuku:** None. **T.D. Pereira:** None. **A.M. Badura:** None. **S.S. Wang:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 584.20/OO5

Topic: E.02. Cerebellum

Title: Selective activation of cerebellar granule cells and molecular layer interneurons has distinct impact on performance in watermaze tasks

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Abstract: The ability to accurately integrate and represent information about environmental features is termed spatial cognition. It comprises declarative and procedural memory components. The procedural component relies on the body-centered reference frame providing knowledge about the sequence of movements, whereas the declarative component integrates information about relationships between landmarks based on allocentric reference frames (i.e. regardless of the animals location). Declarative knowledge of distances and spatial arrangements of objects is supported by active location updating processes creating an accurate spatial map that enables self-localization in the absence of external cues. Without continuous updates, this system is susceptible to accumulating directional errors. Cerebellar structures participate to a great extent in procedural memory, relaying error corrected signals to cortical structures by conveying integrated sensory information, thereby refining movements. Studies using a mouse

model with impaired synaptic long-term depression (LTD) at the parallel fiber-Purkinje cell synapse support the notion that the cerebellar circuit serves to foster the procedural optimization of goal-directed navigation through LTD, whereas the declarative component is not affected by impairment of LTD. Our aim was to dissect the relative contribution of cerebellar granule cells (GCs) and molecular layer interneurons (MLIs) to memory formation for space. We used adeno-associated virus to express channelrhodopsin 2 in respective celltypes of the cerebellar circuitry during two different spatial navigation paradigms: the Morris watermaze (MWM) and the starmaze. The MWM requires both memory components - procedural and declarative. The starmaze, however, relies mainly on declarative capacities. Optogenetic activation of GCs and MLIs resulted in different patterns of performance decrease in the two paradigms indicating their distinctive roles in spatial memory. Whereas optogenetic activation of GCs in C57Bl/6 mice suppressed efficient learning in both mazes, activation of MLIs led to a selective decrease in the late phase of the starmaze navigation performance. Thus, GCs convey information relevant for both components of memory, whereas MLIs may play a distinctive role in the declarative component of spatial navigation.

Disclosures: T. Surdin: None. **M.D. Mark:** None. **S. Herlitze:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 584.21/OO6

Topic: E.02. Cerebellum

Support: Wellcome Trust
EMBO

Title: Probing the functional interactions between distinct elements of the cerebellar cortex and deep nuclei circuitry in awake behaving mice

Authors: *M. BEAU[1], D. KOSTADINOV[2], Y. CHUNG[2], M. HAUSSER[2]
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Abstract: The cerebellum is a structure crucial for motor control and learning. It has been extensively studied in a variety of reductionist motor paradigms, but how it is engaged in complex tasks with more degrees of freedom, and how distinct elements of cerebellar circuitry interact with each other in real time to drive behaviour remain largely unexplored. In particular, the exact interplay between the Purkinje Cells (PCs), the output neurons of the cerebellar cortex, and neurons of the deep cerebellar nuclei (DCN) is an open question in the field, which requires simultaneous recordings of functionally connected cerebellar cortex and DCN neurons.

We are addressing this problem using Neuropixels silicon probes, which allow sampling from 384 densely-spaced channels along a linear recording shank that can span both the cerebellar cortex and DCN. A typical recording session yields >100 well-isolated units across the 4 mm of recording depth. Using a combination of cell-type specific optogenetic tagging, prior knowledge about the firing properties of distinct cerebellar cell types, and post-hoc histological validation of our recording sites, we can cluster these recorded units and putatively identify the major cerebellar cell classes. In the cerebellar cortex, PCs, molecular layer interneurons and granule cells were readily identifiable. Typical recordings yielded 20 ± 4 PCs, 30 ± 10 MLIs and 10 ± 2 GCs (n = 4 mice). Importantly, we can detect PC complex spikes, despite the variability of their waveforms. DCN recordings revealed 15 ± 5 units that were tentatively classified into excitatory and inhibitory neurons based on their firing rates. Correlation analysis of the spike times of these recorded units revealed a variety of functional relationships between nearby cells, as well as neurons spanning the cerebellar cortex and DCN, confirming our ability to record from the entirety of the cerebellar circuitry.
We are now using this system to probe the dynamics of interactions between these cell types during a motor task with several degrees of freedom. In this task, head-fixed mice are trained to use a steering wheel with their forepaws to translate a virtual object presented on monitors. We are now combining this behavioural paradigm with our Neuropixels recordings as well as custom video tracking of limb movements to gain novel insights into how sensorimotor parameters are encoded by distinct cerebellar circuit elements, and guided by interactions between these elements, at single spike resolution. This approach will provide insights into how the cerebellar corticonuclear interactions govern complex sensorimotor behaviours.

Disclosures: M. Beau: None. **D. Kostadinov:** None. **Y. Chung:** None. **M. Hausser:** None.

Poster

584. Cerebellum: Cortex and Nuclei II

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Program #/Poster #: 584.22/OO7

Topic: E.02. Cerebellum

Support: NIH Grant

Title: Transcriptional role of mef2 in cerebellar granule neurons

Authors: *S. P. MAJIDI[1], N. C. REDDY[1], T. YAMADA[1], L. HU[2], T. CHERRY[3], M. E. GREENBERG[2], A. BONNI[4]
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Abstract: Accurate control of gene expression is critical for the proper development of the diverse cell types that comprise the mammalian brain. Transcription factors (TFs) are an important class of gene regulators distinguished by their ability to bind specific DNA sequences, which gives rise to their ability to regulate complex patterns of gene expression. The myocyte enhancer factor 2 (Mef2) family of TFs has held a longstanding interest in the field of neuroscience due to its strong expression in brain tissue and its critical role in a host of processes in the mammalian nervous system, including activity-dependent regulation of synapse and spine number, dendritic morphogenesis, and learning and memory. Previously, our lab has revealed that a sumoylated form of Mef2a regulates synaptic differentiation of cerebellar granule neurons. In addition to Mef2a, granule neurons also co-express a second Mef2 family member, Mef2d. Despite the high expression of Mef2d in granule neurons, the contribution of Mef2d to granule neuron development has not yet been elucidated. Furthermore, its genome-wide transcriptional role during granule neuron development remains to be studied. To gain insight into the role of Mef2d during granule neuron development, we have performed *in vivo* morphologic assays following conditional knockout of Mef2d in granule neurons. Additionally, in order to shed light on the genome-wide binding and transcriptional activity of Mef2d in the developing cerebellum, we have employed chromatin immunoprecipitation- and RNA-sequencing of granule neurons. Following conditional knockout of Mef2d, RNA-sequencing reveals that gene expression of a substantial portion of Mef2d-bound sites are dysregulated. We are interested in employing strategies in granule neuron culture to further explore the role of Mef2d in regulating transcription at Mef2d-bound sites. These studies should provide further insight on how Mef2 regulates the development of cerebellar granule neurons.

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Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 585.01/OO8

Topic: E.04. Voluntary Movements

Support: Wellcome Trust

Title: Similarity of execution and observation neuronal population activity in macaque motor and ventral premotor cortex

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Abstract: Motor cortical neurons can modulate their activity during both action execution and observation, yet only the former state is accompanied by overt movement. Single neurons can show very similar activity during the two conditions (classical mirror neurons (MNs)), or opposite patterns of discharge (suppression MNs). Here, we used principal component analysis (PCA) to compare population-level activity during execution and observation and assess the degree of overlap in their cortical representations.
One male rhesus macaque was trained to perform or observe cued reach-to-grasp movements on two objects, affording precision grip and whole-hand grasp. We obtained multi-electrode recordings (Thomas Eckhorn recording drives) of extracellular single-unit responses in contralateral (left) primary (M1) and ventral premotor (F5) cortex during task performance. Pyramidal tract neurons (PTNs) were identified in both areas via antidromic stimulation between two chronically implanted tungsten electrodes in the ipsilateral medullary pyramid.
From clustered single-unit spikes, we compiled trial-averaged peri-event time histograms to form timepoints x units matrices for execution and observation, separately for different task epochs, objects, and neuronal sub-populations. For each matrix, we performed PCA to obtain the top 5 principal components (PCs), representing trajectories through neural space. We then calculated the proportion of explained variance relative to the total variance of each condition's subspace. To quantify shared variance, we projected observation data onto the first 5 execution axes and summed the variance captured. We then quantified overlap between execution and observation as the ratio of this sum to the variance explained by the first 5 observation PCs.
We recorded 51 PTNs (36 M1, 15 F5), and 173 unidentified single units (UIDs) (106 M1, 67 F5), many of which clearly modulated their activity during one or both conditions. During grasp, the first PCs typically captured greater variance in execution than observation in M1, but not F5, suggesting a more precise locking of M1 single units to the execution of grasp. Execution and observation subspaces were generally well aligned during the delay period prior to the go cue, which indicated the upcoming trial type. F5 representations during reach and grasp showed an overall greater overlap between execution and observation than M1, suggesting greater similarity in F5 between the two conditions.

Disclosures: S.J. Jerjian: None. **G. Vigneswaran:** None. **R.N. Lemon:** None. **M. Sahani:** None. **A. Kraskov:** None.

Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 585.02/OO9

Topic: E.04. Voluntary Movements

Support: NIH Grant NS084948

Title: A newly learned controller is inflexible and computationally demanding

Authors: *S. A. HUTTER, J. A. TAYLOR
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Abstract: Over the past two decades, the field of motor learning has primarily focused on the process of adapting a previously learned control model, presumably through the update of an internal forward model. Surprisingly, learning a novel controller (i.e, a mapping between goals and actions) has received relatively little attention. Only a handful of studies have investigated learning new controllers and these have found what appears to be a fundamental difference between learning de novo compared with compensating for a perturbation (Shmuelof et al., 2012; Telgen et al., 2014). Furthermore, a novel controller may be more dependent on decision-making processes (Chen et al., 2017), which have been shown to rely on model-free and model-based learning processes when learning an arbitrary stimulus-response mapping (Haith and Krakauer, 2013). Building on this work, we set out to determine the requirements of learning a controller for a novel motor task. We modified the "grid-sailing task," developed by Fermin and colleagues (2010) to expose how model-free and model-based processes learn to navigate a path with a new stimulus-response mapping (i.e., controller). Here, subjects navigated a cursor across a grid by pressing keys on a keyboard, and we manipulated the mapping between key-presses and movement of the cursor to be either direct and intuitive (i.e. the top button moves the cursor up, the left button moves the cursor left, etc.) or arbitrary and unintuitive. Note, we define intuitive mappings as those that conform with prior expectations based on interactions with spatial navigation. First, we contrast the flexibility and reliability of the established controller (direct key-mapping) with the newly learned controller (arbitrary key-mapping). Flexibility of the controller was assessed with a transfer test, where subjects navigated to novel locations on the grid, while reliability of the controller was assessed on the trained route after experience with the transfer test. Additionally, we hypothesized that reaction time differences between the established and arbitrary controller in the above tasks will elucidate any additional processes required to use the novel controller. This reaction time should depend on complexity for the novel controller, while the established controller should have considerably reduced cost of complexity. We find that the new controller is less flexible, less reliable, and requires greater processing time on transfer trials than the established controller.

Disclosures: S.A. Hutter: None. **J.A. Taylor:** None.

Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 585.03/OO10

Topic: E.04. Voluntary Movements

Title: Exploring motor repertoires induced by optical stimulation of corticospinal neurons

Authors: *N. SALAH[1,2], Y. LIU[3], Z. HE[4]
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Abstract: Volitional motor control requires cortical input that is largely mediated via the corticospinal tract, which forms a direct connection between the cortex and the spinal cord. Methods of assessing the involvement of this pathway in motor control have been falling short due to the scarcity of efficient targeting techniques that would enable exclusive manipulation of corticospinal neuron (CSN) activity. Moreover, CSNs have only been studied in animals either in anesthetized or head-fixed states when looking only at a subpopulation of neurons involved in this pathway. Here, a method where exclusive optogenetic manipulation of the entire CSN population in awake, freely behaving mice has been established. A patterned illumination system developed by Mightex has been modified by enlarging the field of view to target the entire forelimb area of the motor cortex. Moreover, the system incorporated the flexibility to target a wide range of CSN population sizes spanning tens of micrometers all the way to the entire CSN population. It also provided high flexibility in designing stimulation patterns where populations could be activated simultaneously or sequentially. Hence, a craniotomy was done to expose the motor forelimb area and a cranial window was implanted on top of which the system was fixed. In doing so, a spatially organized map of motor repertoires resembling ethologically-relevant behavior has been revealed. These were coordinated movements that were exhibited by single-point opto-stimulation at both the mesoscale and microscale levels. Furthermore, the hypothesis that the CSNs possess a modular organization has been explored by sequentially activating regions eliciting certain motor repertoires, and some evidence has been shown here supporting this in some CSN populations, but not all. Therefore, exploiting the full capacity of the system which enables simultaneous imaging and stimulation in the future would lay down the framework of dissecting motor circuits and further studying the mechanisms underlying such variability in the motor outcome across the CSN population.

Disclosures: N. Salah: None. **Y. Liu:** None. **Z. He:** None.

Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

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Topic: E.04. Voluntary Movements

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CONACyT PhD fellowship: 597738

Title: Cortico-striatal contribution to the execution of a chain of sequences

Authors: *A. SANCHEZ-FUENTES, K. RAMÍREZ-ARMENTA, J. RAMÍREZ-JARQUÍN, F. TECUAPETLA
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Abstract: It's has been suggested that the basal ganglia receives an internal signal from different cortices in order to start/stop an action sequences, e.g. from the supplementary motor area (in rodent M2) and prefrontal cortices. To date, it's not fully understood how the different cortices may guide the striatal activity to start/stop and switch between actions sequences. In this work we ask how the corticostriatal projections contribute to execute a chain two actions sequences.
Methods: A task in which animals do a chain of two sequences of lever press, presenting in blocks, stimulus-response trials (S-R; where animals are guided to switch between two levers) and self-paced trials (S-P; where animals switch between sequences without any guide) was developed. The neuronal activity of antidrormic corticostriatal neurons was identified [primary (M1), secondary motor cortex (M2) and prelimbic cortex (PL)] while animals execute this chain of sequences. To test whether the cortico-striatal synapses are contributing to initiate/execute the chain of sequences we implemented the optogenetic inhibition of cortico-striatal terminals once the chain of sequences has been learn.
Our preliminary results, from electrophysiology extracellular recordings in cortex show that cells in M1 decrease its activity during the performance of the chain of sequences. PL cortex increased its activity at the boundary of the chain of sequences. The M2 cortex activity increases at the beginning of the first and second sequence. Our preliminary results from the optogenetic inhibition of the cortico-striatal terminals show that the inhibition of the terminals from M2, during the initiation of the chain of sequences, accelerated the performance and the transition to the second sequence in the chain (interestingly it only happened for S-R trials). The optogenetic inhibition of corticostriatal M1 terminals only showed effects when we inhibit during the performance.
These results support the idea that the different corticostriatal synapses have specific contributions for the execution of a chain of sequences.
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Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

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Title: Fast, flexible, real-time closed-loop manipulation of voluntary whisking behavior

Authors: *K. SEHARA[1], V. BAHR[2], B. MITCHINSON[3], S. E. DOMINIAK[1], M. STAAB[1], M. A. NASHAAT[1], M. PEARSON[4], M. E. LARKUM[1], R. N. S. SACHDEV[1]
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Abstract: One of the major goals of behavioral neuroscience is to uncover constantly-changing relationships between the animal's behavior, neural activity and the context of behavior. The advent of virtual reality and optogenetic techniques enabled one to easily manipulate behavioral context and neural activity. One element that has been largely absent from these approaches is the ability to rapidly manipulate neural activity based on the context or on sequence of behaviors emitted by the animal. Here we use a neuromorphic chip-based event-driven camera system to implement real-time tracking of whisking behavior in mice trained to move their whiskers to touch a piezo element for reward. Our system tracks the position of single whiskers that are painted with UV paint to enhance tracking. The motion of the whisker is converted into series of thresholded events on the neuromorphic chip, which are used to rapidly estimate the position of the whisker. The system can trigger a TTL output within a short (~2 ms) latency when the whisker reaches the "target region" that we can interactively specify online. Using this system, we can detect each protraction or retraction of a whisker, and can deliver a reward or generate optogenetic stimuli for activating or inactivating cortex. Our methods are fast, flexible and sensitive enough to be used on single slender whiskers of the mouse that can move at frequencies up to 25 Hz, and can therefore be used for tracking whiskers or, in principle, any other part of the animal's body (i.e. the forepaw, hindlimb etc). Closed-loop, fast-feedback and flexible methods such as ours comprise a valuable tool set for manipulating behavior, and for understanding how

activity of neural circuits adapt to changing value of behavior and to a rapidly reconfigured environment.

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Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

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Program #/Poster #: 585.06/OO13

Topic: E.04. Voluntary Movements

Support: Wellcome Trust
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Title: Perturbation of ipsilateral motor cortex is detrimental to healthy human motor learning

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Abstract: The role of the ipsilateral hemisphere in motor learning has long been debated. One hypothesis is that activity in the ipsilateral motor cortex (M1) makes positive contributions to motor learning (Waters et al., 2017; Berlot et al., 2018), and so increasing this activity should be beneficial. However it has also been shown that the M1s are mutually inhibitory(Ferbert et al., 1992; Chen, 2004), and high levels of activity in the ipsilateral, contralesional hemisphere post-stroke - resulting in higher levels of inter-hemispheric inhibition (Murase et al., 2004)- correlate with worse functional impairments (Ward et al., 2003) in at least some cases. In line with this, interventions to reduce ipsilateral activity have been shown successful in improving post-stroke recovery (e.g. Fregni et al., 2005).
In this within subjects study we investigated how modulating activation of ipsilateral M1 in healthy participants influenced motor learning, consolidation and physiology within the contralateral M1. Participants (n=20, 9 female, mean age = 25.5, age range = 20-32) underwent excitatory anodal, inhibitory cathodal or sham transcranial direct current stimulation (tDCS) to right M1 during learning of a serial reaction time task (SRTT) with the right hand. Transcranial magnetic stimulation (TMS) measures of cortical excitability, $GABA_A$receptor activation (SICI 2.5ms) and glutamatergic activation (ICF) in the left M1 were taken at baseline and three time points after stimulation.
We found that both real stimulation conditions resulted in significant worsening of online

learning compared to sham, but had no effect on offline performance or consolidation of skill. These results indicate that perturbing activation in the ipsilateral M1 of healthy participants, whether in an excitatory or inhibitory manner, is detrimental to motor learning. In line with previous work, we observed that anodal ipsilateral tDCS also caused a significant, but transient, decrease in $GABA_A$receptor activation (Bachtiar, Johnstone et al., *in review*), and increase in glutamatergic receptor activity relative to sham.
Ipsilateral anodal tDCS-induced increase in SICI 2.5ms correlated with stimulation-induced change in skill, such that greater decreases in contralateral M1 GABA were associated with better learning (Stagg et al., 2011). A parsimonious explanation for these results would be that modulating ipsilateral excitability leads to behavioural worsening, but that this can be offset, at least in part, by changes in contralateral GABA activity during learning. These results offer the beginning of a mechanistic explanation for the complex role of the ipsilateral M1 in motor learning.

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Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

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Topic: E.04. Voluntary Movements

Support: NIH NINDS U01 NS0905905
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Title: Motor cortex descending projections drive orofacial behaviors through specific brainstem premotor networks

Authors: *N. MERCER LINDSAY[1], P. M. KNUTSEN[4], H. J. KARTEN[2], D. KLEINFELD[3]
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Abstract: Focal activation of motor cortex has been shown to enact behaviorally meaningful motor output including defensive behaviors, ethological limb movements, and chewing (Graziano et al., *Neuron*, 2002). However, the details of how the cortical circuitry interfaces with the brainstem premotor circuits is unknown. We studied the hierarchical nature of this control with respect to motor acts that involve vibrissa, jaw, and forelimb muscles.
The spinal trigeminal nuclei pars oralis (SpVO) and rostral interpolaris (SpVIr) contain premotor neurons known to directly synapse on vibrissa, jaw, and forelimb motor neurons (Takatoh et al., *Neuron*, 2013; Stanek et al., *eLife*, 2014; Soledad Esposito et al., *Nature*, 2014). This positions

SpVO and SpVIr as ideal candidates to understand the specificity of cortex-to-brainstem-to-muscle feed forward networks.
We use the transsynaptic tracer pseudorabies to confirm the presence of premotor neurons of the intrinsic vibrissae, digastric, and biceps brachii muscles in the spinal trigeminal nuclei. We show a dense population of premotor neurons for all three muscles in dorsal SpVO and a distinct population of mostly intrinsic vibrissae and biceps brachii premotor neurons in ventral SpVIr. Using a combination of modern viral techniques, we show that 1) motor cortex projections collateralize to both SpVO and SpVIr and 2) the density of motor cortex synaptic inputs shifts with respect to location of labeled neurons in motor cortex. We then virally labeled either SpVO- or SpVIr-projecting motor cortex neurons with a red-shifted channelrhodopsin (ReaChR; Lin et al., *Nat Neurosci*, 2013). We observe that long, i.e., ~ 10 s, trains of light evoked sustained, coordinated activity in a combination of vibrissa, jaw, and forelimb muscles (SpVO-projecting) or vibrissa and forelimb muscles (SpVIr-projecing), consistent with the types of premotor neurons found in each location. Last, we use Thy1-ChR2 mice to show that focal activation of all layer 5 motor cortex outputs results in broad muscle synergies that are specific to a motor act, e.g., forelimb to mouth or chewing.
All together our data illustrates the functional specificity of motor circuits beginning in the cortex and continuing into the premotor populations. We conclude that neurons in motor cortex broadly control muscles through many collateral pathways but with a specialized interface that results in behavioral specificity.
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Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

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Topic: E.04. Voluntary Movements

Support: Deutsche Forschungsgemeinschaft (SFB 889, project C9)

Title: Multimodal signal processing for grasp planning in the primate brain

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Abstract: Correctly interpreting the environment is essential for all animals in order to perform meaningful actions. Information perceived with different senses greatly facilitates the interaction

with objects or other animals, especially for manipulative actions.
Many brain areas are involved in the sensorimotor transformation process in control of hand grasping, ranging from object recognition to grasp preparation and grasp execution. However, the interaction between these different cortical areas and whether grasp planning activity depends on which sense has been used to perceive an object has not been studied in detail.
In order to shed new light on this topic, we trained two male rhesus macaques (7-8 years old, *macaca mulatta*) to perform a delayed-grasping task, in which they had to lift objects of different size and shape that have been perceived before either by vision or touch. During this task, we recorded spiking activity simultaneously from four relevant cortical areas: the anterior intraparietal area AIP, premotor area F5, primary motor area M1, and the primary somatosensory area S1.
Preliminary analysis from one animal revealed differences in spiking activity between visual and tactile trials. These differences were most prevalent during the preparatory period of the task, after the object was seen or touched but before the animal was instructed to grasp and lift it. Neurons in the grasp-related areas (F5 and M1) showed strong, object selective preparatory activity during the visual trials, as expected. However, this effect was much weaker during tactile trials and disappeared almost completely shortly before grasp execution, hinting towards a sensory modality-specific representation of objects or action intentions in these areas. In AIP, we found a weak memory effect during visual trials and close to no memory effect during tactile tasks. In somatosensory cortex, we observed no significant memory effect during visual trials and only a weak one during tactile trials.
Furthermore, behavioral analysis revealed that the animal was capable of preforming the correct grasp for each object without problems, making the assumption that the animal simply does not memorize the objects or grasps unlikely. Therefore, preparatory object information is either stored elsewhere in the brain (in areas we did not record from, such as prefrontal or temporal areas) or is encoded within these areas in a less straight-forward fashion, which both will require further investigation to resolve.

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Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

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Topic: E.04. Voluntary Movements

Support: CIHR MOP-125915

Title: Sex-related differences in the relationship between dementia risk and cognitive-motor integration performance

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Abstract: Cognitive-motor integration (CMI) involves concurrent thought and action which requires the interaction of large networks in the brain. The objectives of our research are to 1) investigate the effect that dementia risk has on the ability to integrate rules into action, and 2) to examine the neural basis of CMI impairment in individuals with dementia risk. Given evidence that early-stage dementia involves neural network dysfunction, we propose that problems with CMI can be used to detect dementia in its early stages. To this end, we previously tested females that are at high- and low-dementia risk (based on family history) on four increasingly visually-dissociated visuomotor tasks using two linked touchscreens (a standard condition requiring direct interaction and three dissociated non-standard conditions of visual feedback reversal, plane-change, and plane-change + feedback reversal). We observed that women at high risk for dementia compared to low-dementia risk showed deficits in movement accuracy and precision (error scores) for non-standard CMI tasks, and that poorer rule-based movement performance was correlated with neural network alterations typically seen in individuals with AD.[1, 2] To extend findings to the entire population and to test for sex-related differences in the relationship between dementia risk and cognitive-motor integration, the current study tested age-matched males at low- and high-risk for dementia on the standard and non-standard CMI tasks. Preliminary findings reveal no significant behavioural differences between high-risk (n=13) and low-risk (n=12) males. Analyses of sex-related differences revealed that low-risk males and females do not differ in their timing or error scores. Interestingly however, there is a significant difference in error scores in two of the cognitively demanding tasks (plane-change and plane-change + feedback reversal), where high-risk females performed worse than high-risk males. These data suggest that the underlying brain networks that control thinking and moving at the same time are different between men and women[3], and that dementia risk may affect female CMI performance to a greater extent. Future work will examine the exact nature of these task-related brain networks and their relationship to individual genetics using collected imaging and genetic data. 1. Hawkins KM, Sergio LE. 2014. J Alz. Dis. 42:607-621. 2. Hawkins KM, et al. 2015. J Alz Dis. 44:867-878. 3. Gorbet DJ, Sergio LE. 2007. Eur J Neurosci. 25(4):1228-1239.

Disclosures: A. Rogojin: None. **D.J. Gorbet:** None. **K.M. Hawkins:** None. **L.E. Sergio:** None.

Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 585.10/OO17

Topic: E.04. Voluntary Movements

Title: Sequence learning improves horizon and speed of motor planning

Authors: *G. ARIANI, N. KORDJAZI, J. DIEDRICHSEN
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Abstract: The ability to plan sequential movements prior to or while executing is an essential feature of skill development. While much of the motor learning literature has focused on planning mechanisms for single discrete movements (e.g. arm reaching), the interplay between planning and execution in the context of motor sequences has remained largely under-investigated. We sought to characterize two mechanisms underlying performance improvements in sequence production: 1) how the possibility of planning sequential movements in advance affects performance, and 2) how this planning ability evolves with sequence learning.
We designed two experiments where participants had to produce sequences of finger presses quickly and accurately in response to numerical stimuli (e.g., 1 = thumb, 5 = little). Experiment 1 manipulated the viewing window (i.e., the number of visible digits ahead of the current finger press) in 14-digit sequences to examine a spatial aspect of planning: how the availability of information about upcoming actions affects performance. Experiment 2 varied preparation time (i.e., the delay before making the first press) for 5-digit sequences using a forced reaction time task to examine a temporal aspect of planning: how the availability of time to plan affects performance. Moreover, to investigate how sequence learning interacts with motor planning and execution processes, both experiments had a period of training over a few days in which participants practiced producing reoccurring as well as random sequences.
Experiment 1 shows that performance improves significantly with the availability of viewing up to 2 digits ahead on the first day of training, and up to 4 digits ahead on the last day of training. Experiment 2 shows that performance in both single finger selection and sequence execution improves with longer preparation times: provided enough time, participants plan up to 3 digits ahead in the sequence, and sequence-specific planning improves as a result of learning. We take these results as evidence that the motor system takes advantage of time and visual information to plan sequential upcoming actions in parallel, and that both planning speed and horizon increase with learning.
We conclude that performance benefits in sequence production as a function of training can be explained by a combination of increased planning horizon, faster single finger selection, and improved parallel motor planning. Finally, we propose a computational model with multiple drift-diffusion processes that captures our behavioral results and sheds light on the interactions between planning, execution, and learning in sequence production tasks.

Disclosures: G. Ariani: None. **N. Kordjazi:** None. **J. Diedrichsen:** None.

Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

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Program #/Poster #: 585.11/OO18

Topic: E.04. Voluntary Movements

Support: NIH Grant: DA R01 027222

Title: Sex, age, and strain differences result in different behavioral responses to ritalin (methylphenidate) exposure

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Abstract: Both appropriate and inappropriate use of methylphenidate (MPD) has spiked in the last three decades by both sexes and all ages throughout the world. This study was conducted in order to determine if there is sex, age, and genetic strain differences in response to MPD exposure. The effect of variable MPD doses on the behavior of male and female adolescent (post-natal day 39-49) and adult (post-natal day >60) rats of three different genetic strains: Sprague-Dawley (SD) rats, Wistor-Kyoto (WKY) rats, and spontaneously hyperactive rats (SHR) were studies. Twenty-four male and twenty-four female groups were used. The 48 groups each had an N=8. The results show that adult male and females express more significant ($p<0.05$) hyperactivity as compared to adolescent rats. This difference may be due to ongoing brain development in male and female adolescents. Significant ($p<0.05$) differences in response to MPD were observed among the three genetic strains. There were also significant differences to MPD exposure between males and female rats. Female young and adult rats of all three strains responded with significantly more excitation in their behavior activity when exposed to MPD than their male counterparts. The difference between the adolescent female and male rats suggests that the difference between the sexes is only partially related to the gonadal system. In other words, factors aside from a mature reproductive system (not seen in adolescents) are implicated in the differential response to MPD exposure between sexes. All of these factors reinforce the importance of further studies to characterize differential individual responses to MPD, as this may play a role in potential misuse and dependency.
Acknowledge: This research was supported by a National Institutes of Health (NIH) grant, DA R01 027222

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Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 585.12/PP1

Topic: E.04. Voluntary Movements

Support: NSF Grant

Title: Dorsal premotor contributions to auditory timing: Causal transcranial magnetic stimulation studies of interval, tempo, and phase

Authors: *J. M. ROSS[1], J. R. IVERSEN[3], R. BALASUBRAMANIAM[2]
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Abstract: It has been suggested that networks involved with movement planning play an essential role in timing perception, but specific contributions of premotor cortex are unknown. The Action Simulation for Auditory Prediction (ASAP) hypothesis proposes that the dorsal auditory stream is involved in predictive beat-based timing through bidirectional interchange between auditory perception and dorsal premotor (dPMC) prediction. Although dPMC involvement in beat-based timing is supported by brain imaging, a causal role of dPMC has not yet been tested. We used a transcranial magnetic stimulation (TMS) protocol that down-regulates cortical activity, continuous theta burst stimulation (cTBS), to test for causal contributions of left dPMC to time perception in three experiments. These experiments observed interval timing perception, musical tempo perception, and musical phase perception. Perceptual acuity was assessed pre- and post-cTBS using a test of sub-second interval discrimination and the tempo and phase subtests of the Adaptive Beat Alignment Test (A-BAT), which tests the ability to detect mismatches between the beat of musical stimuli and a superimposed click track. We show ($N = 30$) that cTBS down-regulation of left dPMC interferes with interval timing ($t(29) = -2.083$, $p = .046$, Cohen's $d = 0.38$) and the ability to detect mismatches in musical tempo ($t(29) = -2.318$, $p = .028$, Cohen's $d = .42$). We did not find disruption of musical phase timing in this study ($t(29) = -1.265$, $p = .216$, Cohen's $d = .23$). Our data support causal involvement of premotor networks in timing, specifically of the left dPMC to accurate interval and musical tempo perception.

Disclosures: J.M. Ross: None. **J.R. Iversen:** None. **R. Balasubramaniam:** None.

Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

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Program #/Poster #: 585.13/PP2

Topic: E.04. Voluntary Movements

Support: Royal Society – Kohn International Fellowship NF170650

Title: Neurobehavioural imaging of natural motor learning in a complex human skill

Authors: *S. HAAR, C. M. VAN ASSEL, A. A. FAISAL
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Abstract: Motor skill learning is a key feature of our development and our daily lives, from a baby learning to roll, to an adult learning a new sport, or undergoing rehabilitation after a stroke. Human motor abilities are highly diverse, and as we keep learning new motor skills in all those skills we see tremendous diversity between individuals. While most of us can learn any skill, only some of us have the potential to excel in it. The process of real-world skill learning is long, complex, and difficult to quantify. As a result, it is rarely studied and very little is known about the behavioral and neural process of motor skill learning that makes some of us better learners. Such knowledge can change the way we teach kids, train athletes, or do rehabilitation. Here we use state of the arts methods to do holistic behavioral and brain recordings in order to study the longitudinal learning processes of real-world motor skill. The skill which we work on is playing pool table billiard, and we track the subjects over a period of 3 months, using inertial measurement units to record full body movements, eye-tracking glasses to record eye movements, and EEG to record brain activity. Using this rich data, recorded while naive subjects train in a novel task, we aim to unravel the key behavioral and neural processes that drive motor skill learning. Our results from the early phase of learning, while subjects are practicing repeated trials of the same shot, show a gradual change in the variability structure of arm and hand movement. We see a decrease in the variability of the right wrist rotation in parallel with a gradual increase the covariance between the rotation and the extension of the right wrist. We also see a gradual decrease in the covariance between the joint velocities of the right shoulder and the right elbow, which is in line with improvement in performance. These gradients suggest a biomarker of initial learning. While those are in line with the motor adaptation literature, which shows a gradual decrease in variability throughout adaptation processes, the covariance increase suggests far more complex changes in variability structure. Since skill learning is a longer and more complex process than adaptation, the continuation of this curve and how it changes across different shots is not clear and the accumulating data of this longitudinal experiment would give us insight to such principles of motor learning in complex human skill. This evaluation of changes in the complexity of behavior and its neural correlates (measured directly with EEG and

indirectly by eye movements), enable a systematic and integrative understanding of real-world motor skill learning and its neuronal requirements and constraints.

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Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

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Topic: E.04. Voluntary Movements

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Japan Society for the Promotion of Science 15H03044
Japan Society for the Promotion of Science 15H05871
Japan Society for the Promotion of Science 15H05880

Title: Transcranial static magnetic stimulation over human primary motor cortex can modulate implicit motor learning

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Abstract: Transcranial static magnetic stimulation (tSMS) is a recently introduced noninvasive brain stimulation technique that can modulate brain function, as well as transcranial magnetic and DC stimulations. However, it is not known whether tSMS can alter robust human behavior or not. Here, we investigated the hypothesis that motor learning might be interfered by tSMS applied over the primary motor cortex (M1), which was reported to temporarily suppress cortical excitability. For motor task, we chose a serial reaction time task including the pre-fixed sequences in the random series to evaluate an implicit motor learning, which M1 has been identified as a key structure for the acquisition and early consolidation. Forty-four healthy right-handed volunteers participated in the study. tSMS was placed over the right M1 (C4 of the 10-20 electroencephalography system) or dorsolateral prefrontal cortex (DLPFC: F4) which is associated strongly with conscious recall of the sequence (explicit learning). The control group received a sham stimulation. We tested their performance before (Pre) and after (Post) practice. The performance was also evaluated 24 hours later (Day2) to examine offline learning. Two participants recalled 8 items out of the twelve-item sequence during the free-recall test and thus

were excluded from analysis. A two-way ANOVA revealed main effects of stimulations and times; however, there was no statistically significant interaction. Although motor performance significantly improved with all groups in Post session, offline learning effect in Day2 session revealed in only M1 group. Offline learning was evident only in the group with M1 stimulation. These findings suggested that modulation of M1 using tSMS, which is reported to suppress cortical excitability, may actually enhance offline motor learning in an implicit task.

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Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

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Topic: E.04. Voluntary Movements

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Title: Improving motor learning via phase-amplitude coupled theta-gamma tACS

Authors: *H. AKKAD[1], J. DUPONT-HADWEN[2], S. BESTMANN[2], C. J. STAGG[1]
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Abstract: Theta (θ) oscillations are thought to underlie at least some aspects of long-range network functional connectivity, and within a given brain region interact with local gamma (γ) frequency oscillations in a specific type of cross frequency coupling known as phase-amplitude coupling (PAC). PAC is thought to be an important mechanism by which the brain is able to modulate local activity, and has been postulated to have behavioural importance. Recent work suggests that transcranial alternating current stimulation (tACS) can engage endogenous oscillatory circuits in a behaviourly relevant manner.
We investigated the effect of θ-γ PAC on motor learning using tACS over the right primary motor cortex (M1) in a randomized, single-blind, sham-controlled study. When applied exogenously using tACS, θ-γ coupling should exert its excitatory effects during the positive part of the waveform (peak), thus increasing neural firing. Therefore, we hypothesized that applying peak-coupled θ-γ tACS over M1 might enhance learning compared to trough-coupled theta-gamma or sham stimulation. We used a ballistic thumb abduction training task requiring abduction of the left (non-dominant) thumb with maximal acceleration. We used two θ-γ waveforms: a 6Hz θ rhythm modulated the amplitude in the γ-band either at the peak (phase 0°-180°) or trough (phase 180°- 360°) of the θ wave; we will refer to these as θ-γ-peak (TGP) and θ-γ-trough (TGT), respectively. Both real stimulation conditions had a 2mA peak-to-peak

amplitude and a stimulation duration of 20 minutes. 60 subjects were equally randomized to receive TGP, TGT or sham stimulation in a between-subject design during training on the thumb abduction task.
All groups significantly improved their motor performance over the course of the experiment. These improvements were larger in TGP tACS compared to both sham and TGT stimulation, though the latter did not reach significance, whereas TGT and sham showed no significant difference. TGP-tACS resulted in a mean maximal acceleration gain that was ~26% larger than in the TGT or sham groups. Additionally, towards the end of stimulation, the TGP group improved their mean acceleration from baseline by an effect size of Cohen's d= 0.98 compared to sham.
Our findings suggest that θ-γ PAC is important for motor learning and that tACS has the capacity to modulate it. Additionally, our intervention appears to facilitate a substantial gain on motor learning. We are currently replicating our findings in an independent sample, now using a double-blind design and blinded analysis approach. The replication study is pre-registered in full on the Open Science Framework (https://osf.io/452f8/registrations/).

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Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

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Topic: E.04. Voluntary Movements

Support: Acibadem University ABAPKO Grant 2016.04.01

Title: Non-invasive EEG based assessment of laparoscopic clinical simulation based training with first time users

Authors: F. UCRAK[1,2], *M. B. BAYRAM[3], I. A. OZCAN[3], M. E. AKSOY[4], B. ERKMEN[2]
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Abstract: INTRODUCTION: Laparoscopic surgery requires superior and more distinct psycho-motor skills compared to the open surgical procedures. While quantitative determination of the skill level is difficult, the use of electroencephalography (EEG) to measure competency gains has potential to create an unbiased criterion for determining the permanent performance of laparoscopic surgical simulation training. Measurement of brain waves by EEG can

quantitatively indicate how much of the brain capacity is used. There is no precise and complete work in this area, especially in laparoscopic simulations, using two hands in coordinated fashion, tasks that must be completed in a limited amount of time. For this purpose, EEG data collected during laparoscopic surgical simulation training was quantified spectrally and evaluated statistically.
METHODS: In the framework of the protocol approved by the ethics committee, 10 male (right dominant, 22±2.43 years, mean±SD) university students who had no previous experience in laparoscopic surgical simulator participated in the experiment with written consent. The peg transfer test, which is a laparoscopic surgical simulation training module, was performed on two separate dates, exactly one week apart. Each day, the test was performed consecutively for 3 times. EEG was recorded through 32 channels simultaneously. Spectral analysis of EEG data was performed using the NPXLab™ Suite (written by Luigi Bianchi). The results were statistically evaluated with IBM SPSS™ software.
RESULTS: When two time points of relative power were observed in anatomically, significant increases were found in the alpha and theta bands in the T3-T5 channel; alpha bands in the T5-O1 and P4-O2 channels. The increase in the alpha band showed the relaxation and the increase in the theta band might mean that creativity, emotional connection, intuition and relaxation taking place. The change in the T3-T5 region indicated that the subjects remembered the previous experiment and were calmer and the change in the T5-O1 region allowed subjects to understand the process more easily, while the change in the P4-O2 region showed that they used the non-dominant side and spatial memories more actively. It is observed that when the subjects came for the second day, they had higher concentration, performed the tests more calmly and remembered the operation, based on their successful test runs, compared to their first day.
CONCLUSION: EEG rhythms have a direct relationship with physical motor activities and motor planning. Our preliminary findings may be used as a marker for a quantitative formation of laparoscopic surgical simulation training criterion.

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Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 585.17/PP6

Topic: E.04. Voluntary Movements

Title: Low-frequency modulation of discrete goal-directed force contractions

Authors: *S. L. BRACKSIECK[1], E. SAJJADI[2], A. CASAMENTO MORAN[1], B. YACOUBI KEYHANI[1], E. CHRISTOU[1,2]
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Abstract: Fluctuations in force during steady force tasks are mainly explained by low frequency oscillations (<0.5 Hz). Although this low-frequency modulation of the force output is a strong predictor of the within-contraction variability, it remains unknown whether it predicts the variability of repeating discrete dynamic force contractions. Thus, our purpose was to characterize the modulation of the force output across discrete dynamic goal-directed force contractions. Nine young adults (23.6±4 years) performed a 50-s dynamic task with the dominant ankle. Within this time, participants repeated force contractions at a frequency of 0.8 Hz aiming at a force amplitude of 10% and 40% maximum in separate trials. This manipulation of force increases the voluntary drive to the motor neuron pool. We quantified the modulation of the force output by quantifying the power spectrum density of the peak force. To be able to examine the power spectrum density of discrete data points, we transformed the discrete peak forces across trials into a continuous signal. We examined the power spectrum at a 0.05 Hz resolution. All of the subjects exhibited a clear peak in the modulation of peak force at ~0.1 Hz for both the 10% (0.09±0.01 Hz) and 40% (0.1±0.01 Hz) amplitude. The low-frequency modulation of peak force was related to the variability of peak force across contractions for 10% (R^2=0.71, P<0.01) and 40% (R^2=0.69, P<0.01). As expected, the peak force variability increased from 10% to 40% maximum (0.03±0.005 N^2) and related to an increase in low-frequency oscillations from 0-0.2 Hz (R^2=0.56, P<0.01). Thus, similar to steady contractions, the force output variability of discrete contractions is influenced by a low-frequency modulation. Given that these low-frequency oscillations are present during steady contractions and fast discrete dynamic contractions, it likely suggests a central origin.

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Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 585.18/PP7

Topic: E.04. Voluntary Movements

Title: Conflict and suppression during action preparation: Suppression of task set, not responses

Authors: *J. XU, L. ELPHAGE, A. M. HAITH
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Abstract: In our daily activities, we often need to withhold an automatic, habitual response in order to carry out the desired action. Prevailing theories suggest that such selection is achieved through a combination of *conflict monitoring* (detecting whether there are conflicting candidate responses) and *response suppression* (inhibiting specific actions) to allow the correct, deliberate response to be generated (Botvinick et al., 2001; Ridderrinkof, 2002; Wiecki & Frank, 2013). Here, we present evidence against this framework, suggesting instead that conflict is detected and resolved at the level of stimulus-response *mappings*, rather than at the level of individual *responses*.
In our task, an arbitrary symbolic cue (Phoenician letter, or colored 'X') instructed participants which of four potential directions to aim a reaching movement (e.g. blue = left). We created a conflict by presenting this cue in a spatial location that was sometimes incongruent with the instructed direction. In order to track the evolution of participants' response preparation, we had participants perform this task under timed-response conditions - forcing them to emit responses at a range of preparation times (PTs) ranging from 0 to 600ms. In incongruent trials, when participants were forced to respond at very low PTs (200-400ms), participants reliably (and erroneously) moved towards the spatial location of the stimulus. At higher PTs (>500ms), however, they were able to consistently select the correct response. At intermediate PTs (300-400ms), however, participants transiently increased their probability of responding to one of the two locations that did not follow either map. This pattern is consistent with the idea that the prepotent response was suppressed before the deliberate response was selected. However, this signature of suppression was apparent even in congruent trials, in which there was no conflict between candidate responses. Our results challenge the view that response preparation is influenced by monitoring of conflict between candidate responses and selective suppression of prepotent responses. We suggest that response preparation and response initiation may be sensitive to different forms of conflict, with response preparation influenced primarily by conflict between task sets (SR mappings), and response initiation influenced by conflict between candidate responses.

Disclosures: L. Elphage: None. **A.M. Haith:** None.

Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 585.19/PP8

Topic: E.04. Voluntary Movements

Title: Individuals with an ACL reconstruction have altered neuromotor function

Authors: *C. N. ARMITANO, S. MORRISON, D. M. RUSSELL
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Abstract: The anterior cruciate ligament (ACL) is a key structural component in stabilizing the knee joint during purposeful movement. However, damage and subsequent ACL reconstruction does not always often result in a return to normal function. Indeed, wide spread motor problems can emerge as a result of the absence of a natural ACL. For example, individuals with a reconstructed ACL often exhibit increased variability and irregularity coupled with changes in coordination during gait. What has not been assessed to date is whether ACL damage also leads to slowing of responses under postural conditions. The current study was designed to compare differences in reaction time under both seated and postural (i.e. standing) conditions. It was also of interest to examine how ACL reconstructed individuals responded under the more challenging postural task. Fifteen adults with unilateral ACL reconstruction and 15 age-matched healthy controls participated in this study. Baseline assessment of neuromotor function including measures of proprioception, balance, strength, and walking ability were performed. Simple and choice reaction time response were assessed under seated (i.e. control) conditions and during a postural stepping task. The results revealed similarities between both groups with regards to the baseline measures of proprioception, balance, strength, and gait as well as the seated reaction time tasks. However, during the postural stepping task, individuals with ACL reconstruction had significantly slower reaction times compared to the healthy controls. This finding indicates that these persons had a reduced ability to respond quickly under more challenging postural conditions. This finding of slower responses when stepping for the ACL reconstructed adults may be a compensatory response to the previous injury and/or residual symptoms post-ACL reconstruction. Overall, these findings indicate that reconstruction of the ACL ligament impacts neural mechanisms, altering individuals' ability to respond under challenging balance tasks.

Disclosures: C.N. Armitano: None. **S. Morrison:** None. **D.M. Russell:** None.

Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 585.20/PP9

Topic: E.04. Voluntary Movements

Title: Eye movements in real baseball batting by elite players

Authors: *Y. KISHITA[1], M. KASHINO[2,1]
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Abstract: In baseball, it takes only about half a second for pitched balls to reach home plate. In such a short amount of time, baseball batters judge whether to hit or not and then swing the bat at the right time and place. A previous study (McLeod, 1987) suggests that it takes about 200 ms to adjust their swing to novel visual information. The swing itself takes about 200 ms as well. For

these reasons, baseball batters predict the ball's trajectory and start their swing when the ball is still far from home plate. Moreover, ball velocity in visual angle reaches more than 500 deg/s around home plate. For this reason, it is considered that batters cannot keep their eye on the ball through its full fright. Our question is: How do good batters hit the ball in such tough conditions, What is a reasonable visual-motor strategy? We examined eye movements of baseball batters hitting random pitches of two types: a fast ball (about 130 km/h) and a curve ball (about 110 km/h) in order to elicit eye movements that would take place in real baseball games. Batters were required to judge the pitch a fast ball or curve ball before swinging the bat. Participants included professional players, skilled non-professional players, and a former college league player. Batters hit a series of pitches wearing a helmet equipped with an eye tracker (500 fps). In most cases, batters kept their gaze with fixational-like eye movements on the ball in the early stages, and then started predictive saccades toward the ball. The saccadic eye movements landed 0 - 200 ms before bat-ball contact in most cases, and the relationships between the ball and predictive saccades in terms of time and space differed according to batting results. In successful trials, predictive saccades led the gaze to where ball be or would be. However, in false trials (swing away or miss), the gaze reached the wrong place. In some cases, misjudgments of the types of pitch (fast ball or curve ball) led the gaze to the batter's predicted trajectory of the pitch. These results suggest that predictive eye movements reflect the prediction of ball trajectories and types of pitches.

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Poster

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Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 585.21/PP10

Topic: E.04. Voluntary Movements

Support: JST CREST JPMJCR14E4

Title: Cognition-action behavior of top athletes in experimental batting explains their performance in real games

Authors: *D. NASU, A. KOBAYASHI, M. YAMAGUCHI, N. SAIJO, M. KASHINO, T. KIMURA
NTT Communication Sci. Labs., Kanagawa, Japan

Abstract: Human action often involves cognitive processes, i.e., sensory processing, prediction and decision making. Sports provide a case in point, and top athletes should possess high abilities in both cognition and action. In sports, such a cognition-action process frequently occurs within a split second. For example, in baseball/softball, a batter has to predict the trajectory of a

pitched ball, decide whether to swing and hit it within about 500 ms from the time the pitcher's releases. Previous studies have shown the importance of cognitive and motor processes in baseball/softball. However, they examined each process separately, which leaves the overall picture veiled. In particular, the relationship between the two processes and how much each contributes to the batting performance in real games are still unclear.
Here, we paired a cognitive task and a real batting task to investigate the cognition-action structure in softball batting. Elite women softball players, some of whom had been members of the national team, participated in the experiments. Balls were thrown by real pitchers at two speeds, one relatively higher than the other. Batters responded by pressing a button in the first task (cognitive task). Then they tried to hit a ball in the next task (batting task). In both tasks, batters did not know how fast the ball would be thrown. Since different ball speeds change the time to contact (TTC), participants had to predict the ball speed/TTC by discriminating ball speed and to swing according to their prediction. To clarify the cognition-action structure, we introduced a path analysis using structure equation modeling. Our model consisted of six variables, obtained from the two tasks and the season batting average.
Our model showed good fitting (χ^2 test, $p = 0.58$; CFI = 1.00; TLI = 1.07; RMSEA = 0.00) and produced three findings. First, the discrimination accuracy (false alarm rate) and speed (reaction time) obtained from the cognitive task were significantly related to the time shift of swing onset in the batting task, indicating that batters who showed accurate and early discrimination shifted their swing onset according to ball speed/TTC. Second, batters who shifted their swing onset showed superior batting performance (i.e., high exit velocity and low miss ratio) in the batting task. Third, the experimental performance was significantly related to batting average in the 2017 season. Overall, we described the cognition-action structure for top athletes quantitatively and showed that the relationship between these two processes could explain a batter's performance in real games.

Disclosures: D. Nasu: None. **A. Kobayashi:** None. **M. Yamaguchi:** None. **N. Saijo:** None. **M. Kashino:** None. **T. Kimura:** None.

Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 585.22/PP11

Topic: E.04. Voluntary Movements

Title: Availability of pitching motion information in batting timing control revealed by virtual reality

Authors: *T. KIMURA, D. NASU, M. YAMAGUCHI, M. KASHINO
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Abstract: Recently, we found that skillful softball batters adjust the timing of their trunk motion in bat swings according to ball speed when the pitcher randomly throws slow or fast balls. The timing adjustment starts about 300 ms after the pitcher releases the ball, suggesting that expert batters make very early decisions about swing timing based on information related to the pitching motion. In this study, we manipulated the pitching motion using a virtual reality (VR) batting system to assess its effect on batting timing control in female expert batters, including national team players. We used a head-mounted display-based VR system for application to softball batting. With this system, batters can experience highly realistic virtual hitting of a pitched ball: pitched balls are depicted based on previously recorded ball trajectories, thrown in time with the motion of a pitcher avatar based on simultaneously recorded motion capture data, and launched depending on their interaction with the bat. In this virtual batting measurement, we found that the trunk rotation timing in some players started earlier for mismatched combinations (slow balls thrown with fast ball pitching motion) compared with matched combinations (fast ball motion and a fast ball). In a separate measurement, where they were asked to push a button as soon as they had judged the ball to be a fast ball, they showed increased uncorrect responses in the mismatched combination. Interestingly, none of the participants were conscious of such mismatched combinations. These results empirically indicate one cognitive feature in the implicit (unconscious) brain processing of skillful bat control; namely, some batters unconsciously utilize information related to the pitching motion when deciding whether or not to swing (go/no go). Our VR system will provide novel insights into athletic performance and its neural mechanisms in a batting scenario, which had been hard to obtain in conventional measurements.

Disclosures: T. Kimura: None. **D. Nasu:** None. **M. Yamaguchi:** None. **M. Kashino:** None.

Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 585.23/PP12

Topic: E.04. Voluntary Movements

Support: JSPS JSPS KAKENHI 18K17889

Title: Increased gain in online correction of a reaching task in ball game athletes

Authors: *T. IJIRI[1], H. KOBAYASHI[2], K. NAKAZAWA[1]
[1]Dept. of Life Sci., [2]Grad. Sch. of Arts and Sci., The Univ. of Tokyo, Tokyo, Japan

Abstract: Introduction Catching or hitting a flying ball requires us to adjust the trajectory of hand or bat within an very short time to minimize spatiotemporal error. During arm reaching movement, the movement can be adjusted to sudden target displacement in a much shorter latency than that of conventional button-press choice reaction task (Kadota and Gomi 2010). The

initial component of the correction is known to be implicit and unconscious (Prablanc and Martin 1992). Although the fast and implicit visuomotor processing could be crucial for athletes' high performance, there is no research that examined the characteristics of the motor response of athletes at short latency described above. Here, we investigated the latency and gain of unconscious movement correction athletes and non-athletes.Methods: Ten male subjects (mean age of 24.0 years old; range of 21-35; 5 ball-game athletes and 5 non-athletes; all right handed) participated in this study. Subjects were asked to move their right index finger to a visual target shown at the center of PC monitor. In one-third of the trials, the target jumped leftward or rightward 60 ms after the finger movement initiation (TJ trial). The task was performed under two conditions: subjects were instructed to adjust their movement to the new target location (pro-task) and in the opposite direction to the new target location (anti-task). The subjects performed 90 trials for each condition (180 trials in total). A reflective marker was attached on the tip of index finger and the marker position was measured with a motion capture system (oqus300, Qualisys) at 500 Hz. Latency of movement correction was defined as the time from the target displacement to moment when the lateral/medial acceleration of the index finger became different from that of control trial (no target jump). Gain of unconscious movement correction in anti-task was defined as the difference of peak acceleration amplitude between left and right TJ trials.Results: The mean latency of movement correction in pro-task was 103.0 ± 15.0 ms in athletes and 110.5 ± 3.6 ms in non-athletes. In anti-task condition, The mean gain of movement correction was 5.90 ± 1.1 m/s^2 in athletes and 2.3 ± 0.9 m/s^2 in non-athletes.Discussion: Our results suggest that the latency of movement correction was not different between athletes and non-athletes, on the other hand, the gain of movement correction was highly increased in ball game athletes. We also acquired diffusion weighted MRI for each subject to further analyze a neural mechanism underlying the fast corrective response. The structural connection between V5/MT+ and lateral geniculate nucleus or superior colliculus will be examined in the future study.

Disclosures: T. Ijiri: None. **H. Kobayashi:** None. **K. Nakazawa:** None.

Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 585.24/PP13

Topic: E.04. Voluntary Movements

Support: ERC Starting Grant number: 335328
NFR Grant number: 239963

Title: Efficient cortical coding of 3D posture in freely behaving rats

Authors: *B. MIMICA, B. A. DUNN, T. TOMBAZ, S. BOJJA, J. R. WHITLOCK
Kavli Inst. for Systems Neurosci., NTNU, Trondheim, Norway

Abstract: In order to meet physical or behavioural demands of their environments, animals constantly update their body posture, but little is known about the neural signals this ability is dependent on. To better understand the role of cortex in relation to natural pose and movement, we tracked heads and backs of eleven freely foraging rats in 3D while recording from posterior parietal cortex (PPC) and frontal motor cortex (M2), areas reliably shown to encode orienting movements or movements of individual effectors, such as the eye, arm and hand. This enabled us to determine arena-centered (i.e. allocentric) relevant variables, namely the animals' spatial locations, but also to disambiguate head-direction from movement-direction. In addition, we estimated head rotations around three axes (azimuth, pitch and roll) relative to the body (i.e. in egocentric coordinates) together with postural states of the back alone. Our analysis of coding properties of individual neurons revealed remarkable specificity in tuning, mainly to the combination of different head angle positions relative to the body, but sometimes also to azimuthal flexion and the pitch of the back independently. These coding properties were shown to persist even in complete darkness. Importantly, detailed GLM analyses revealed single units in both regions were predominantly tuned to postural features of the head, back and neck, but only to a lesser degree to their movements. Likewise, the observed coding scheme was distributed in an efficient manner, where more cells were likely to be tuned to features which were less likely to occur during unrestrained movement, thereby not over-representing the default-state posture. Representations of the head and back were organized topographically, and the analysis of signal correlations across areas suggest that, on average, PPC preceded M2 activity by ~50ms. Tuning in both areas was sufficiently robust to allow reconstruction of ongoing pose with ~90% accuracy. Together, these data provide a first-time view of PPC and frontal motor areas' activity related to body pose in unrestrained individuals.

Disclosures: B. Mimica: None. **B.A. Dunn:** None. **T. Tombaz:** None. **S. Bojja:** None. **J.R. Whitlock:** None.

Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 585.25/PP14

Topic: E.04. Voluntary Movements

Support: ERC Grant 335328

Title: Task-dependence of movement coding in mouse parietal cell populations

Authors: *T. TOMBAZ[1], B. A. DUNN[1], R. J. A. CUBERO[1], P. MAMIDANNA[2], K. HOVDE[1], B. MIMICA[1], J. R. WHITLOCK[1]
[1]Kavli Inst. for Systems Neurosci., NTNU, Trondheim, Norway; [2]Inst. for Theoretical Physics, Werner Reichardt Ctr. for Integrative Neurosci., Eberhard Karls Univ., Tubingen, Germany

Abstract: A fundamental question in neuroscience is the elucidation of neural mechanisms underlying natural behaviors, which emerge when freely moving animals interact with the environment. To ensure survival, the brain must be able to rapidly generate flexible behaviors to meet the demands of different contexts. However, the extent to which movement coding in cortex depends on an animal's given context is not well understood. To investigate the specificity or generality of behavioral coding at the single-cell and network levels, we recorded neural population activity in posterior parietal cortex (PPC) while freely behaving mice performed different tasks. We chose this area because it interfaces with sensory and motor processing streams in cortex, and because it has well-established functions in the spatial coordination of motor behavior. Specifically, we performed *in vivo* calcium imaging in layer 2/3 and layer 5 of PPC while animals engaged in goal-oriented behaviors in a pellet-reaching task, and during spontaneous foraging in an open field with a running wheel. Single cell analyses showed that 40-50% of PPC neurons stably represented various behaviors in each task, such as grasping food or rearing. While a large fraction of the population was active in both tasks, this was not informative of the extent to which the network state discretized behavioral contexts. To address this, we applied the t-student stochastic neighbor embedding (t-SNE) dimensionality reduction method, which embedded deconvolved spike trains of the imaged ensemble in a two-dimensional manifold. This revealed clusters of neural activity states that could be easily visualized and used to indicate discrete behaviors. Moreover, the activity clouds between different tasks were completely non-overlapping, despite the similarity of individual behaviors across tasks (e.g. turning). Our results demonstrate that individual PPC neurons are tuned to multiple actions in different tasks, but the way in which cell populations represent these actions differs fundamentally depending on the context in which they were embedded.

Disclosures: T. Tombaz: None. **B.A. Dunn:** None. **R.J.A. Cubero:** None. **P. Mamidanna:** None. **K. Hovde:** None. **B. Mimica:** None. **J.R. Whitlock:** None.

Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 585.26/PP15

Topic: E.04. Voluntary Movements

Support: NFR Grant number: 239963
ERC Starting Grant number: 335328

Title: Neural representations of discrete, sequential behaviors in the rodent posterior parietal and frontal motor cortices

Authors: *B. DUNN[1], T. TOMBAZ[2], B. MIMICA[2], K. HOVDE[1], J. R. WHITLOCK[3]
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Abstract: There is an emerging view that posterior parietal (PPC) and secondary motor (M2) cortices represent behaviors at different scales: from elemental poses that comprise behavior, to the choice of the behavior itself. It is not clear, however, how these areas encode the different sequences of dynamic posture underlying the rich diversity of naturally-occurring behaviors. To address this, we observed freely-behaving rats while recording from neurons in PPC and M2 using silicon probes, or while performing calcium imaging using miniscopes in freely-running mice. We tracked the head and body of the animals in 3D and, using machine learning methods, segmented the animals' movement data into sequences that capture the common movement patterns found across animals. We identified a combination of neurons whose tuning was independent of the behavioral sequence in which a movement was embedded, while others responded preferentially to specific sequences. In light of these findings, we propose that PPC and M2 not only encode basic features of behavior, but string postural sequences together to form meaningful sequences of actions.

Disclosures: B. Dunn: None. **T. Tombaz:** None. **B. Mimica:** None. **K. Hovde:** None. **J.R. Whitlock:** None.

Poster

585. Voluntary Movements: Cortical Planning and Execution: Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 585.27/PP16

Topic: E.04. Voluntary Movements

Support: German Research Foundation (DFG) SCHE 1575/3-1

Title: Uncorrelated low-dimensional population response and noise correlation network structure in the macaque fronto-parietal grasping network

Authors: *B. DANN[1], H. SCHERBERGER[1,2]
[1]German Primate Ctr., Goettingen, Germany; [2]Dept. of Biol. and Psychology, Univ. of Göttingen, Goettingen, Germany

Abstract: Recently developed multi-electrode arrays and corresponding recording systems enabled analyses of how neuronal populations perform cognitive and behaviorally relevant

computations. Several studies on monkeys and mice performing perceptual choice and delayed movement tasks revealed that neuronal population activity in prefrontal, parietal, and temporal cortex could be well understood as a dynamical process in a low-dimensional space with far less dimensions than neurons. It has been hypothesized that the low-dimensional population structure is tied to the underlying network connectivity, but since it is impossible to measure the structural connectivity of the corresponding neuronal population, this relationship has not been studied directly. However, noise correlations capturing the trial-to-trial co-fluctuations estimated for the same specific task conditions and with high temporal precision can be assumed to reflect structural connectivity as an approximation. This allows a direct comparison of the noise correlation network structure with the low-dimensional population response to provide a first glimpse of their relationship.We used parallel recordings from about 48-90 neurons in the fronto-parietal grasping network while two monkeys performed a free-choice or instructed delayed grasping task. The low-dimensional single trial population structure was extracted using a customized dimensionality reduction method based on linear discriminate analysis. We found that seven dimensions captured more than 80 percent of all task-related single trial population activity. The fine-scale noise correlations network structure was extracted using pairwise cross-correlations that were corrected for correlations induced by task-related activity. For this cross-correlated surrogate activity with the same low-dimensional population structure was simulated and subsequently subtracted.Intriguingly, the contributions to the seven dimensions of population activity and the number of significant noise correlations per neuron were heavy-tailed distributed showing a high degree of heterogeneity of neuronal contributions. However, the number of significant noise correlations per neuron was uncorrelated with the contributions to any of the population activity dimensions (mean $R^2 <$ 10E-2). Based on these results we hypothesize a continuum in the population, in which some neurons strongly encode task relevant information but contribute little to the network communication (‘information hubs’), whereas other neurons hardly encode task-related information but are crucial for network coordination (‘coordinator hubs’).

Disclosures: B. Dann: None. **H. Scherberger:** None.

Poster

586. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Human

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 586.01/PP17

Topic: E.04. Voluntary Movements

Title: Modulation of corticomotor excitability in response to localized cooling

Authors: Y. ANSARI[1], A. REMAUD[2], *F. TREMBLAY[1]
[1]Sch. of Rehabil. Sci., Univ. of Ottawa, Ottawa, ON, Canada; [2]Clin. Neurosci. Lab., Bruyere Res. Inst., Ottawa, ON, Canada

Abstract: Thermal stimulation (TS) has been proposed as a method to facilitate motor recovery after stroke, but its neural basis remains poorly defined. Recently, we showed (Ansari et al., 2018) that distal focal cooling or warming stimulation targeting a single digit produced a variable modulation in corticomotor excitability, as reflected in motor evoked potentials (MEPs). These results raised the question as to whether extending the area of TS could produce more consistent effects. Here, we report our observations regarding the impact of a ~3X increase in cooling area, going for single- to multi-digit application. Participants (n=22, young and senior, 12 females) consisted of a subset of the pool who participated in our original study with single digit cooling (Ansari et al., 2018). The protocol was identical and consisted in measuring skin temperature and MEPs (target muscle, FDI) at baseline (BL), during cooling at 1-min (C1) and, then, post-cooling at 5-min (PC5) and 10-min (PC10). The cooling stimulation was produced using a large Torex® gel pack sleeve that covered the four digits up to the metacarpal bones, sparing the thumb finger. For BL measurements, a neutral gel pack at 24° covered the fingers, whereas, for those with cooling, a gel pack at 10° was applied. Increasing the area of cooling produced comparable changes in skin temperature with a peak decline at C1 of -11.1 and -10.4 °, respectively. No main effect or interaction was detected on MEPs ($F<1.0$, $p>0.49$), indicating that extending the cooling area did not influence variations in amplitude. However, a trend was noted for MEP latency, which tended to be prolonged with multi-digit when compared to single-digit cooling. Much like in our first study, individual responses were variable with ~half the participants showing MEP depression (9/22) at C1 and one-third showing facilitation (7/22). The rest showed no modulation (6/22). These proportions were comparable to those previously reported for single-digit cooling. Interestingly, variations in MEP amplitude measured at C1 for single-digit were correlated ($r=0.50$, $p=0.02$) with those measured for multi-digit cooling at the individual level. These results indicate that extending the area of cooling stimulation in the distal hand did not produce more consistent effects on corticomotor excitability. Also, our results confirm that modulation in response to distal focal cooling are variable from one person to another but seems fairly consistent for a given individual with repeated applications, regardless of the area of stimulation.

Disclosures: Y. Ansari: None. **A. Remaud:** None. **F. Tremblay:** None.

Poster

586. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Human

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 586.02/PP18

Topic: E.04. Voluntary Movements

Support: JSPS short-term Fellowship FY2017

Title: The inhibition of voluntary muscle relaxations depends on similar mechanisms to the inhibition of muscle contractions

Authors: *J. DE HAVAS[1,2], S. ITO[1], H. GOMI[1]
[1]Human & Information Sci. Lab., NTT Communication Sci. Labs., Atsugi, Kanagawa, Japan; [2]JSPS Intl. Res. Fellow, Tokyo, Japan

Abstract: Successful control of the body depends on both our ability to execute planned movements, and on our ability to rapidly cancel movements that are no longer required. Most movements are executed via muscle contractions. However, voluntary movements are also often the result of a combination of gravity and a voluntary muscle relaxation. Such voluntary relaxations have been found to be preceded by increased activity in prefrontal and motor regions of the brain, suggesting they result from a motor plan. Determining whether voluntary muscle relaxation can be inhibited once initiated, and if so, what time-course and muscle activity is associated with this inhibition, will be informative with regards to establishing how voluntary relaxations are planned and executed by the brain. To this end, we employed a modified version of the Stop Signal Task to compare inhibition of muscle relaxations and contractions in humans. Participants sat in front of a screen with their elbow on a padded table, supinated and bent at an angle of ~40° from the horizontal. They were instructed to move their arm downwards as rapidly as possible whenever a green circle appeared on the screen (Go trials), and remain stationary whenever a green square appeared (No-go trials). During 'relaxation blocks' they moved the arm downwards by relaxing the biceps muscle, but during 'contraction blocks' the movement was achieved by contracting the triceps muscle. Participants were instructed that if they heard a tone (Stop trials) they should cancel the movement and maintain the current arm position. Tone onset time in relation to the go signal varied between 0 and 500ms (stop signal delay). Electromyography was recorded from right biceps brachii and triceps brachii (lateral head). Results from n = 5 (4 female, mean age = 32.6, SD = 9.34 yrs) participants indicated that there was no significant difference in RT for the go trials across contraction and relaxation conditions (500 vs. 537ms; $t(4) = 1.56$, $p = 0.19$). Both voluntary contraction and relaxation commands could be inhibited ~50% of the time. The probability of successfully inhibiting a response increased reliably as stop signal delay decreased. By plotting probability of moving against stop signal delay it was possible to calculate stop signal reaction time (SSRT). We found no significant difference in SSRT across contraction and relaxation conditions (237 vs. 220ms; $t(4) = 0.9$, $p = 0.42$). In both conditions cancelling movement was associated with transient increases in muscle activity, time-locked to the stop signal. The results suggest there may be similar mechanisms underlying how voluntary muscle relaxations and contractions are initiated and controlled.

Disclosures: J. De Havas: None. **S. Ito:** None. **H. Gomi:** None.

Poster

586. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Human

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 586.03/PP19

Topic: E.04. Voluntary Movements

Title: Cerebellar-motor cortex connectivity: One or two different networks?

Authors: *D. SPAMPINATO[1], P. A. CELNIK[3], J. ROTHWELL[2]
[1]Biomed. Engin., [2]Univ. Col. of London, London, United Kingdom; [3]Physical Med. & Rehab, Neurol, Johns Hopkins Univ., Baltimore, MD

Abstract: Recently it has been argued that two distinct interneuron networks in the primary motor cortex (M1) contribute distinctly to two varieties of physiological plasticity and motor behaviors (Hamada et al., 2014). Although one of the interneuron groups is thought to be dependent on cerebellar (CB) activity, direct physiological distinction regarding CB-M1 interactions (CBI) to these subpopulations remains poorly understood. In a series of experiments, we assessed whether M1 coil orientation, thought to test different neuronal populations, is differentially influenced by cerebellar stimulation. In experiment 1 (n = 10), we tested the effect of coil orientation (posterior-anterior, PA; anterior-posterior, AP) and inter-stimulus intervals (ISI: 3, 5 and 7 ms) on CBI; assessed with a conditioned TMS pulse over the cerebellum prior to TMS over the contralateral M1. We found there was a significant ISI x coil orientation interaction, specifically PA-CBI was most prominent at 5ms ISI (p = 0.02), whereas AP-CBI at 7ms ISI (p = 0.01). In a follow-up experiment, we sought to determine whether this result reflects distinct processing of cerebellar inputs within M1. To do this, we measured AP- vs. PA-CBI at their preferential ISI, prior to and following standard paired associative stimulation (PAS). Importantly, we administered the repeated pairs of electrical stimuli to the median nerve and PA-TMS at an interval of 21.5 (i.e. PAS at 21.5) since this technique is capable of modulating the plasticity of PA-M1 excitability without affecting cerebellar activity. We found that PA-CBI changed following PAS 21.5 (p = 0.04), but did not modulate AP-CBI (p = 0.47), indicating that CB-M1 interactions are different for the two M1 neural networks. In a final experiment (n = 12), we assessed whether M1 coil orientation affects CBI in the context of two motor behaviors that weight differently cerebellar vs. M1 contributions. Here, we tested how learning two distinct motor learning tasks (weighting sensorimotor calibration vs. a sequencing task) affected AP- vs. PA-CBI measured at their preferential ISI. To determine how learning affects AP- vs. PA-CBI, we compared CBI before, during and after training. We found that learning a sensorimotor calibration modulated PA-CBI specifically early during learning (p = 0.02), whereas AP-CBI changed only late (p = 0.01). Additionally, during sequence learning, PA-CBI also changed only early (p = 0.01), whereas AP-CBI was not modulated. Together, these

results suggest that there are two independent CB-M1 pathways that contribute distinctly to different forms of motor learning.

Disclosures: D. Spampinato: None. **P.A. Celnik:** None. **J. Rothwell:** None.

Poster

586. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Human

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 586.04/PP20

Topic: E.04. Voluntary Movements

Title: Do the facial primary motor cortices communicate directly with each other?

Authors: F. GINATEMPO[1], N. G. MANZO[2], *J. C. ROTHWELL[3], F. DERIU[1]
[1]Biomed. Sci., Univ. of Sassari, University of Sassari, Italy; [2]Human Neurosciences, Sapienza Univ. of Rome, University of Rome, Italy; [3]Inst. Neurol, London, United Kingdom

Abstract: The crucial role of the corpus callosum in the execution of movements involving both body sides, particularly the hands, is well known[1], whereas proximal muscles are more likely to involve cortico-reticulospinal pathways[2]. Studies investigating the function of the primary motor cortex innervating lower facial muscles (fM1) showed that cortical projections to the facial motor nucleus are bilateral. However, how and at which level bilateral movements of facial muscles are coordinated is still unknown. Aim of this study was to probe the function of interhemispheric connections between fM1s, using TMS protocols. In 9 healthy subjects, interhemispheric inhibition (IHI) and facilitation of fM1 were investigated bilaterally using a paired pulse, twin coil, TMS protocol, in the depressor anguli oris (DAO), upper trapezius (UT) and first dorsal interosseous (FDI) muscles. Test motor evoked potentials (MEP) were conditioned using conditioning stimuli (CS) between 90% and 130% of the resting motor threshold (RMT) at 7 different interstimulus intervals (ISI) ranging 4-12ms. To exclude a possible stimulation of the contralateral DAO, the effect of the CS alone and of paired pulse TMS at 1 and 2ms ISIs were investigated (CS intensities of 110% -130% RMT). Latency and amplitude of conditioned MEPs were analysed. IHI was observed in the UT and FDI using a CS of 120% RMT at ISIs of 8ms (-23% p=0.02) and a CS of 120% and 130% RMT at 8-12ms ISIs,(-29% p=0.004), respectively. By contrast IHI was not detectable in the DAO, where a facilitated conditioned MEP with larger amplitude (+55%; p=0.009) and shorter latency (2.1, 1.4 and 1ms for 4, 2, 1ms ISIs; p=0.001) than the test MEP was detected at ISIs of 1, 2 and 4ms and pulse intensities from 110% to 130% RMT. No significant differences were found between the test DAO MEP and those obtained with the CS alone or with paired pulse TMS at 1,2 and 4 ms ISIs. While this study confirms that IHI occurs in both the hand and axial muscles, with lesser effect in the proximal muscles, it shows for the first time no evidence of a clear control exerted by the corpus callosum on fM1. It is likely that the coordination of lower facial muscles mainly

involves brainstems circuits rather than interhemispheric connections. This hypothesis is supported by studies showing that facial muscles are not able to contract asymmetrically[3] and are not affected by lesions of the corpus callosum[4]. [1] Ferbert A, Priori A et al. J Physiol. 1992;453:525-46. [2] Brinkman J, Kuypers HG. Science. 1972; 5;176(4034):536-9. [3]Cattaneo L, Pavesi G. Neurosci Biobehav Rev. 2014;38:135-59. [4]Guandalini P, Franchi G, Spidalieri G. Brain Res. 1990;5;508(2):273-82.

Disclosures: F. Ginatempo: None. **N.G. Manzo:** None. **J.C. Rothwell:** None. **F. Deriu:** None.

Poster

586. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Human

Location: SDCC Halls B-H

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Program #/Poster #: 586.05/PP21

Topic: E.04. Voluntary Movements

Support: European Commission. Horizon 2020. Call: H2020-MSCA-IF-2015 Topic: MSCA-IF-2015-EF Type of Action: MSCA-IF-EF-ST Proposal Number: 700512. Proposal Acronym: CortIMod

Title: Brain preparation to self-paced movements using TMS-EEG: New insights into the role of preparatory cortical inhibition

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Abstract: Motor cortical processes in preparation for movements can be studied probing changes in cortical excitability with transcranial magnetic stimulation (TMS). Many studies have focused on analyzing how the brain reacts to TMS at times right before muscles activate. For practical reasons, such studies have typically been done using simple reaction time paradigms or variations thereof. Paradoxically, while non-invasive recordings of brain electrical activity in preparation for movements indicate that cortical circuits involved in the generation of forthcoming actions are increasingly more active in the last hundreds of ms before an action is released, Motor Evoked Potentials (MEPs) resulting from single-pulse TMS appear to show something different: an inhibitory period is observed at around the time when the imperative command to move is given. Classically, such inhibition has been suggested to prevent premature responses. However, this and other proposed interpretations of the observed changes in MEPs in preparation for movements may be a mere result of assessing these neurophysiological changes using only highly time-constrained conditions to probe evolutions in cortical excitability.

Here we present results from an experiment in which subjects perform self-paced bilateral button presses while TMS pulses are delivered over the motor cortex. We show MEP changes in task-relevant/-irrelevant muscles together with changes in EEG-derived TMS-evoked potentials (TEPS). Also, we present results regarding the timings of movements relative to the TMS events to study possible influences that stimuli have on the self-chosen movement times. We observe a significant ($p<0.01$) reduction of MEPs ~200ms before the times of button presses in both task-specific and task-irrelevant muscles. At the same time, late TEP components (P60/N100) show a significant ($p<0.05$) reduction in amplitude, while early components remain unchanged. Additionally, we observe a shift of movement times towards the TMS time points when stimuli are delivered around 350-200ms before movements are recorded. Our results reflect unexpectedly tight resemblances with physiological and behavioural recordings obtained in simple reaction time paradigms, thus questioning interpretations of the influence of external cues in driving cortical activities, and in the way we estimate the brain generates voluntary movements in a self-paced way

Disclosures: J. Ibanez Pereda: None. **A. Reka:** None. **R. Hannah:** None. **L. Rocchi:** None. **J.C. Rothwell:** None.

Poster

586. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Human

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 586.06/PP22

Topic: E.04. Voluntary Movements

Title: Effects of behavioral tasks on neural activity relevant to motor preparation

Authors: T. KUBO, Y. MATSUMOTO, *T. URAKAWA, O. ARAKI
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Abstract: Cortical networks relevant to specific behavioral tasks have been reported in a number of previous studies [e.g., 1], in which motor preparation (MP) has been less focused on. Meanwhile, previous MP studies have exclusively investigated neural activities relevant to MP, in which differences in the behavioral task were not taken into account [e.g., 2]. The present electroencephalographic (EEG) study attempted to clarify whether neural activities relevant to MP itself would depend on the behavioral task. In experiments, two behavioral tasks were employed: 1) POSITION TASK, in which a motor action (a movement of the finger) was required based on the spatial position of a target number on a visual image and 2) PARITY TASK, in which the motor action was required based on parity of the target number. In each task, two conditions (the movement condition, MC; the no-movement condition, NMC) were set to capture neural activity relevant to the MP itself (MC vs. NMC). Under this experimental paradigm, we tried to determine whether and how a difference in the behavioral task would

affect the MP itself. In analyses, phase synchronization analysis between EEG electrodes was performed using the phase lag index (PLI). Results obtained showed that the PLI significantly increased between the central and parietal electrodes at the frequency of the delta to theta range in the POSITION TASK. This synchronization persistently occurred during the MP. As for the PARITY TASK, the PLI was significantly higher among the frontal, parietal, and occipital electrodes at the frequency of the alpha to beta range. This synchronization occurred exclusively during an early phase of MP. Our findings suggest that neural activity relevant to the MP itself differs based on the behavioral task. The frequency-dependent functional connectivity and occurrence timings during the MP period were all specifically modulated in a task-dependent manner.

References

[1] Husain M, & Nachev P. Space and the parietal cortex. *Trends in Cognitive Sciences*, 11, 30-36, 2007. [2] Kajihara T, Anwar M N, Kawasaki M, Mizuno Y, Nakazawa K, & Kitajo K. Neural dynamics in motor preparation: From phase-mediated global computation to amplitude-mediated local computation. *Neuroimage*, 118, 445-455, 2015.

Disclosures: T. Kubo: None. **Y. Matsumoto:** None. **T. Urakawa:** None. **O. Araki:** None.

Poster

586. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Human

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 586.07/QQ1

Topic: E.04. Voluntary Movements

Title: Beta-band intramuscular coherence in the tibialis anterior predicts temporal gait adaptation on a split-belt treadmill

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Abstract: INTRODUCTION: Synchronization of motor unit firing from the same muscle is thought to indicate the presence of common synaptic inputs. Specifically, coherence between motor unit firing in the beta frequency band (13-30 Hz) has been shown to be dependent on intact supraspinal control. The functional role of common drive to leg muscles in gait adaptation is unclear. The objective of this study was to (1) examine changes in tibialis anterior (TA) intramuscular coherence during split-belt treadmill adaptation and re-adaptation, and (2) determine if TA intramuscular coherence measure predict adaptive kinematic changes. METHODS: 18 healthy young adults walked on a split-belt treadmill. Each session consisted of a pre-adaptation, adaptation, post-adaptation, and 2 re-exposure periods. During the pre-adaptation period, subjects walked symmetrically at a slow (0.5 m/s) and a fast (1.0 m/s) speed

for 5 mins each. During the adaptation period, walking was challenged by altering the speed of each leg at a 1:2 speed ratio (0.5 m/s for the slow belt, 1.0 m/s for the fast belt) for 15 mins. During the post-adaptation period, subjects walked with both legs at 0.5 m/s for 10 mins. Each re-exposure paradigm consisted of a 10-min adaptation at the split-speed condition and a 10-min washout period (0.5 m/s). Kinematics were recorded with reflective markers on the lower extremity, and EMG was collected using two pairs of surface electrodes placed at the proximal and distal ends of TA. RESULTS: Beta frequency coherence was significantly greater early in adaptation compared to late adaptation period (early swing, $p < 0.001$; late swing, $p = 0.011$). This difference in coherence was less during the 2nd split-belt adaptation (early swing, $p = 0.023$; late swing, $p = 0.192$), and insignificant during the 3rd exposure (early swing, $p = 0.106$; late swing, $p = 0.460$). Higher amount of beta band coherence early in adaptation in early swing phase was associated with smaller double-support (DS) asymmetry ($p = 0.006$, $r = -0.619$, $r^2 = 0.383$), but not with step-length symmetry values ($p = 0.064$). Early adaptation DS symmetry was positively associated with difference in DS symmetry between early and late adaptation ($p < 0.001$, $r = 0.870$, $r^2 = 0.758$). CONCLUSION: Association between higher beta coherence during early swing in the TA with smaller DS asymmetry may reflect a functional role of the common drive to the TA. Individuals with higher common drive adjust their temporal parameters faster on the split-speed condition.

Disclosures: S. Sato: None. **J.T. Choi:** None.

Poster

586. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Human

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 586.08/QQ2

Topic: E.04. Voluntary Movements

Title: Neural correlates of impaired speech and hand motor timing processing in Parkinson's disease

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Abstract: Parkinson's disease (PD) is a neurological disorder associated with the degeneration of dopaminergic neurons in the basal ganglia primarily affecting the motor system. Studies have shown that patients with PD exhibit slower responses during a wide range of motor reaction time tasks, which is accounted for by their abnormal temporal processing during the planning phase of movement compared to neurologically intact control subjects. In addition, PD patients show deficits in tasks involving temporal judgment and generate shorter timing intervals in self-paced tapping tasks. These findings support the notion that temporal processing mechanisms of

movement are compromised in PD due to dysfunctional fronto-striatal circuits. Electrophysiological studies have found Beta band desynchronization as a neural signature of impaired temporal processing in PD during the planning phase of limb movement. However, our understanding about how PD may affect motor timing processing during speech is not clear. The present study examined the neural and behavioral mechanisms of motor timing deficits during speech production and hand movement in PD patients. Event-related potentials (ERPs) were recorded in 15 PD patients and 15 age-matched control subjects while they were visually-cued to prepare to produce a steady vocalization of a vowel sound or press a button, and to initiate the cued movement following the onset of a go signal on the screen. Experiment was conducted in two counterbalanced blocks in which the timing interval between the visual cue and go signal was temporally-predictable or unpredictable. Findings showed PD Patients were slower than control subjects for both speech and hand movement regardless of stimulus timing. ERP findings showed attenuation of pre-movement ERP activities over the frontal and parietal regions for PD vs. control subjects regardless of stimulus timing and response modality. These findings suggest that the attenuation of pre-movement ERP activities is a neural correlate of motor timing deficits during the planning phase of speech production and hand movement in PD.

Disclosures: K. Johari: None. **R. Behroozmand:** None.

Poster

586. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Human

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 586.09/QQ3

Topic: E.04. Voluntary Movements

Support: MEXT/JSPS KAKENHI 25702033
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Title: Visual stimulation facilitates cervical interneuron systems mediating corticospinal excitation to motoneurons in arm muscles

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Abstract: Modulatory actions of visual inputs to cervical interneurons (IN) are poorly understood in humans. In the present study, we examined whether photic stimulation (PS)

modulates corticospinal excitations to arm muscles, which would be mediated by cervical IN systems. Healthy subjects, who all gave informed consents, were seated with recording of electromyograms from the right biceps brachii (BB) muscle. Flash stimulator for PS [white light, 50 μs duration, 2 J (relative energy)] was placed 60 cm in front of the subject's eye. Transcranial magnetic stimulation (TMS) over the contralateral primary motor cortex and electrical stimulation of the ipsilateral ulnar nerve at wrist (NERVE) were delivered separately or in combination. A 10 ms inter-stimulus interval (ISI) for the combined stimulation (TMS behind) was used to give converging inputs on the upper cervical segments. PS was sometime delivered 60 ms before TMS. Combination of TMS and NERVE gave rise to facilitation of motor evoked potentials (MEPs) in the BB. When the combined stimulation was delivered with PS, the NERVE-induced facilitation of the MEP significantly increased in comparison to the control condition without PS. Furthermore, with recording of single motor units and constructing post-stimulus time histograms, it was observed that a short-latency excitatory peak (i.e., di- or oligosynaptic effects), but not the shortest one, following the combined stimulation was significantly facilitated by PS. PS facilitates short-latency cortico-spinal excitations in an arm muscle, which was facilitated by peripheral nerve stimulation under a short ISI. The present findings therefore suggest that PS facilitates cervical IN systems, which receive converging inputs from pyramidal tract and peripheral nerve, and project to arm motoneurons.

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Poster

586. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Human

Location: SDCC Halls B-H

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Program #/Poster #: 586.10/QQ4

Topic: E.04. Voluntary Movements

Support: NIH Grant R21NS093695
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Title: Motor planning muscle activation patterns and reaction time

Authors: *S. DELMAS, A. CASAMENTO-MORAN, S. H. PARK, B. YACOUBI, E. A. CHRISTOU
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Abstract: Reaction time (RT) is the short time interval between the appearance of a stimulus and initiation of a motor response. Within reaction time (RT), two processes occur, selection of motor goals and motor planning. An important but unresolved question is whether perturbation to the motor planning component of RT slows the response and alters the neural drive to the

muscle. The purpose of this study was to determine how the modulation of muscle activity, an index of the neural drive to the muscle, during a RT response changes with motor plan perturbation. Twenty-four young adults (20.5 ± 1.1 years, 13 women) participated in this study. Participants performed 15 trials of an isometric reaction time task with ankle dorsiflexion using an oscillating anticipatory strategy (oscillating force from 10-20% MVC). We compared RT and modulation of muscle activity when the stimulus appeared at the trough or at the peak of the sinusoidal task. When the stimulus appears at the trough, it is compatible with the response requirements in that the anticipation motor plan and response require a force increase. In contrast, when the stimulus occurs at the peak, the anticipation motor plan and response are incompatible in that the anticipation motor plan is to decrease force whereas the response requires an increase in force. We recorded the electromyographic (EMG) activity of the primary agonist muscle (tibialis anterior; TA). We quantified RT as the delay between the onset of stimulus and agonist EMG. We found that RT ($P = 0.003$) was longer when the stimulus occurred at the peak compared with the trough. During the time of the reaction, the EMG power from 10-35 Hz was less at the peak than the trough ($P = 0.019$), whereas the EMG power from 35-60 Hz was similar between the peak and trough ($P = 0.92$). These results suggest that a perturbation to motor planning lengthens RT and alters the neural drive to the muscle by decreasing the relative amount of power from 10-35 Hz.

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Poster

586. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Human

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 586.11/QQ5

Topic: E.04. Voluntary Movements

Support: Leibu Fonds & FNRS, Belgium

Title: Neurophysiological biomarkers of the psychological flow in realworld tightrope walking

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Abstract: The experimental search of psychological “flow” can be accomplished by the combined recording of the electromyographic (EMG), electroencephalographic (EEG) and electrocardiographic (ECG) signals during highly skilled motor performance. This singular brain state emerges from an action requiring clear goal and a perfect match between specifics skills and challenge (Csikszentmihalyi, 1975; Mao et al., 2016; Cheron, 2016). Amongst different

sports, the tightrope walker activity appeared as particularly attractive because the highly restrictive field of action requiring optimal balance control permanently exerted at the edges of the fatal fall. As the high density EEG recording represents the dynamics of the brain states resulting from synchronous neuronal activity of local field potentials distributed into temporal and spatial coordinated networks of neurons, we have here quantify in the dynamic EEG signals (ERS, ERD and phase locking) the part of this activity devoted to its downstream impact on motor behaviour and the part of neuronal modulation involved in the constant monitoring of the vital internal organs (e.g. heart and gut) which are permanently regulated by the central nervous system and also implicated in the emergence of the flow. For this the EMGs of lower limb muscles and the ECG signals served as trigger for evoked related potentials (ERP), evoked related spectral perturbation (ERSP) and intertrials coherency (ITC). This analysis was firstly accomplished on Oliver Zimmerman's brain before and during walking on a long cable (100 m) placed at an altitude of 15 meters. In the second time, slack-line performers were analysed with the same methodology in laboratory. The effects of virtual reality stimulation representing tightrope visual sensation were also studied. The neuronal generators of the different EEG oscillations were studied by means of inverse modelling (swLORETA) showing along these performances the respective contribution of different cortical areas, the basal ganglia and the cerebellum.

Disclosures: G. Cheron: None. **A. Leroy:** None.

Poster

586. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Human

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 586.12/QQ6

Topic: E.04. Voluntary Movements

Support: FRQS 34547

Title: Corticospinal excitability changes during a complex locomotor task in humans

Authors: *C. DAMBREVILLE, C. NEIGE, C. MERCIER, A. BLANCHETTE, L. BOUYER
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Abstract: INTRODUCTION. In animal models, the primary motor cortex (M1) is known to be involved during complex locomotor tasks such as ladder walking and obstacle avoidance. In humans, while M1 has been shown to contribute to normal walking, only one study looked at additional contribution of the corticospinal system to more complex walking (stepping onto lateral targets) and the reported effects were small. As this task only required small adjustments in the locomotor pattern, it may not have unravelled the full ability of the corticospinal system to adapt ongoing gait movements. The aim of the current study was therefore to measure if a more

complex walking task (requiring step length adjustments) would lead to a larger modulation of corticospinal excitability in humans.
METHODS. Sixteen young healthy participants walked on a treadmill at a speed of 1.0 m/s while facing a large screen during 2 tasks (counterbalanced order): regular walking (the 'simple' condition) and stepping onto virtual targets projected on the screen (the 'complex' condition). During the complex condition, 3 target distances were presented in random order (80, 100 and 120% of individual step length). Real time foot position was also projected onto the screen in the form of small spheres and participants had to adjust their step length to hit the targets. To assess corticospinal excitability, motor evoked potentials (MEPs; n=25 per condition) were induced by single pulse TMS during early swing (Tibialis Anterior hotspot). MEP size (area under the rectified MEP) was compared across conditions.
RESULTS. MEP size increased in all participants during the complex task (mean increase of 100+/-65%, $p<0.0001$; Glass' Δ effect size: 2.27). A learning effect was also observed: target hits raised from 82% to 93% ($P< 0.01$) over the task. Also, the first 10 MEPs were larger than the last 10 ($p<0.02$), but remained larger than during simple walking ($p<0.01$), suggesting possibly a greater need for corticospinal drive during initial learning. There was no correlation between the increase in MEP size and success score ($r= 0.16$; $p=0.27$). To control for the difference in visual inputs between the simple and complex condition, four participants performed a 3rd condition where they were instructed to walk normally while a single, very long target was presented on the screen. There was no statistical difference in MEPs size between this condition and simple walking ($p=0.625$).
CONCLUSION. As hypothesized, M1 was more recruited during the complex gait task. Interestingly, a learning effect was also measured, supporting an additional contribution of the corticospinal system to the early phase of complex motor learning during gait.

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Poster

586. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Human

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 586.13/QQ7

Topic: E.04. Voluntary Movements

Title: Predicting corticospinal excitability from oscillatory activity over motor cortex

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Abstract: Transcranial Magnetic Stimulation (TMS) over primary motor cortex can probe changes in corticospinal excitability by allowing us to record the changes in the amplitude of the electromyographic response to the stimulation, known as the motor-evoked potential (MEP). One of the problems with using MEPs to monitor corticospinal excitability is the trial-wise variability in the size of the MEP under the same experimental conditions. Moreover, the differentiation between cortical and spinal sources of this variability has still not been disentangled. In this study, we seek to identify cortical sources of MEP variability by combining TMS with electroencephalography (EEG). We will explore correlations between EEG activity in various oscillatory bands and corticospinal excitability while participants are at rest and while they are preparing a movement in a delayed response task. We will focus particularly on Beta power, given that both MEP size and EEG beta band power decrease preceding a movement. Using single-pulse and paired-pulse TMS paradigms we will test whether the beta oscillatory activity reflects the same intracortical inhibitory mechanisms for motor preparation that alters the size of the MEP or if the inhibition and variability of the MEP preceding movements is dominated by a separate process from cortical oscillations.

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Poster

586. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Human

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 586.14/QQ8

Topic: E.04. Voluntary Movements

Support: R56 NS097480
NIH R37NS21135

Title: Contributions of the ipsilateral hemisphere to motor control

Authors: *C. MERRICK[1], T. C. DIXON[3], J. J. LIN[4], I. GREENHOUSE[5], A. BRESKA[1], P. B. WEBER[6], D. KING-STEPHENS[6], K. D. LAXER[6], E. F. CHANG[7], J. M. CARMENA[8], R. T. KNIGHT[2], R. B. IVRY[9]
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Abstract: The decoding success of unilateral movement from ECoG or single unit activity is similar for contralateral and ipsilateral motor cortex (Ganguly, 2009). While this observation offers a promising neuroprosthetic alternative for patients with hemiparesis from cortical stroke,

the functional relevance of ipsilateral motor representations in the healthy brain remains unclear. One hypothesis is that, during motor planning of unilateral movements, the activation of an action goal results in the parallel preparation of independent control signals for each limb, with subsequent processes determining which limb is used. In this manner, some degree of preparation for motor execution would have taken place prior to movement selection. From this perspective, ipsilateral representations need not be related to the actual movement, but rather the planned, but not initiated movement of the contralateral limb. We refer to this as the "inferred motor plan."

This hypothesis was tested in two individuals (male, aged 22; 25 years) undergoing intracranial monitoring for the localization of an epileptogenic focus. The patients performed an instructed-delay reaching task using the arm ipsilateral to the subdural grid. The critical manipulation was the position of the non-moving, contralateral hand. In one configuration, it was placed near the midline, adjacent to the ipsilateral limb (Near). In the other condition, the contralateral hand was moved to an eccentric position (Far). In this manner, the movements performed by the ipsilateral hand were essentially identical in the two conditions. However, the inferred motor plan for the contralateral hand would differ for the Near and Far conditions.

Similar to prior work, target location could be classified above chance using the ipsilateral hemisphere. We then built a finite impulse response (FIR) model to predict the activity in the ECoG electrodes based on task features (e.g., target onset, movement onset). If ipsilateral activity represents the inferred motor plan of the stationary hand, we would expect activity to change in a target dependent fashion and, most important, to be modulated by the stationary hand position (e.g., the plan to a given target will differ in the Near and Far conditions). Indeed, model comparison using the mean R^2 across ten validation sets revealed that the best model for a subset of electrodes in both patients included a multiplicative interaction term (i.e., position of contralateral hand X target location). This suggests, in line with the parallel planning hypothesis, there may be information about the inferred motor plan of the stationary contralateral hand in the ipsilateral hemisphere.

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Poster

586. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Human

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 586.15/QQ9

Topic: E.04. Voluntary Movements

Support: BBSRC

Title: Putative propriospinal modulation of premotor and motor cortical output during grasping

Authors: *K. L. BUNDAY[1,2], Z. POH[2], S. AZZOPARDI[2], M. DAVARE[3]
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Abstract: The primary motor cortex (M1) and ventral premotor cortex (PMv) play a major role in the control of grasping. Anatomical studies have revealed that these regions project to the spinal cord, directly and indirectly, and likely interact with the propriospinal network (PN). The PN is a pre-motoneuronal network located at mid-cervical levels (C3-C4), which transmits and alters descending cortical commands for targeted reaching and grasping. How the PN interacts with motor output from M1 and PMv during different grasps in humans is not well understood. The PN can be studied indirectly by conditioning motor evoked potentials (MEPs), elicited by transcranial magnetic stimulation (TMS), and H-reflexes, elicited by peripheral nerve stimulation (PNS), with sub-threshold PNS. In experiment #1, sub-threshold PNS was applied to the ulnar nerve at the wrist to condition Flexor Carpi Radialis (FCR) MEPs, elicited by M1 TMS, during an isolated FCR contraction (iFCR) or FCR contraction with precision grip (PG) or whole hand grasp (WHG). Central and peripheral conduction times were used to time the arrival of descending and ascending volleys at the spinal cord at 5 different inter-stimulation intervals (ISIs), namely 0, -3, -4, -5 and -6 ms. Negative ISIs indicate that PNS is delivered prior to TMS, allowing time for PNS volleys to travel to higher cervical segments (e.g. C3-C4), and 0 ms indicating that PNS and TMS-evoked volleys converge monosynaptically at spinal motoneurons (C6-C8). In experiment #2, TMS was applied over PMv to condition PN modulated H-reflexes, elicited by PNS to the median nerve applied at the elbow, during iFCR, PG and WHG. Here, TMS and sub-threshold PNS ulnar nerve volleys were timed to arrive at PN levels (C3-C4) at 5 different ISIs, namely 0, 2, 4, 6 and 8 ms. H-reflexes were either elicited alone (baseline), or conditioned by ulnar nerve PNS (ISI: 4ms), or by ulnar PNS and PMv TMS. In experiment #1, we found a significant interaction between ISI and grasp. Specifically, at 0 ms ISI, MEPs were significantly larger during PG than WHG and, at -4 ms ISI, MEPs were significantly larger during WHG compared to PG. In Experiment #2, we found that PMv TMS differentially modulated H-reflexes during iFCR and PG, but only for late ISIs. This contrasts with our previous findings that, at rest, PMv interacted with PN at early ISIs. Our results suggest that while monosynaptic corticospinal pathways contribute to precisions grip, motor cortical output during whole hand grasp can be modulated by the PN. Interestingly, PMv appears to modulate the PN directly or indirectly (e.g. via M1) depending on whether these interactions are tested at rest or during contraction, respectively.

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Poster

586. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Human

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Program #/Poster #: 586.16/QQ10

Topic: E.04. Voluntary Movements

Support: WAY (FP7-ICT-288551)

Title: ERNing performance improvements: Error related negativity (ERN) is associated with errors in lifting performance during an object manipulation task

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Abstract: In order to successfully interact with objects, our brain must predict the outcome of motor acts based on a copy of the motor command - often termed an efference copy. One of the ways these predictive models are updated is through reactive adjustments based on sensory feedback during movement. In this circumstance, to adjust an ongoing movement and recover from deviations in prediction, the motor system requires an error signal of a very short latency. The presented analysis was conducted to examine whether error related negativity (ERN) corresponds with errors in an object manipulation task; the ERN component was selected as it is associated with a brain system for detecting errors and engaging in corrective behavior. To accomplish this, we examined electroencephalograms (EEG) and kinematic data from participants (N = 12) during two lifting conditions. In the first condition, object weight was expected, heavier than expected, or lighter than expected, based on the previous lift. In the second series of lifts, the object's surface friction at the point of finger contact changed between trials. The object friction was expected, greater than expected, or less than expected, based on the previous object lift. The epoch of interest (EOI) when examining the EEG data was 0-300ms from timepoint zero (T=0). For the weight series trials, T=0 was defined as the start of load phase, defined as the period between digit placement and lift-off. For the friction series, T=0 was defined as first contact with the object. ERN was defined as the largest negative peak within the EOI at electrode sites Fz and Cz. ERN peak amplitude was significantly larger when the participant lifted an unusually heavy object or an unusually light object, compared to an object of expected weight. Similarly, ERN peak amplitude was larger for trials in which the friction-weight relationship greater or less than expected. Behavioral data verified those trials associated with increased ERN had significant errors in prediction. Specifically, changes in load phase duration, maximum grip force (maximum GF applied by the thumb and finger), and/or maximum load force (summed maximum lifting forces of the thumb and index finger) were observed when compared to those trials in which an expected weight/surface were encountered. In summary, we

provide initial evidence that ERN may be present during trials in which errors are experienced in a natural motor task, possibly serving as the source of error signal used by the motor system to make online adjustments to movement in response to sensory feedback.

Disclosures: G.R. Lynch: None. **L.A. Baugh:** None.

Poster

586. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Human

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 586.17/QQ11

Topic: E.04. Voluntary Movements

Support: VA RR&D N1759-P
VA RR&D N9274-S
University of Florida Graduate School Fellowship

Title: Interactions between intracortical and interhemispheric inhibition in chronic stroke

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Abstract: The interhemispheric competition hypothesis (IHC) posits a causal relationship between imbalanced interhemispheric inhibition (IHI) and motor function following stroke where contralesional (CH) IHI exceeds ipsilesional hemisphere (IH) IHI. The IHC model is not strongly supported by evidence. It remains unclear whether imbalanced IHI following stroke reflects excessive CH-to-IH or deficient IH-to-CH inhibition. Moreover, the interaction between GABA-mediated intracortical inhibition and IHI is under appreciated. Here we investigated functioning of intra-cortical and inter-hemispheric circuits using ipsilateral silent period inhibition (iSP), to measure IHI, and short-intracortical inhibition (SICI) in both hemispheres of 19 individuals with chronic stroke (age: 62±7 years; chronicity: 4.7±4.4 years; upper-extremity Fugl-Meyer Motor Assessment (UEFMA): 51.5±14/66 (range: 20-66), 15 males) to better understand interhemispheric interactions and their relationship to motor recovery. We assessed motor impairment, correlating UEFMA with grip MVC difference (Non-paretic MVC - Paretic MVC, r = -.689, p =.0003) to produce a continuous measure of motor impairment and associated motor asymmetry. CH SICI revealed a strong association with MVC difference (r = .627, p = .004), indicating systematic CH disinhibition with more severe motor impairment. A laterality index of iSP inhibition [(iSP CH - iSP IH)/(iSP CH + iSP IH)], where positive values indicate greater CH-to-IH IHI, revealed a significant quadratic relationship (r^2 = 448) with MVC

difference indicating lower motor impairment is associated with relative IHI balance, but greater motor impairment with IHI imbalance either CH > IH or IH > CH. Finally, the iSP laterality ratio was significantly associated with IH SICI ($r = -.492$, $p = .038$) indicating the presence of IH inhibition in individuals with stronger CH-to-IH IHI but IH disinhibition in individuals with stronger IH-to-CH IHI. Concurrent evaluation of SICI in both hemispheres, IHI, and motor impairment helps characterize the heterogeneity among individuals post-stroke revealing only a subset of chronic individuals represented by the IHC model. Importantly, our data reveal imbalanced IHI results from overactivity in either hemisphere which likely contributes to inconsistencies in the current literature including why IHC-based interventions (i.e., rTMS, NIBS) have not demonstrated broad efficacy. Finally, our data identify a group of individuals with dysfunction of IH SICI in the presence of balanced IHI highlighting the importance of GABAa-mediated intracortical circuits to motor recovery.

Disclosures: C. Patten: None. **Q. Ding:** None. **W.J. Triggs:** None.

Poster

586. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Human

Location: SDCC Halls B-H

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Program #/Poster #: 586.18/QQ12

Topic: E.04. Voluntary Movements

Title: Differences in motor unit discharge characteristics between ankle plantarflexors and dorsiflexors during steady contractions

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Abstract: The three primary ankle muscles for sagittal plane control - the tibialis anterior (TA; a dorsiflexor (DF)), the soleus (SOL; a plantarflexor (PF)) and the gastrocnemius (GA; a plantarflexor (PF) and knee flexor) - each provide different biomechanical functions during standing, locomotion, and transitional tasks such as sit-to-stand. For example, during standing, SOL provides primarily sustained postural support whereas TA and GA primarily provide fast responses to maintain balance. In addition, the three muscles differ in their composition of muscle fiber types in a manner that is consistent with the biomechanical functions. SOL contains primarily low threshold slow type muscle fibers in the human, whereas TA and GA have a higher percentage of fast type muscle fibers.
Differences in neural control of the TA, SOL, and GA likely exist to support their functional roles. These muscles are differentially affected after central nervous system injury, and previous work using transcranial magnetic stimulation has shown differences in corticospinal efficacy.

Various analyses of motor unit discharge from proximal and distal arm muscles during voluntary contractions have revealed differences in neural control that are consistent with their biomechanical functions. The purpose of this study was to characterize motor unit discharge from the TA, SOL, and GA during voluntary PF and DF torque generation during sitting and standing, in order to compare neural control among the muscles and tasks.
Six participants without neurologic injury participated in the study. For sitting tasks, the tested ankle was secured to the Biodex System 4 Pro for measurement of PF and DF joint torque during isometric torque generation. One 64-channel EMG grid was placed over the TA, 2 grids were placed on the medial and lateral surfaces of the SOL and 2 grids were placed over the medial and lateral GA. Separate steady isometric contractions of DF and PF were performed at efforts ranging from 10 - 50% of maximum voluntary torque and were held for 20 - 45 sec. For standing tasks, participants performed static standing with eyes closed, with each leg on a separate force plate, as well as anterior and posterior leans, to preferentially engage the plantarflexors and dorsiflexors, respectively. Multi-channel surface EMG data were decomposed into motor unit spike trains using an automated algorithm (Negro et al, 2016).
Differences were seen among the three muscles in terms of motor unit coherence, discharge rates, discharge variability, and properties of the motor unit action potentials. These preliminary findings provide information with which to infer differences in neural control of these muscles.

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Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

Location: SDCC Halls B-H

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Topic: E.04. Voluntary Movements

Support: BT/PR/6615/MED/14/85782005, Department of Biotechnology, Govt. of India to NJ
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Title: Cortical, callosal and thalamic inputs to the neck and jaw motor representations in rats

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Abstract: In the rodent primary motor cortex, the regions where microstimulation evokes movements of different body parts are organized in a topographic manner. Although the rat whisker and forepaw motor representations have been studied in detail, information about the sources of inputs to the neck and jaw motor representations is sparse. Here we describe cortical,

callosal and thalamic inputs to the neck and jaw representations in the motor cortex of rats. Intracortical microstimulation was performed to delineate these motor representations in adult Long Evans rats, and small injections of the retrograde fluorescent tracers were made. Following appropriate survival period, rats were perfused, the cortex was flattened, and sectioned in a tangential plane. The thalamus was sectioned in a coronal plane. The sections of the brain were processed for fluorescence microscopy and other architectonic markers. Locations of fluorescently labeled neurons in the cortex and thalamus were plotted, and precisely aligned with the help of the motor map, the somatosensory isomorph and the architectonic borders that were drawn using sections stained for Nissl substance and acetylcholinesterase. The results show that both neck and jaw motor representations receive ipsilateral cortical inputs from the motor cortex, the primary somatosensory cortex and the higher somatosensory areas, although the locations of the labeled neurons showed only partial overlap. The neck representation received relatively more inputs from the posterior parietal cortex, retrosplenial, orbital and piriform cortices. Callosal inputs to both the neck and the jaw representations were from the homotopic motor cortex and the surrounding regions. There were also sparse inputs from the contralateral somatosensory cortex. Only the neck motor cortex received strong inputs from the contralateral orbital cortex. Thalamic inputs to the neck and jaw motor representations were from the ventroanterior and ventrolateral nuclei, ventormedial nucleus, posterior nucleus, and the centrolateral and paracentral nuclei. The neck motor cortex received relatively more thalamic inputs from mediodorsal nucleus, whereas the jaw motor cortex received more inputs from the ventroposterior medial nucleus. Results suggest that the neck primary motor cortex has more of an integrative role in motor control as compared to the jaw motor cortex.

Disclosures: H. Mohammed: None. **N. Jain:** None.

Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

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Title: Macaque premotor cortex activity and behavior support embodied choice model of decision-making

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Abstract: Decision-making is often described as a serial process in which sensory information is evaluated to arrive at a choice, followed by a motor report of that choice. In contrast to this 'serial model', Cisek and Pastor-Bernier (2014) and Lepora and Pezzulo (2015) argue that action dynamics are taken into consideration during the sensory decision-making process - the 'embodied choice model'. Here, we investigated whether monkeys' behaviors were consistent with either model, and which model was represented neurally in premotor cortex (PM). We trained two monkeys to discriminate the dominant color of a red-green checkerboard by reaching to a corresponding colored target. Targets appeared on the left and right, and we manipulated action cost by presenting them at two distances, randomly assigned for each target on each trial. After a variable delay, the checkerboard appeared and the subject could make a reach report. The serial model predicts that a subject would determine the dominant color (red or green) before executing a reach (left or right), and performance would be unaffected by the target distances. In contrast, the embodied choice model predicts that performance would be biased towards the choice with a lower action cost, especially for difficult decisions. We found that monkeys were more likely to reach to the closer target when the checkerboard was more difficult (effect of distance on performance, logistic regression, $p < 0.001$). This behavior is consistent with the embodied choice model and with previous findings in humans (Marcos et al., 2015). Next, we looked for neural correlates of these action costs. We recorded single and multi-units in PM (266 for monkey T, 104 for monkey O). If action costs were reflected by changes in the starting points of population firing rate dynamics, we should be able to find differential activity for the distance configurations. After target onset, when action cost information was first available, neural activity was identical regardless of target configuration (linear regression of target distance on firing rates, $p > 0.01$). On the other hand, firing rates of PM neurons covaried with reach direction, checkerboard difficulty, reaction time, and target distance, suggesting that action cost information may be integrated with perceptual information during the same epoch. Future work will further investigate how these action costs are incorporated into the dynamics. In summary, these results show that monkey behavior and neural activity in PM support the embodied choice model of decision-making, rather than the serial model.

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Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

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Title: Reward-dependent modulation of correlated neural variability mediates trial-by-trial motor learning

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Abstract: Recent studies have suggested that neural systems may be able to use reinforcement to regulate variability associated with execution of movements on a trial-by-trial basis. This raises an intriguing possibility that the brain might also be able to use reward to regulate variability associated with motor planning. To test this idea, we analyzed neural activity in cortical circuits of monkeys during a motor timing task in which behavioral variability is largely due to noise in motor planning. Animals performed a 2x2 context-dependent time interval production task in which they were instructed to proactively produce either an 800 or 1500 ms time interval using either a saccade or a ballistic button press.
Produced intervals were variable and significantly correlated across trials (up to 20 trials). As expected from the so-called scalar property of interval timing, the magnitude of variability scaled with the target interval for both effectors. Surprisingly, long-term correlations were effector-specific, and variability within each context reduced systematically when the previous trial was rewarded. In other words, scalar variability, which reflects noise in the nervous system, was subject to reward-dependent modulation.
To investigate the neural mechanisms through which reward impacts behavioral variability, we recorded from neurons in the dorsomedial frontal cortex(DMFC) where neural activity predicts animal's timing behavior. Since correlated neural variability is a key determinant of variance across a population of neurons, we hypothesized that reward might regulate behavioral variability by acting upon correlated neural variability. Therefore, we compared the variance across a population of simultaneously recorded neurons following rewarded and unrewarded trials within and across different effectors. We quantified the trial-by-trial correlated variability

by removing long-term correlations in neural activity and measuring the projection of the residuals on the dimensions of maximum variance (i.e., principal components). Consistent with our hypothesis, rewarded trials led to a reduction of correlated neural variability in a context-specific manner. This finding suggests that noise correlations in cortex are modulated by reward and may play a role in regulating behavioral variability in motor planning. More broadly, our results suggest that correlated neural activity in cortex might provide a substrate for trial-by-trial reinforcement learning.

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Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

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Program #/Poster #: 587.04/QQ16

Topic: E.04. Voluntary Movements

Support: JSPS KAKENHI 16J05329

Title: The role of anterior corpus callosum in bimanual coordination in head-fixed rats

Authors: *M. IGARASHI, Y. AKAMINE, J. R. WICKENS
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Abstract: Rodents demonstrate dexterous coordination of forelimbs when feeding. Manipulation of food objects involves repertoires of paw usage including symmetric and asymmetric bimanual movements. Recently, we developed a high-resolution kinematic tracking system for studying bimanual coordination in rats. The system recorded forelimb position during spontaneous food handling and consumption in head-fixed conditions. Here, we demonstrate the use of this system in automatic movement classification, and its application to investigating cortical crosstalk in bimanual movements. Five male Long Evans (LE) rats were trained to bimanually handle donut-shaped food rewards. Forelimb movements were recorded by high-speed cameras (200 frames/sec), and stored as 3-D kinematic datasets. An automatic segmentation and classification algorithm was applied to identify three categories of movements: unimanual, symmetric bimanual, and asymmetric bimanual. Quantification of the classified movements revealed that feeding behavior is dominated by symmetric bimanual movements (56.55%) with less prevalent asymmetric bimanual movements (32.18%) and unimanual movements (11.27%). We further investigated the role of cortical crosstalk in bimanual coordination. Forelimb movements were recorded in nine male LE rats during food handling. To block the cortical crosstalk, 500 nL of 2% Lidocaine was injected into the anterior corpus callosum (aCC) through which pass commissures from cortical forelimb motor areas. Test sessions consisted of three repeated daily cycles of baseline (Saline), and aCC inhibition (Lidocaine) conditions. The kinematic tracking

system and classification method revealed that the frequency of occurrence of symmetric bimanual movements was reduced by aCC inhibition. In contrast, asymmetric bimanual movements were increased. Other parameters related to the global scale of motor skills, such as mean food drop rate and consumption times, remained unchanged. Collectively, these results suggest that the symmetric dominance in bimanual movements in rodents is modulated by cortico-cortical crosstalk via the anterior corpus callosum.

Disclosures: M. Igarashi: None. **Y. Akamine:** None. **J.R. Wickens:** None.

Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

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Topic: E.04. Voluntary Movements

Support: NINDS Grant F32NS093709
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Title: Mirror neuron populations lead non-mirror neuron populations during execution of a reach, grasp, and manipulate task

Authors: *K. A. MAZUREK[1], M. H. SCHIEBER[2]
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Abstract: Mirror neurons discharge both when an individual executes a particular action and when the same individual observes another individual perform the action. In populations of mirror neurons (MNs) and non-mirror neurons (non-MNs) recorded during a reach-grasp-manipulate (RGM) task, hidden Markov models (HMMs) have detected sequences of four hidden states corresponding in time approximately to four sequential behavioral epochs in each trial: the initial, reaction, movement, and final hold epochs. Here, we compared the timing of these hidden state transitions in simultaneously recorded populations of MNs versus non-MNs during execution trials of the RGM task.
Two male Rhesus monkeys each executed the RGM task and then observed an experimenter performing the same task. Neural activity was recorded from floating microelectrode arrays implanted in premotor cortex (PM) and primary motor cortex (M1). MNs were identified as single- or multi-units that modulated significantly during both execution and observation trials, whereas non-MNs were identified as units that modulated during execution but not observation trials. Units recorded in each of 3 sessions from each monkey were separated into four subpopulations: PM MNs, PM non-MNs, M1 MNs, and M1 non-MNs. HMMs were trained to detect hidden states separately using each subpopulation without temporal information about the task events separating the four behavioral epochs in each trial.

We then selected those execution trials in which HMMs had detected all four hidden states in both MN and non-MN populations and performed pairwise comparisons of state transition times in the MN versus non-MN populations. Pooling such trials across monkeys and sessions, we found that hidden state transitions in MN populations occurred before the corresponding transitions in non-MN populations. The same was true whether PM and M1 populations were considered together or separately. Our findings challenge the notion that MNs simply monitor actions and suggest instead that during action execution MN populations represent behavioral states before non-MN populations.

Disclosures: K.A. Mazurek: None. **M.H. Schieber:** None.

Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

Location: SDCC Halls B-H

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Program #/Poster #: 587.06/QQ18

Topic: E.04. Voluntary Movements

Title: Cortical activity during a motor task in behaving mice

Authors: *N. C. GIORDANO[1], C. ALIA[2], A. CATTANEO[3], M. CALEO[2]
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Abstract: How the motor cortex controls planning and execution of voluntary movements is not completely understood. This basic knowledge is crucial to understand reorganization and plasticity of cortical outputs after a damage, such as ischemia. After a lesion, the destruction of neural networks indeed stimulates a reorganization of the connections in response to anatomical or functional deficit. However, to assess the brain mechanisms supporting behavior, both in physiological conditions and after a lesion, monitoring the activity of single neurons *in vivo* is needed. This requires the analysis of the coordinated activity of different cell types during a motor act in behaving animals.
Here we used head-restrained mice trained to lick a reward delivered at random intervals. The neuronal signals were acquired through a 16-channel silicon probe. During the task, we performed *in vivo* electrophysiological recordings in two distinct areas known to be involved in the control of licking, the anterior lateral motor cortex (ALM), a potential premotor area, and the posterior-medial motor cortex (PMM), that is part of the primary motor cortex. However, there is a paucity of information on how, across layers, the single units in the ALM and PMM behave in the neural circuitry for the control of voluntary licking.
Licking events were divided in single isolated events or multiple repetitions.
We found that, during single isolated licking events, in ALM many neurons show modulation coinciding with the licking event itself. Moreover, most neurons' activity, especially in the

deeper cortical layers, anticipates the specific action long before the movement onset (100-200 msec). This is consistent with the ALM involvement in planning directed licking. We defined these neurons perimovement and preparatory neurons, respectively. We further showed that during the multiple licking events the perimovement neurons always discharged when the action occurred, while the preparatory neurons showed activity only before the first event and not before every single licking event in the sequence. The analyses on how the information is represented in the PMM are still ongoing.
These preliminary results reveal cell type specific processes within ALM for globally representing the movement; there is a precise transformation of preparatory activity into movement commands. These findings might be relevant for further study on the synaptic reorganization after damage of the premotor cortices.

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Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

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Topic: E.04. Voluntary Movements

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Title: Distribution of layer 5 sensorimotor cortex neurons projecting to mesencephalic nuclei

Authors: *V. LOPEZ-VIRGEN, R. OLIVARES-MORENO, G. ROJAS-PILONI
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Abstract: Layer 5 pyramidal tract neurons (PTN) are canonical elements of the cerebral cortex. PTN are the main excitatory output to subcortical structures like striatum, superior culliculus, pons, red nucleus and spinal cord. Nevertheless, little is known about the organization of PTN that may encode different cortical outputs to subcortical structures. The aim of the present work is to characterize the distribution of the thick-tufted layer 5 pyramidal neurons of the sensorimotor cortex (M2, M1, S2 and S1) projecting to tectum, red nucleus (RN) and pons. Eight wild-type C57BL/6 mice adults were simultaneously injected using retrograde tracers in tectum, RN and pons (Cholera toxin conjugated with Alexa-488, Biotinylated dextran amine and Fluoro-Gold). Five days after, the animals were perfused and 50-µm coronal sections were obtained from sensorimotor cortex (2.46 mm to -0.80 mm relative to bregma) and from the injection sites (-3.4 mm to -4.04 mm relative to bregma). Large scale fluorescence images with cellular resolution were obtained to quantify the number of retrogradely labeled cells.No differences in the size of injection size between tectum, red nucleus and pons were observed. All projecting

neurons were distributed broadly in the sensorimotor cortex (M1, M2, S1 and S2) and no significant differences in the density were found in the different areas. However, the density profiles in M2 shown that red nucleus projecting are located more superficial than pons projecting neurons. In S1 and S2 the results indicated that the projection neurons to the pons are located more superficial than RN projecting neurons. Despite the layer 5 cortical neurons projecting to tectum, RN and pons are intermingled in the sensorimoror cortex double and triple retrogradely labeled neurons represents less than 5% of the total projecting neurons. The anatomical segregation of PTN suggests that subcortically projecting sensorimotor cortex layer 5 neurons are also functionally segregated.

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Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

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Title: Representation of multiple grip types in the primary motor cortex of capuchin monkeys

Authors: *A. MAYER[1], M. K. BALDWIN[2], D. F. COOKE[3], B. R. LIMA[4], J. J. PADBERG[6], G. LEWENFUS[5], J. G. FRANCA[7], L. A. KRUBITZER[8]
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Abstract: Capuchin monkeys are New World primates that are distinguished by their manual abilities and tool use behavior. They can execute 16 different types of precision grips, as well as manufacture, select, and spontaneously use tools in the wild. Despite being an ideal model for studying complex manual behaviors, very little is known about the functional organization of the primary motor cortex (M1) of these primates. In the present study, we investigated M1 using long-train intracortical microstimulation (LT-ICMS) in four adult capuchin monkeys, which

consisted of 500 ms trains of 0.4 ms biphasic square wave pulses (a negative 0.2 ms phase followed by a positive 0.2 ms phase) delivered at 200 Hz. All evoked movements were recorded on digital video and analyzed offline, and all stimulation sites were correlated with architectonically defined cortical borders.
Our results reveal movement representations of the entire body within M1, including foot and tail movements. Around 22% of all evoked movements involved finger movements. The majority consisted of finger flexions (67% of all finger movements) and their stimulation sites were clustered within M1. No clear spatial arrangement between these and finger extension movements could be observed.
Using the criteria described by Spinozzi et al. (2004), 14% of evoked finger flexions could be categorized as "power grip", where all fingers move towards the palm, and 31% could be categorized as "precision grip", in which the thumb moves towards one or more other fingers. Among these, different subtypes of precision grip could be identified. The most common was the opposition between D1 and D2 (75% of all "precision grips"). Interestingly, the execution pattern of movement of these digits varied between animals. In one case, the precision grip was achieved mostly by the adduction of D1 towards D2 such that the medial surface of the first touched the lateral surface of the second. In another case, D2 was mostly touched by the tip of D1. The other variations of precision grips we observed consisted in the opposition between D1 and D2+D3 (19%), and between the tips of D1 and all other fingers (6%). Thus, the complex manual behavioral repertoire of the capuchin monkey is well represented within M1, where specific types of hand grips can be revealed using LT-ICMS.

Disclosures: A. Mayer: None. **M.K. Baldwin:** None. **D.F. Cooke:** None. **B.R. Lima:** None. **J.J. Padberg:** None. **G. Lewenfus:** None. **J.G. Franca:** None. **L.A. Krubitzer:** None.

Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 587.09/QQ21

Topic: E.04. Voluntary Movements

Support: Howard Hughes Medical Institute

Title: Normal and perturbed neural dynamics in motor cortex during a reach-to-grab task

Authors: *B. SAUERBREI, J.-Z. GUO, J. ZHENG, W. GUO, M. KABRA, N. VERMA, M. MISCHIATI, K. BRANSON, A. HANTMAN
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Abstract: Reaching, grasping, and object manipulation play a central role in the lives of mammals with prehensile forelimbs. The musculoskeletal complexity of the limb poses a

challenging control problem for the central nervous system, which must orchestrate precisely-timed patterns of activity in many muscles to perform a wide diversity of tasks. The motor cortex is thought to be critical for producing these patterns, and single-unit recording studies have demonstrated that the activity of cortical neurons is correlated with muscle tension and limb kinematics. Direct experimental control over cortical circuits for reaching, however, has proven challenging. Here, we achieve rapid, reversible, and bidirectional control over reaching behavior in head-fixed mice using optogenetics in conjunction with electrophysiology. In order to isolate the cortical commands for movement, we compare neural dynamics during normal, voluntary reaching with dynamics during optogenetically-induced, involuntary reaches. Involuntary reaches are executed with much shorter reaction times than voluntary reaches, suggesting that motor cortex is downstream of brain regions involved in the decision to reach. Neural dynamics during involuntary reaches recapitulate the dynamics during voluntary reaching. Briefly silencing motor cortex during movement execution perturbs limb kinematics, but the cortical network rapidly recovers from this perturbation, driving successful completion of the task. Furthermore, the cortical network and behavior are robust to cell-type-specific perturbation of excitatory populations. These results suggest that ongoing neural dynamics in motor cortex are both necessary and sufficient to orchestrate reach-to-grasp movements.

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Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 587.10/QQ22

Topic: E.04. Voluntary Movements

Support: MIUR
FP6-IST-027574-MATHESIS

Title: Does the medial reach-to-grasp network host mirror neurons?

Authors: *R. BREVEGLIERI, F. E. VACCARI, A. BOSCO, M. GAMBERINI, P. FATTORI, C. GALLETTI
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Abstract: Mirror neurons are a particular type of cells that discharge both during action execution and during observation of the same action performed by other agents (Rizzolatti et al. 1996, Cogn Brain Res). So far, they have been found in several nodes of the lateral grasping network, a circuit that includes the ventral premotor cortex and the inferior parietal lobule. In the present work, we looked for mirror neurons in the superior parietal lobule, a node of the medial

reach-to-grasp network (Galletti and Fattori, 2018 Cortex) never explored in this regard. We recorded the neural activity of 100 neurons in the medial posterior parietal area V6A of two male *Macaca fascicularis* during grasping movements and during observation of the same action performed by the experimenter. In all tested neurons we also checked the neural response to the passive object observation, when grasping was not required to the animal. The overhealming majority of V6A neurons (86/100) were modulated only when the monkey executed the action, suggesting that these cells were able to discriminate between own and other's actions. A minority (14%) of neurons showed mirror features, discharging also during observation of actions performed by the experimenter. However, differently from the classic mirror neurons, V6A mirror neurons responded also to the passive object observation, like the 'canonical-mirror' neurons of the lateral grasping network, recently described in literature (Bonini et al. 2014, J Neurosci). V6A mirror neurons showed dissimilar responses when the monkey performed the action and when it observed the same action performed by the experimenter, so they seem not to be involved in action understanding, differently from the classic mirror neurons found in the lateral grasping circuit. In addition, because the neural responses of V6A mirror neurons to object observation were different according to the contexts (object observation before own action, before action performed by the experimenter, and when no grasping was required), we suggest that these neurons encode the relevance of the object in the action to be performed. In conclusion, area V6A is well equipped to monitor own actions but is not able to build an internal representation of observed actions.

Disclosures: R. Breveglieri: None. **F.E. Vaccari:** None. **A. Bosco:** None. **M. Gamberini:** None. **P. Fattori:** None. **C. Galletti:** None.

Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 587.11/QQ23

Topic: E.04. Voluntary Movements

Support: CIHR MOP-12675
CIHR FDN-143209

Title: Single neuron defined cortico-subcortical mesoscale networks are associated with specific motor actions in awake chronic mice

Authors: *D. XIAO, J. M. LEDUE,, M. P. VANNI, T. H. MURPHY
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Abstract: How information processing in motor pathways drives a specific action is still largely unknown. Difficulty comes from recording neural activity over large spatial scales while

monitoring motor output and single neuron responses. We provide a chronic recording system which integrates with wide-field calcium functional imaging, multi-site sub-cortical cellular electrophysiology and peripheral nerve recording in head-fixed mice which undergo self-initiated bouts of running and or facial movements. Facial motor nerve impulses were measured by paired fine wire recording. Mesoscale GCaMP imaging was used to assess regional cortical activity. Nerve or single neuron spike-triggered averaging allowed the identification of cortical regions that are preferentially related to specific actions. Multiple tetrodes were then implanted in regions of interest to record extracellular spike activity. Spontaneous firing of facial motor neurons is linked to specific patterns of cortical mesoscopic activity, and unexpectedly was found to be associated with unique patterns of cortical activation which extended to higher-order associative cortical areas, including RS, PTA, ACC and mPFC. These higher-order associative cortical areas along with subcortical areas in striatum and brainstem were linked with spontaneous facial movement that occurred during the self-initiated movement bouts. Preliminary results also indicated cortico-hippocampal networks associated with rhythmic nose movement and a cortico-VTA network was associated with spontaneous running. We suggest that these large scale brain networks coordinate spontaneous running and whisking associated movements. Our chronic recordings remained stable for weeks, demonstrating that this method can be employed to investigate the dynamic and distributed neuronal ensemble interactions that underlie processes of motor control and sensorimotor learning in behaving mice. Our findings are consistent with specific behavioral actions involving large scale brain networks.

Disclosures: D. Xiao: None. **J.M. LeDue,:** None. **M.P. Vanni:** None. **T.H. Murphy:** None.

Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 587.12/QQ24

Topic: E.04. Voluntary Movements

Support: JSPS KAKENHI 16K17369
JSPS KAKENHI 25780453

Title: Encoding of contralateral and ipsilateral hand movements by neurons and local field potentials in the primary motor cortex in monkeys

Authors: *Y. NAKAYAMA, O. YOKOYAMA, E. HOSHI
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Abstract: The primary motor cortex (M1) of primates is considered to play a crucial role in executing contralateral hand movements. However, the input-output organization of the M1 involved in controlling hand movement still remains elusive. In the present study, we recorded

neuronal activity and local field potentials (LFPs) from the M1 while monkeys (*Macaca fuscata*) performed a button-press movement with either the right or left hand. Three types of movement-related neuronal activity were observed: (1) with only the contralateral hand (contralateral neuron), (2) with only the ipsilateral hand (ipsilateral neuron), and (3) with either hand (bilateral neuron). The proportion of contralateral neurons was much larger than that of ipsilateral neurons, and quantitative analyses also revealed that neuronal selectivity was biased toward contralateral hand movement. We also found a movement-related power increase in the high-gamma (80-120 Hz) and theta (3-8 Hz) bands of LFPs. These power increases were classified into the following three types: (1) a greater modulation during contralateral hand movement than during ipsilateral one (contralateral LFP), (2) a greater modulation during ipsilateral hand movement than during contralateral one (ipsilateral LFP), and (3) a comparable modulation during contralateral and ipsilateral hand movement (bilateral LFP). The proportion of contralateral LFPs was much larger than that of ipsilateral LFPs in both high-gamma and theta bands. These results suggest that in the M1 both input signals from other cortical areas and output commands to the spinal cord have contralateral biases. Taking together with our previous findings that the caudal cingulate motor area (CMAc) and supplementary motor area (SMA) are involved in selecting which hand to use (Yokoyama et al., 2016; Nakayama et al., 2015), the M1 may play a role as a cortical center that receives inputs about contralateral hand movement pre-selected in other cortical areas such as the CMAc and SMA, and sends motor commands to the spinal cord to execute contralateral hand movements.

Disclosures: Y. Nakayama: None. **O. Yokoyama:** None. **E. Hoshi:** None.

Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

Location: SDCC Halls B-H

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Program #/Poster #: 587.13/QQ25

Topic: E.04. Voluntary Movements

Support: National Defense Science & Engineering Graduate Fellowship (NDSEG) Program
NIH NINDS Grant 1R56NS097480

Title: Stability and independence of the ipsilateral representation of reaching movements in motor cortex

Authors: *T. C. DIXON[1], C. M. MERRICK[2], R. T. KNIGHT[2], R. B. IVRY[2], J. M. CARMENA[3]
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Abstract: The canonical understanding of motor control is that each side of the body is primarily controlled by the contralateral hemisphere of the brain. However, multiple streams of evidence suggest an additional role for the ipsilateral motor cortex. The level of dissociation between these contralateral and ipsilateral signals and their roles in control remain open topics. Here we investigate one candidate explanation for the ipsilateral representation of reaching movements. Built on past findings that the cortex can simultaneously represent multiple alternative motor plans, we test the hypothesis that plans for accomplishing a common reaching goal are prepared in parallel for both limbs contralaterally. In this framework, correlations between the non-selected and selected reach plans would thus provide a spurious representation of the reaching movement in ipsilateral cortex despite being truly related to the contralateral limb. We tested this hypothesis using ensemble spiking activity from bilateral primary motor (M1) and dorsal premotor (PMd) cortices in one macaque monkey performing a simple reaching task. Spiking data were recorded using multi-channel acute recording probes (V-probe: Plexon, Inc). Kinematics of both upper limbs were monitored (PhaseSpace, Inc) while the subject received feedback in a 3D virtual reality environment. Using an instructed-delay reaching task, the subject was required to obtain specific starting configurations of the hands before reaching with a single limb to one of six targets. In this manner, we explicitly dissociated the selected movement from ostensible non-selected plans for the stationary limb. We analyzed the data using a population decoder approach, predicting motor behavior from neural activity, to test 2 key predictions of this hypothesis: 1. Ipsilateral decoders will generalize poorly to trials where the non-selected (stationary) hand is placed in a different position. 2. Modulation of neural activity during ipsilateral reaching will be similar during contralateral reaching. Consistent with the first prediction, our results show that ipsilateral decoders were more sensitive to changes in the starting position of the non-selected hand than contralateral decoders. However, no substantive similarity between activation profiles during ipsilateral and contralateral reaching was observed. These results suggest that parallel preparation of reaching plans with either limb does not account for a significant portion of the ipsilateral modulation in motor cortex during movement. Rather, the ipsilateral activation may simply be an independent signal that is more sensitive to changes in body posture.

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Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 587.14/QQ26

Topic: E.04. Voluntary Movements

Support: DARPA-BTO program-TNT

Title: Cholinergic modulation enhances the performance of skilled motor behaviors

Authors: *X. PENG, D. C. DONEGAN, J. L. HICKMAN, C. G. WELLE
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Abstract: Cortex receives widespread cholinergic modulation which desynchronize neural activity in cortex, increase arousal and improve sensory discrimination. Despite the well-documented ascending cholinergic projections to motor cortex, little is known about the role of cholinergic inputs on the performance of skilled motor behaviors. This work aims to determine the influence of cholinergic inputs during the performance of dexterous forelimb reach in the adult mouse, and to identify corresponding changes in neural dynamics of motor cortex. In mice trained to perform a dexterous forelimb reach task, temporally-precise stimulation was delivered to basal forebrain (BF) immediately following a successful reach. Stimulation was delivered by optical activation (20 Hz, 10 ms pulse) of channelrhodopsin (ChR2) driven by the ChAT promoter in cholinergic neurons, or in all neuronal subtypes of BF through AAV2-driven expression. Paired cholinergic activation enhanced the success rates above baseline performance. Interestingly, optical stimulation of all neuronal subtypes in BF enhanced motor performance more effectively than stimulation of cholinergic neurons alone, suggesting that multiple BF ascending projection systems may be relevant for modulating skilled motor behaviors. These results suggest that the BF projection system may modulate the motor cortex and can be exploited to enhance the performance of dexterous motor behaviors. In order to further investigate the effects of BF inputs on motor cortical circuits, population neural dynamics were monitored in freely moving animals using a miniscope to image neural calcium activity (GCaMP6) in M1. Preliminary data shows that individual neurons from layer II~III forelimb motor regions respond selectively to components of the reach movement. Future experiments pairing BF stimulation with successful reach will provide insights in the influence of BF inputs on motor cortical neural ensembles in the dexterous reach task.

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Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 587.15/RR1

Topic: E.04. Voluntary Movements

Support: Simons Collaboration on the Global Brain
HHMI
CIHR Fellowship
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Title: Mesoscale analysis of decision making, motor planning and movement initiation

Authors: *L. D. LIU[1,2], T. WANG[1], S. CHEN[1], O. MARSCHALL[3], S. DRUCKMANN[1,4], N. LI[2,1], K. SVOBODA[1], X.-J. WANG[3]
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Abstract: Cognition depends on the brain's ability to integrate past and current information and manipulate information internally in the absence of external stimuli. A fundamental challenge is to understand how the structure and dynamics of neural circuits support such internal states. Measurements of activity have been mostly limited to a handful of neurons in one brain region. However, even simple cognitive tasks engage multiple interacting brain regions and we currently lack a comprehensive picture of neural activity across the brain for any behavior. Recent advances in electrophysiology (e.g. Neuropixels probes) provide the opportunity for simultaneous recordings of neural activity across multiple brain regions. We use one or two neuropixels probes for recordings from up to a dozen brain areas at the same time. Our goal is to produce comprehensive, whole brain 'activity maps' for mice during decision-making and motor planning.
We present data from a delayed response behavioral task, in which mice make a sensory discrimination, followed by a delay epoch during which they plan a directional movement, and then followed by a response epoch during which they execute the movement. We have previously identified the anterior-lateral motor cortex (ALM) as an area critical for motor planning and movement initiation. Using an anatomy-based scheme based on the Allen Mouse Brain Connectivity Atlas (and our own data) we target brain areas that form multi-regional networks with ALM. Each electrode track is recovered in three dimensions and mapped into a standardized brain atlas. We are developing pipelines for processing, analyzing, and sharing large-scale neurophysiology data. In this preliminary report, we show data from ALM, thalamus, basal ganglia, superior colliculus, brainstem, and cerebellum. This work will advance our understanding of modular, multi-regional neural dynamics underlying cognition.

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Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

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Topic: E.04. Voluntary Movements

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Title: The effects of sensory loss on the neurobiomechanics of sensorimotor behavior

Authors: *F. I. ARCE-MCSHANE[1], N. G. HATSOPOULOS[1,2,3], C. F. ROSS[1], B. J. SESSLE[4]
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Abstract: Oral somatosensation plays a key role in breathing, feeding, and speech such that altered sensation in pain disorders and sensory loss following neurological injuries have devastating effects on the quality of life. However, how neurons in the primary orofacial sensorimotor cortex use afferent information from the tongue, jaw, and facial muscles to effect functionally critical, coordinated movements is still largely unknown. To address this question, we examined the effects of sensory loss on the performance of a tongue-protrusion task and on the activity of neurons in the primary motor (MIo) and primary somatosensory (SIo) areas of the orofacial sensorimotor cortex in monkeys (*Macaca mulatta*). Temporary sensory loss to the anterior two-thirds of the tongue was induced by using a bilateral lingual nerve block while recording the spiking activity from microelectrode arrays implanted chronically in MIo and SIo. A monkey performed two blocks of 100 trials of the tongue-protrusion task prior to the nerve block application and a block of 100 tongue-protrusion trials every half-hour following anesthesia. Immediately after the nerve block, the monkey's success rates dropped transiently to 30% but returned to pre-nerve block success rate within the hour. However, the decrease in peak force and the increase in the time to reach the required force after injection of the nerve block persisted for over three hours. In pre-nerve block trials, the monkey generated greater forces than required. In the nerve block condition, the monkey was able to generate the required force though at a slower pace. Spiking activity of MIo and SIo neurons also differed between pre-nerve block and post-nerve block as seen in changes in the mutual information carried by single units and the coherent activity between pairs of neurons. Our results demonstrate that while sensory loss to the anterior two-thirds of the tongue transiently impaired the gross performance of the sensorimotor task, the finer details of task performance rely heavily on the availability of relevant sensory inputs.

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Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 587.17/RR3

Topic: E.04. Voluntary Movements

Support: R01NS089652

Title: Sensory-motor sequence generation and learning of tongue movements

Authors: *D. XU[1], Y. CHEN[2], A. M. DELGADO[2], D. H. O'CONNOR[1]
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Abstract: Behaviors are generally composed of basic motor outputs coordinated in sequences. Learning new motor sequences and tuning them requires an animal to voluntarily output actions and alter future actions based on sensory feedback. The pinnacle of sensory-motor sequence learning is embodied by human language, where the control and modulation of tongue motion is essential. Rodents also show rich and complex motor sequences using the mouth and tongue. To study the neural basis of sensory-motor sequence learning, we developed a novel behavior in head-fixed mice where they learned to perform sequences of licks to a set of defined target locations. Tongue motions were captured by high-speed video and kinematics quantified by artificial deep neural nets. Contact forces between tongue and lick port were recorded by custom made sensors. Mice learned this task within a week and could perform a sequence of licks to 7 directions within ~1 second. During behavior, we recorded single-unit activity (n >= 250) from various brain regions (S1, M1, M2, striatum, thalamus) using silicon probe electrode arrays. Units across and within regions showed diverse task-related activities, many of which were tuned to aspects of motor sequences beyond the directions of individual licks. To examine any causal role of various brain areas, we performed closed-loop optogenetic silencing experiments, focusing on preparation, initiation and continuation of motor sequences. By combining rich behavioral quantifications, high-density electrophysiology and loss-of-function screening, we aim to characterize how an ongoing sensory-motor loop is evolved and tuned, and the underlying mechanisms of learning.

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Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

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The McGovern Institute

Title: Cortical dynamics associated with multiple timescales of sensorimotor adaptation

Authors: *N. MEIRHAEGHE[1], H. SOHN[2], M. JAZAYERI[3]
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Abstract: Humans can efficiently adapt to changes in their environment. To do so, the brain must flexibly adjust its neural dynamics to meet the ever-changing behavioral demands. In the framework of dynamical systems, such adaptive dynamics can be achieved in two ways. First, controlling external inputs enables the system to visit unexplored neural states. Alternatively, changes in synaptic couplings within the system modify its latent dynamics, effectively creating new activity patterns in state space. Because synaptic modifications occur on relatively slow timescales, we hypothesize that fast behavioral adaptation relies on input-control strategies, while slow adaptation relies on adjustments of latent dynamics.To test these hypotheses, we build on a time reproduction task in which monkeys measure a sample time interval between the first two beats of a rhythm and produce a saccadic eye movement on the third omitted beat. In this task, animals' responses are biased toward the mean of the previously encountered intervals. This bias can be quantitatively captured by a Bayesian model that integrates a noisy measured interval with the animals' knowledge about the prior distribution of sample intervals. Moreover, our previous work has shown that prior knowledge exerts its influence on behavior through an adjustment of both inputs to and latent dynamics of premotor cortical areas. Leveraging these observations, we sought to test the role of inputs and latent dynamics in two modified versions of the time reproduction task that elicit fast and slow adaptation. For fast adaptation, we covertly changed the distribution of sample intervals from a narrow prior to a single-interval. Animals adapted rapidly over a few hundred trials within a single behavioral session. For slow adaptation, we covertly switched a wide prior to a narrow prior. In this case, behavior adapted slowly across 5-6 sessions over the course of thousands of trials. We speculate that fast adaptation is achieved via adjusting the inputs to premotor areas without changing the latent dynamics, while slow adaptation depends on gradual reshaping of latent dynamics within premotor cortex. Our ongoing work seeks to test these hypotheses by comparing cortical dynamics in premotor areas during fast and slow adaptation.

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Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

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Topic: E.04. Voluntary Movements

Support: CNRS-PEPS
ANR-GRASP
EU grant 269921 (BrainScaleS)

Title: Directional selectivity across macaque motor cortical layers during reach planning and execution

Authors: *B. E. KILAVIK
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Abstract: Neurons in motor cortex are selective to different parameters during movement planning and execution, such as the direction of arm reaches. While this directional selectivity has been extensively studied, we still know very little about how it is distributed and dynamically modulated across different cortical depths.
In this study, directional selectivity was explored in a macaque monkey performing a pre-cued center-out reaching task. The target location was cued visually, several seconds before the directionally non-informative go-signal. During the delay, the monkey had to memorize the target location and could prepare the movement. Recordings were made simultaneously from superficial and deep cortical layers with multi-contact linear array (laminar) probes, in arm regions of dorsal premotor cortex (PMd) and the transition zone between PMd and primary motor cortex (M1).
Directional selectivity was explored in two task periods: following the visual cues and around movement execution. Following the cues, the neuronal population in superficial layers had an earlier phasic response than that in deep layers. However, directional selectivity developed stronger in the deep neuronal population, following the earlier, but less directionally selective superficial response. Just before the movement onset and during its dynamic phase, selectivity was stronger in superficial layers. The neurons in deep layers became equally directionally selective as the superficial population only as the hand arrived in the target, towards movement end.
These different dynamics of directional selectivity might be related to the different predominance of cortico-cortical vs. sub-cortical projections of superficial vs. deep cortical layers, respectively. Cue information seems present in superficial motor cortical layers before the directionally specific planning commences, predominantly involving deeper layers. This suggests that motor cortex participates in the decision process linking visual cues and motor preparation. Eye movements demonstrated a strong reliance on visual feedback before and during the dynamic phase of the reach, possibly causing the increased selectivity of superficial neurons in these movement epochs. The increased selectivity of neurons in deep layers towards movement end might reflect final motor command adjustments to successfully enter the peripheral target.

Disclosures: B.E. Kilavik: None.

Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

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Program #/Poster #: 587.20/RR6

Topic: E.04. Voluntary Movements

Support: Wellcome Trust Senior Fellowship Grant 106149

Title: A robust brain-spinal interface using local field potentials and epidural stimulation

Authors: *M. AMBROISE, A. JACKSON
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Abstract: Previously we have demonstrated a brain-spinal interface (BSI) to restore volitional grasping movements to monkeys after temporary pharmacological paralysis (Zimmerman and Jackson, Front Neurosci 2014). We have since made several modifications in order to improve long-term reliability and facilitate implementation in a low-power, implantable neuroprosthesis. First, we derive control signals from local field potentials (LFPs) as they provide better long-term stability and require lower sampling rates than action potential recordings, and use unsupervised areal velocity decoding (Jackson and Hall, IEEE TNSRE 2017) to detect intended movement from low-frequency cycles in the multichannel LFP. Second, we deliver epidural instead of intraspinal stimulation, using a multi-contact electrode positioned around the ventral and dorsal surface of the cervical enlargement. Here we report results of testing this improved BSI in a macaque monkey.

The monkey was trained to perform a movement task that required grasping a lever and pulling downwards by extending the elbow, prior to surgical implantation with electromyogram (EMG) electrodes in six hand and forearm muscles, two 32-channel floating microelectrode arrays in dorsal and ventral premotor cortex, a chamber over primary motor cortex and an 8-channel cuff electrode around the C7 segment of the spinal cord. Areal velocity was calculated in real-time by projecting the multichannel premotor LFP onto a plane using Principal Component Analysis. During normal task performance, this areal velocity signal increased at the onset of volitional movement.

Over 7 testing sessions, we located hand area of primary motor cortex (by intracortical microstimulation) and injected muscimol to induce reversible paralysis lasting several hours. When the BSI was enabled, areal velocity exceeding a threshold triggered spinal stimulation (50 Hz trains of biphasic stimuli delivered between dorsal and ventral sites, 100-200 μA, 0.2 ms per phase) that elicited robust EMG responses and strong muscle contractions. With the BSI enabled, the monkey performed an average (±SD) of 6.0 ± 3.1 successful trials per minute. By contrast, with the BSI disabled the monkey could perform only 0.5 ± 0.6 successful trials per minute, despite an areal velocity signal that indicated the animal's continued attempts to move.

These results demonstrate the potential for our BSI to restore simple voluntary upper-limb movements. Moreover, our use of LFP decoding and epidural stimulation offers the possibility of implementing this approach in a robust and reliable implanted neuroprosthesis suitable for clinical application.

Disclosures: M. Ambroise: None. **A. Jackson:** None.

Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 587.21/RR7

Topic: E.04. Voluntary Movements

Support: Wellcome Trust
ERA-NET NEURON

Title: Epidural and transcutaneous spinal cord stimulation facilitates descending inputs to upper-limb motoneurons

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Abstract: Renewed interest in spinal cord stimulation (SCS) has emerged in recent years following reports of significant gains of voluntary motor function in spinal cord injured patients after SCS-assisted rehabilitation. Although not fully elucidated, the mechanism behind this effect is believed to be a potentiation of spinal network excitability that unmasks spared but weakened descending pathways from the brain. We performed a series of experiments in anaesthetized, neurologically intact monkeys in order to better characterize SCS and elucidate its impact on corticospinal excitability. Spatial selectivity of SCS was investigated using an 8-contact ring electrode placed epidurally or subdurally around the cervical spinal cord while electromyogram (EMG) signals were recorded from upper limb muscles. In separate experiments we delivered transcutaneous stimulation using a high carrier frequency through electrodes on the skin. Trains of suprathreshold SCS at different frequencies were used to characterize temporal facilitation and suppression after ventral or dorsal stimulation. Ventral electrodes elicited more robust movements than the dorsal side, with spinal motor evoked potentials reliably following even high-frequency trains. For dorsal electrodes, short-term facilitation was observed for the first few pulses followed by subsequent suppression for the rest of the train. This effect was most pronounced at high frequencies.
Next, we assessed the interaction between cortical inputs and subthreshold SCS by delivering intracortical microstimulation to the hand area of primary motor cortex during trains of epidural or transcutaneous SCS (at frequencies of 10, 20, 50 and 100 Hz). Cortical-evoked motor

potentials were reliably facilitated by dorsal stimulation, although the effect was more pronounced for epidural versus transcutaneous SCS. Facilitation was maximal for the stimulus frequency of 50 Hz. Interestingly, no suppression of the cortical motor evoked potential was observed, even for the highest SCS frequency.
These results can be explained by a simple model whereby dorsal stimulation activates afferent inputs to motoneurons, raising their excitability, but also presynaptically inhibits subsequent afferent input. SCS at high frequencies thus suppresses subsequent spinal-evoked responses but does not suppress the cortical-evoked response. We further suggest that the facilitation of cortical-evoked responses could be used to optimise patient-specific stimulation parameters prior to SCS-assisted rehabilitation.

Disclosures: T. Guiho: None. **J. Kersey:** None. **S.N. Baker:** None. **A. Jackson:** None.

Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 587.22/RR8

Topic: E.04. Voluntary Movements

Support: DFG RU-1847 Grant GA1475-C1
EC-H2020-FETPROACT-16 732266 WP1

Title: Parietal and premotor planning signals for walk-and-reach movements towards far-located goals in unrestrained rhesus macaques

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Abstract: In sensorimotor neuroscience with non-human primates reaching movements are usually studied in isolation or in combination with tightly constrained hand, eye or head movements. We know little about how goal-directed reaching movements are planned and controlled when whole body movements are involved. In our recently developed Reach Cage (Berger and Gail, 2018 bioRxiv), we now studied how the fronto-parietal reach network encodes walk-and-reach movement goals.
We trained two rhesus macaques to a delayed walk-and-reach task. Movement targets were at four horizontally distributed directions near to the animal (reach) and four further away so that the animal needed to walk towards the targets before reaching (walk-and-reach). The monkeys performed movements towards one of eight targets with variable instructed delay (planning period) after visual cueing of the target. We recorded wirelessly single and multi-unit activity

from the parietal reach region (PRR), dorsal premotor cortex (PMd) and arm area of the primary motor cortex (M1). We analyzed motor-goal encoding during the instructed delay prior to the movement.
Individual units were modulated for both, reach movements and walk-and-reach movements, with an overall lower fraction of walk-and-reach modulated units. To characterize the population activity, we used demixed principle component analysis (dPCA) to identify how much variance is captured by the target distance (reach vs. walk-and-reach) and horizontal position. Distance explains most variance of planning activity (>50% for PMd and PRR and approx. 30% for M1). In all areas we found distance related dPCA components that show significant modulation starting less than 200ms after target onset. This was expected due to the clear differences in movement behavior between reach and walk-and-reach movements. We wanted to know whether the fronto-parietal network encodes distance-invariant target position. If horizontal target position is encoded for near and far-located movement targets, we expect to find components that contain an isolated representation of horizontal position. If encoding would be specific for near-located targets, the full horizontal position variance would be dependent on distance and dPCA would characterize this as an interaction term. Indeed, dPCA could isolate significantly modulated components in all three brain areas that represent only horizontal position. The variance explained by horizontal position is around 10%-20%. The significant modulation for position independent of distance suggests that the fronto-parietal network is co-encoding far-located walk-and-reach targets with near-located reach targets.

Disclosures: M. Berger: None. **A. Gail:** None.

Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 587.23/RR9

Topic: E.04. Voluntary Movements

Support: NRF-2016M3C7A1904988

Title: Encoding of licking direction in a shared space of neuronal ensemble activities in anterior lateral motor cortex in rodents

Authors: *S. CHAE[1], S.-P. KIM[2]
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Abstract: Anterior lateral motor cortex (ALM) in rodents have been known as a cortical area commanding directional licking. Neurons in ALM also retain the directional information before movement execution. A number of studies have revealed the neural mechanisms of a conditional

licking strategy associated with sensory information within ALM as well as between ALM and other brain areas. However, how neurons in ALM neurons transform licking direction information in working memory into licking movements within the ALM still remains unknown. To address this, we analyzed the publicly available datasets in the CRCNS data repository. In the experiment, multi-channel neural spikes from left ALM were recorded in 11 adult mice during a tactile decision task. A pole touched the whisker of the mice for 1.3 s, cueing the direction of reward. After the pole was off, mice waited for 1.3 s (delay period) and licked to the right or left after 0.1-s beep sound (response period). The behavioral results were classified into four cases: Hit right (HR), Hit left (HL), Error right (ER) and Error left (EL), where the error trials occurred when the mice licked in the direction opposite to cued direction. We hypothesized that ALM neurons shared the information of licking direction in the delay period to transform it to movement execution after an action cue (i.e. beep), rather than processing information independently. To estimate shared information among neurons, we used the factor analysis to decompose ensemble activity in every 200-ms of the delay period into the shared signal, which was modulated by low-dimensional latent variables, and private signals with no covariance between neurons. We approximated shared space alignment which was defined as the projection of the shared space in the response period onto the shared space in the delay period. The resulting alignment ranged between 0 and 1 where 1 denoted perfect alignment between two shared spaces and 0 denoted orthogonality between the spaces. A projection matrix was estimated using the data in the Hit trials. We found that the shared space alignment in the Hit trials was significantly higher than a chance level ($p<0.01$). However, when we aligned the shared space of the Hit trials with the Error trials based on the cuing direction (e.g. ER onto HR), the shared space alignment was not significantly different from the chance level. In contrast, when we aligned the shared space of the opposite Hit trials with the Error trials (e.g. EL onto HR), the shared space alignment became significant ($p<0.01$). This result indicates that neurons in ALM may collectively form a specific pattern to encode future licking direction, which is kept aligned during licking.

Disclosures: S. Chae: None. **S. Kim:** None.

Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 587.24/RR10

Topic: E.04. Voluntary Movements

Title: The organization of motor cortex in the Egyptian fruit bat (rousettus aegyptiacus): Specializations of the tongue representation associated with echolocation

Authors: *A. C. HALLEY[1], M. M. YARTSEV[2], L. A. KRUBITZER[1]
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Abstract: The Egyptian fruit bat *Rousettus aegyptiacus* and other members of its genus are the only megachiropteran bats that echolocate, and the only known bats to use tongue clicks rather than laryngeal vocalization to produce active sonar. Very little is known about the organization of motor neocortex in bats generally, or in *Rousettus* specifically, as no previous study has attempted to map the motor cortex in any species of *Chiroptera* using intracortical microstimulation techniques. Here we present the first movement maps in motor cortex made in any bat species. We utilized both short- (50ms) and long-train (500ms) intracortical micro-stimulation (ICMS) techniques applied to a large region of neocortex spanning primary motor cortex, somatosensory cortex, and posterior parietal cortex. Movements were elicited from stimulation of both somatosensory and motor cortices, with hindlimb representations located caudomedially, representations of the face and jaw located rostrolaterally, and wing representations in an intermediate position. Most notably, stimulation of the head and face areas revealed extensive motor representation of the tongue that is topographically organized, such that proximal and distal tongue movements are elicited from stimulating adjacent but distinct areas of the neocortex. While there are no motor stimulation studies on non-echolocating bats to allow for a direct comparison, studies of somatosensory cortex in non-echolocating megabats (Krubitzer & Calford 1992) show no evidence of magnified tongue representation. This suggests a unique evolutionary adaptation in *Rousettus* to allow for finer motor control of tongue movements in the generation of active sonar clicks.

Disclosures: M.M. Yartsev: None. **L.A. Krubitzer:** None.

Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

Location: SDCC Halls B-H

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Program #/Poster #: 587.25/RR11

Topic: E.04. Voluntary Movements

Support: NIH Grant R01NS030853
NIH Grant F32NS100339
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Title: Long-term stability of single channel neural activity during execution of gross and fine reaching in rats

Authors: *D. T. BUNDY[1], D. J. GUGGENMOS[1], M. D. MURPHY[4], M. SAMI[2], R. J. NUDO[3]
[1]Dept. of Physical Med. and Rehabil., [2]Dept. of Neurosurg., [3]Univ. of Kansas Med. Ctr., Kansas City, KS; [4]Univ. of Kansas, Kansas City, KS

Abstract: Following a lesion to motor cortex, reorganization occurs throughout the remaining brain regions and is thought to underlie motor recovery. Unfortunately, the standard neurophysiological and neuroanatomical measures of post-lesion plasticity can only be indirectly related to changes in motor execution. Assessing alterations in task-related neural activity during motor recovery will lead to an increased understanding of how these neuroplastic measures directly contribute to motor execution following recovery. This study examined the long-term stability of neural activity associated with execution of reaching movements in healthy rodents. Male Long-Evans rats (*Rattus norvegicus*, n=5) were trained to perform a reaching task consisting of a 'gross' lever press that allows access to perform a 'fine' pellet retrieval. In each animal, two chronic, 16-channel microelectrode arrays were implanted in motor cortex contralateral to the reaching forelimb, with one array implanted in the caudal forelimb area (rodent primary motor cortex) and a second implanted in the rostral forelimb area (rodent premotor cortex). We recorded multiunit spiking and LFP activity 2-3 times per week from 10 days to 8 weeks post implant and analyzed the consistency of channel-specific task-aligned multiunit firing rate changes. Channels with statistically significant task-aligned firing rate changes were included for further analysis. For each channel, we calculated the multiunit firing rate in a 4s window aligned to either the lever press or pellet retrieval, and calculated the correlation (Pearson's r) of the average firing rate from each recording with the average firing rate from all recordings. Across days, channels, and rats, the average correlation between the daily and overall firing rate was 0.66 for the pellet retrieval and 0.60 for the lever press. We observed decreased correlations in early (days 10-20) and late (>6 weeks) days as rats regained proficiency following the implant procedure and electrodes were encapsulated, and individual units were lost from multiunit recordings, respectively. These results demonstrate that task-related multiunit firing rates are generally consistent with maintained cortical involvement in movement execution; therefore, multiunit firing rate changes can be used for future evaluation during recovery from a cortical injury, particularly in the subacute to chronic periods in which injury-related neuroplasticity is known to occur. Future work will seek to compare the consistency of task-related LFP activity to multiunit spiking activity, particularly for early and late recording sessions.

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Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

Location: SDCC Halls B-H

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Program #/Poster #: 587.26/RR12

Topic: E.04. Voluntary Movements

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NSF MOTO-IGERT
UChicago Big Vision Fund
Tarson Fund

Title: Sensorimotor cortical population responses across natural behaviors

Authors: *J. WALKER[1], F. PIRSCHEL[2], J. N. MACLEAN[4], N. G. HATSOPOULOS[3]
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Abstract: Theoretical and ethological perspectives suggest investigating cortical population responses during complex, natural behaviors will be key to understanding the cortical population code. Multiple groups have found surprisingly low dimensional structure in population responses while studying several cortical areas during highly trained and constrained behaviors. Evidence for low dimensional structure of neural population activity in macaque motor cortex across different tasks has recently been reported, but it remains unclear if such structure persists across naturalistic behaviors. Here we measured structure in sensorimotor cortical population responses during unconstrained natural behavior in the Common marmoset using wireless multielectrode array recordings. We summarized the marmoset's behavioral repertoire and found that more than 90 percent of a waking hour is spent sitting, vertically clinging, engaging in locomotion, leaping, foraging or food manipulation behaviors. Our initial characterizations of sensorimotor cortical population responses during natural behaviors suggest that more dimensions are required to account for the variance in neuronal activity than would be predicted from more constrained behaviors.

Disclosures: J. Walker: None. **F. Pirschel:** None. **J.N. MacLean:** None. **N.G. Hatsopoulos:** None.

Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 587.27/RR13

Topic: E.04. Voluntary Movements

Support: Howard Hughes Medical Institute

Title: Distinct descending motor cortex pathways and their roles in movement

Authors: *M. N. ECONOMO[1], S. VISWANATHAN[2], B. TASIC[3], E. BAS[7], J. WINNUBST[8], V. MENON[9], L. T. GRAYBUCK[4], T. NGUYEN[5], L. WANG[10], C. R. GERFEN[11], J. V. CHANDRASHEKAR[12], H. ZENG[6], L. LOOGER[10], K. SVOBODA[13]
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Abstract: Activity in motor cortex predicts specific movements, seconds before they are initiated. This preparatory activity has been observed in L5 descending 'pyramidal tract' (PT) neurons. A key question is how preparatory activity can be maintained without causing movement, and how preparatory activity is eventually converted to a motor command to trigger appropriate movements. We used single cell transcriptional profiling and axonal reconstructions to identify two types of PT neuron. Both types share projections to multiple targets in the basal ganglia and brainstem. One type projects to thalamic regions that connect back to motor cortex. In a delayed-response task, these neurons produced early preparatory activity that persisted until the movement. The second type projects to motor centers in the medulla and produced late preparatory activity and motor commands. These results indicate that two motor cortex output neurons are specialized for distinct roles in motor control.

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Poster

587. Voluntary Movements: Cortical Planning and Execution: Neurophysiology: Animal II

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Program #/Poster #: 587.28/RR14

Topic: E.04. Voluntary Movements

Support: NINDS Grant 5R01NS062019-06

Title: Grip affordances are encoded in conjunction with grasping movements in M1

Authors: *R. N. TIEN, A. B. SCHWARTZ
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Abstract: Spiking activity in primary motor cortex (M1) correlates with aspects of reaching and grasping movements. The extent to which this movement encoding remains stable over time and

across contexts is largely unknown. Moreover, the possibility that M1 may encode contextual information in addition to ongoing movements has remained mostly unexplored.
Here, we present evidence that in a reach-to-grasp task, M1 neurons encode both the grasping movement and contextual information related to the affordances of the grasped object.
We trained two rhesus macaques to grasp and hold two simple objects and a compound object. The first simple object afforded only a power grip, the second only a precision grip, and the compound object afforded both grips. The goal of the study was to elicit the same grasping movement in different contexts. We recorded M1 spiking activity, the subjects' arm and hand kinematics and muscle activity throughout the behavior.
This paradigm allowed us to compare conditions in which the executed grasping movements were the same while the identities of the grasped object differed (e.g. power grip on the simple object vs. power grip on the compound object). We found that the majority of M1 neurons displayed significantly different firing patterns when different objects were grasped, even when the movements had nearly identical kinematics. In terms of single unit firing rates, the magnitude of firing rate modulation due to object encoding was comparable to or greater than that due to movement encoding, especially around movement onset.
Object identity did not appear to be encoded simply or directly, as most single units displayed complex interactions between object identity and executed movement encoding. This interactive (rather than additive) coding also evolved in time, such that different neurons encoded objects at different times. The net effect was that objects were consistently classifiable from the neural population at well above chance levels from before movement onset through object contact. This contrasts with movement encoding, where classification accuracy for movements increased as the hand approached the object.
Further experiments and classification analyses revealed that object identity representations based on both learned and perceived grasp affordances could be extracted from M1. The results from these experiments will be important to consider in the design of future neuroprosthetic decoders for cortical control of a robotic arm and hand.

Disclosures: R.N. Tien: None. **A.B. Schwartz:** None.

Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

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Topic: E.04. Voluntary Movements

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Title: Multimodal measurement of swallowing using human electrocorticograms, Kinect v2, an electroglottography and a throat microphone in order to reveal swallowing-related neural activities

Authors: *H. HASHIMOTO[1,2], M. HIRATA[1,2], K. TAKAHASHI[3], S. KAMEDA[1], F. YOSHIDA[4,1], T. YANAGISAWA[1,2], S. OSHINO[2], T. YOSHIMINE[1], H. KISHIMA[2]
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Abstract: Research for swallowing-related neural activities in human has been done mainly by a non-invasive manner, PET, TMS, NIRS, fMRI and MEG. These results showed some cortical areas are activated during swallowing, but the spatiotemporal resolution of these results is not high. Therefore, we have used human electrocorticograms (ECoGs) to the analysis of swallowing-related neural activities with high spatiotemporal resolution. For measurement of ECoGs during swallowing, we developed a novel multimodal measurement system.For non-invasive swallowing detection, we used an electroglottograph (EGG), a throat microphone and a simple swallow tracking system (SSTS). Swallowing-related neck impedance changes recorded by EGG and bolus sound recorded by a throat microphone helped us to detect the onset time of swallowing. The SSTS is newly developed by us in order to quantify swallowing-related motion using the Kinect v2 (Microsoft,Redmond, Washington, USA). We defined three mouth-related parameters and two larynx-related parameters. We measured five parameters during water swallowing in ten health participants. Simultaneously, we captured the motion of participants using an RGB camera of the Kinect v2. Changes in mouth-related parameters were observed before swallowing and reached peak values at the time of swallowing. In contrast, larynx-related parameters showed little change before swallowing and reached peak values immediately after swallowing. The SSTS successfully quantified the swallowing process from the oral phase to the laryngeal phase. The SSTS is non-invasive, wireless, easy to set up, and simultaneously measures the dynamics of swallowing from the mouth to the larynx.An electro stimulator supplied digital signals to an EGG, a throat microphone and a 128-channel digital EEG system. The signals made an LED light flash, which was captured by the RGB camera of Kinect v2. The digital triggers and LED light flash enabled us to synchronize multimodal data of an EGG, a throat microphone, SSTS and an EEG. The simultaneous recording of an EGG, a microphone and SSTS enabled us to non-invasively and accurately identify the timing of the swallowing movement. Therefore, we could insert triggers into ECoGs data corresponding to the timing of swallowing. We newly constructed multimodal measurement system during swallowing and analyzed the oscillatory changes related to swallowing using ECoGs data. Time-frequency plots of the subcentral area (Brodmann area 43) demonstrated that high gamma band activity appeared specific to swallowing. This high gamma activities may be the key phenomenon of cortical activities involved in swallowing.

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Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 588.02/SS2

Topic: E.04. Voluntary Movements

Support: NIH Grant 5U01NS098969-02

Title: Differential modulation of neural activity in the ventral lateral nucleus of the thalamus during speech production

Authors: *D. WANG[1], W. J. LIPSKI[1], A. BUSH[1], C. DASTOLFO-HROMACK[2], A. CHRABASZCZ[3], D. J. CRAMMOND[1], S. SHAIMAN[2], R. S. TURNER[4], J. A. FIEZ[3], M. RICHARDSON[1]
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Abstract: Both basal ganglia-thalamo-cortical and cerebello-thalamo-cortical circuits contribute to speech motor control and language processing. The thalamus functions as a relay center in both circuits, yet its involvement in speech production rarely has been studied directly. The posterior part of the ventral lateral nucleus (VLp) mainly receives inputs from the cerebellum and sends outputs to the motor cortex, while the anterior part of the ventral lateral nucleus (VLa) primarily relays information between the globus pallidus and the premotor cortex; both regions are encountered during implantation of deep brain stimulation (DBS) leads into the Vim nucleus. We recorded spoken acoustics and simultaneous local field potentials (LFPs) in the ventral lateral nucleus of the thalamus (VL) in 12 essential tremor subjects while they performed an intra-operative speech task during DBS surgery. On each trial, subjects were asked to name a consonant-vowel-consonant syllable when it appeared on the screen. LFP signals were spectrally decomposed and power values were normalized relative to the baseline period. Recording locations were determined using the Lead-DBS toolbox, and contact locations were categorized to VLa and VLp. High Gamma (70-150 Hz) activation and beta (13-30 Hz) desynchronization were observed during speech, indicating active participation of thalamus in speech production. The increase in high gamma power was locked to speech onset (30/47 contacts, Pearson correlation, $p < 0.01$, FDR corrected), while beta desynchronization was locked to presentation of the visual cue (35/39 contacts, Pearson correlation, $p < 0.01$, FDR corrected), suggesting that oscillations within these frequency bands encode different aspects of the speech task. Furthermore, we observed that the strength of power changes during speech were dependent on

recording location within the nucleus. High Gamma activation during speech was greater in electrode contacts localized to the VLp compared to those localized to the VLa ($p < 0.01$), indicating functional heterogeneity of VL in speech control. These results provide support for the involvement of VL in speech motor control and establish a novel methodological framework to test neurophysiological models of speech production.

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Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 588.03/SS3

Topic: F.01. Neuroethology

Support: DFF 5051-00195

Title: Peripheral constraints on motor learning: Maximal speed of adult vocal muscles is not available during song learning

Authors: *I. ADAM, M. VELLEMA, C. P. ELEMANS
Univ. of Southern Denmark, Odense, Denmark

Abstract: Like human babies, songbirds learn their vocalizations from a social tutor through auditory guided motor learning. To imitate the song of their tutor, they first memorize a template by listening to a social tutor (auditory learning) followed by motor practice (sensorimotor learning). Over the latter period, temporal precision and song complexity increases and finally achieves sub-millisecond timing precision of sound elements. These extraordinary demands on vocal motor skills are met by superfast muscles (SFMs) in the syrinx. Superfast muscles are the fastest known synchronous muscles capable of contraction rates <250 Hz, enabling precise motor execution at the millisecond time scale. We recently showed that the heavy myosin chain linked to this superfast behavior is encoded by the MYH13 gene, which makes up most of the heavy myosin chains in the syringeal muscle of adult songbirds. So far, it is not known how the expression of MYH13 in syringeal muscles is regulated. Moreover, very little is known about the postnatal development of the syrinx, which coincides with vocal learning. Here, we demonstrate that over the course of song learning: (I) MYH13 was upregulated, (II) the duration of syringeal muscle contractions decreased, and (III) the composition of heavy myosin chains changed towards faster myosin forms. Furthermore, we investigated to what extent delayed song learning affected muscle speed and MYH13 expression. Taken together, we show that muscle speed - crucial to achieve precise timing of sound production- increases significantly during vocal motor

learning and that the maximal speed achieved in adult animals is not available during song learning. Our results suggest that increasing vocal performance during song learning may not only reflect neural circuitry maturation but also superfast muscle performance increase. The observed speed increase may result from training by use as consistent with other muscles expressing MYH13. Consequently, postnatal changes in the vocal muscles of songbirds may set constraints on the performance and development of learned vocal motor skills.

Disclosures: M. Vellema: None. **C.P. Elemans:** None.

Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 588.04/SS4

Topic: F.01. Neuroethology

Support: Danish Research Council

Title: Fundamental frequency control by dynamic actuation of the songbird syrinx

Authors: *C. P. ELEMANS[1], A. MAXWELL[2], C. LAUGESEN[2], B. J. KNÖRLEIN[3], D. N. DÜRING[4]
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Abstract: Juvenile songbirds navigate the motor space of their vocal organ - the syrinx - and respiratory system through trial-and-error leaning to generate precise vocal targets and sequences. Studying stereotyped in vivo motor behavior provides limited insight into the biomechanical system because only the final individual solutions are observed, which may not be unique. Understanding how the brain controls song requires therefore systematic quantification of the system's behavior across its multi-dimensional parameter space.
Using novel experimental paradigms, we here combine marker-based 3D motion capture of syringeal elements with simultaneous servomotor actuation of two muscle insertion sites, force measurements and precise pressure control to systematically map the partial syrinx motor space during sound production in vitro. We focused on the control of a biologically important acoustic parameter - fundamental frequency (f_o) - of the produced sounds. Earlier work showed that two muscles (Musculus syringealis ventralis (VS) and Musculus syringealis dorsalis medialis (MDS) directly modulate the length of the vibrating tissue - thought to set f_o Additionally, air pressure and VS activity during song and VS stimulation in vivo and ex vivo affected f_o.
We used individual and combinatory sweeps of VS/MDS shortening and air pressures to map the motor space. Our data shows positive linear relations between pressure and fo and VS shortening

and f_0 Interestingly, co-activating syringeal muscles shifted or increased the fo range in half of the subjects. Additionally we quantified 3D motion of syringeal elements induced by muscle microstimulation ex vivo. These data validate the imposed muscle shortening trajectories using in vitro, and also provide novel insights into biomechanical function of syringeal muscles. Furthermore, our results confirm that the peripheral motor space is redundant in the control of the key vocal parameter fo.
The presented methodology breaks ground towards quantifying the acoustic effects of muscle recruitment, motor output and the calibration and testing of sound production models in bird. As such, we aim to enable experimental access to the entire neuromechanical control loop of vocal motor control.

Disclosures: C.P. Elemans: None. **A. Maxwell:** None. **C. Laugesen:** None. **B.J. Knörlein:** None. **D.N. Düring:** None.

Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 588.05/SS5

Topic: E.04. Voluntary Movements

Support: ERC FP7/2007-2013 no. 339152

Title: Stability in postural tongue control: Response to transient mechanical perturbations

Authors: *T. ITO, J.-L. CAILLET, P. PERRIER
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Abstract: Tongue has properties as a muscular hydrostat that are unique in the human body. It is also a fundamental organ in a variety of basic biological functions for humans, such as breathing, swallowing and speaking. However, the neurophysiological mechanisms enabling its fine motor control have not been yet thoroughly investigated. While the involvement of reflex mechanisms has been largely documented for quick compensations in postural control of limbs and body as a low-level of control function, the functional role or even the existence of autogenous reflex has not been yet clearly established for the tongue. This study aimed to examine responses to transient mechanical perturbations in postural tongue control. Eight native speakers of French were asked to sustain three vowel utterances (/i/, /e/, and /ɛ/) silently, while whispering and while voicing. Articulatory movements were recorded using an electromagnetic-articulometer, together with the acoustic speech signal. Seven sensors were glued to the tongue (tip, blade and dorsum), the upper and lower lip, and the lower incisor (jaw) in the sagittal plane. Transient mechanical perturbations (1N during 1s) were provided to the tongue during the task using a robotic device, via a thin string glued to the blade. We observed an immediate compensatory response against

the mechanical perturbation. The position of each sensor on the tongue did not return to their original position, but the response tends to enable recovering the original shape of the tongue contour. During vowel production the spectral characteristics of the sounds were also modified, but were recovered quickly in synchronization with the compensatory response in motion, which suggests that tongue control was organized so as to maintain the acoustic output. We observed over time two phases in the compensatory response. The amplitude in the earlier phase was strongly related to the amplitude of the initial displacement induced by the perturbation, suggesting the involvement of a purely passive mechanism. The amplitude in the later phase varied according to the task and the location on the tongue, suggesting the involvement of a reflex mechanism which gain was systematically controlled to maintain the tongue shape according to the requirement of the task. The current finding is the first evidence for the existence of tunable reflex-based compensatory mechanisms in postural tongue control. The tongue posture for vowel production can be regulated based not on the specific position, but on the global shape of the tongue contour, which determines vocal tract geometry and, then, speech acoustics.

Disclosures: J. Caillet: None. **P. Perrier:** None.

Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 588.06/SS6

Topic: E.04. Voluntary Movements

Support: NIH Grant R01DC010145
NSF grant BCS 1262297
NIH grant R01DC013979

Title: Oral cavity numbing reduces sensorimotor adaptation to altered auditory feedback

Authors: *I. RAHARJO, H. KOTHARE, J. F. HOUDE, S. S. NAGARAJAN
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Abstract: Sensorimotor adaptation experiments in speech have shown that the speech motor control system learns to compensate for consistent alterations of the auditory feedback. However, these compensatory changes are incomplete, i.e. subjects do not fully oppose the applied alteration. This constraint in adaptation has long been postulated to be caused by the modulatory role of somatosensory feedback. Here, we examined this role by assessing the effect of orosensory numbing on adaptation to altered formant feedback.
We conducted formant adaptation experiments with real-time alteration of formant frequencies. Forty subjects were prompted to produce the word ‘head’ (vowel /ɛ/) 90 times. The repetitions

were split into 20 trials of unaltered feedback (baseline block), 50 trials of +200 Hz shifted feedback in the first formant (F1) (hold block), and lastly followed by 20 trials of unaltered feedback (washout block).
Subjects then swished for a minute depending on their assigned experimental groups (20 subjects each). The lidocaine group swished with a numbing solution (5 ml of 4% lidocaine with 5 ml flavored water), whereas the placebo group swished with a non-numbing solution (5 ml bitter lemon water with 5 ml flavored water). Effectiveness of numbing by lidocaine was verified using nylon monofilament sutures that mapped the tactile threshold of the tip of the tongue at various time points in the experiment. After swishing, the same formant adaptation experiment was then repeated.
Pre-swish and post-swish adaptive responses were calculated in both groups, and were normalised to individual average baseline F1 frequency. A generalised linear model (GLM) revealed that adaptation values were significantly reduced in the post-swish hold block for the lidocaine group ($p<0.0001$), and remained significant in the washout block ($p<0.01$). Reduced adaptive response was not seen in the placebo group. To verify that somatosensory feedback was indeed altered by the lidocaine solution, buckling force data for the filaments was run through a similar GLM model. Tactile sensitivity of the tip of the tongue was reduced significantly for the lidocaine group ($p<0.05$) and remained unaltered for the placebo group.
We observed a reduction in adaptation to altered auditory feedback resulting from oral numbing. This runs counter to the enhancing effects of numbing on immediate compensation for transient auditory perturbations. Nevertheless, our results so far are consistent with our State Feedback Control model of speech motor control.

Disclosures: I. Raharjo: None. **H. Kothare:** None. **J.F. Houde:** None. **S.S. Nagarajan:** None.

Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 588.07/SS7

Topic: E.04. Voluntary Movements

Title: Sound naturalness of wideband speech affects articulatory compensation for altered formant feedback

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Abstract: In order to investigate the importance of auditory feedback during speech production, transformed auditory feedback (TAF) experiments have been conducted, where articulatory compensation while giving perturbation to formant frequency has attracted a lot of attention. Most previous TAF studies have used a formant conversion system with a sampling rate of 8

kHz (or sometimes 10 or 12 kHz). Although a sampling rate of 8 kHz is common for telephone speech, as most linguistic information in speech is conveyed within the narrow-bandwidth of 4 kHz, a wider band speech, e.g. a sampling rate of 16 kHz, is needed to convey nonlinguistic information such as speaker individuality. Our previous studies have shown that compensatory responses to formant perturbation could be significantly larger by improving speech sound naturalness even at a sampling rate of 8 kHz. However, few studies have reported that the improvement of sound naturalness by increasing the sampling rate of feedback speech further affects articulatory compensatory response. In this study, we examine whether the sound naturalness of wideband transformed speech improves articulatory compensation compared to that of narrowband speech. A formant conversion system which we used estimates formant frequencies robustly against a fundamental frequency using our phase equalization-based autoregressive exogenous (PEAR) model. A sampling rate of input speech was set to 16 kHz. We examined a compensatory response to formant perturbation when a sampling rate of output signals was either 8 kHz or 16 kHz by low-pass filter switching. Speech stimuli used a Japanese /he/ syllable. Formants were transformed so that vowel /e/ gradually shifted to sound like /a/ by changing both the first and second formant frequencies (F1 and F2). Results showed that articulatory compensatory responses to F1 were not significantly different between sampling rates, but the aftereffects of 16 kHz for F1 were larger than that of 8 kHz. Moreover, compensatory responses to F2 were significantly larger for 16 kHz than 8 kHz. This indicated that wideband speech naturalness affected the adaptation pattern in F1 and increased compensation magnitude in F2. This result may be related to the sense of agency, but this will be an issue to be addressed in the future.

Disclosures: Y. Uezu: None. **S. Hiroya:** None. **T. Mochida:** None.

Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

Location: SDCC Halls B-H

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Program #/Poster #: 588.08/SS8

Topic: F.01. Neuroethology

Support: NIH Grant MH070712-09

Title: Adaptive song modification is impaired following FoxP2 overexpression in adult zebra finches

Authors: *N. F. DAY, S. N. FREDA, S. A. WHITE
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Abstract: The acquisition and maintenance of complex sensorimotor skills require sensory feedback to optimize motor output. In songbirds such as male zebra finches, an essential animal

model for human speech, sensorimotor learning is required throughout the lifespan to control song behavior. Juveniles imitate a tutor song during initial song acquisition whereas adults correct vocal errors during daily song maintenance. Similar to sensorimotor learning in mammals, vocal plasticity in songbirds is controlled by a basal ganglia thalamo-cortical loop. Speech impairments arising from mutations in the *FOXP2* transcription factor underscore the importance of understanding how individual or suites of genes may influence or impede vocal learning. Within the song-dedicated region of the avian basal ganglia, Area X, *FoxP2* is dynamically regulated based on the the type and quantity of song. In juvenile zebra finches, dysregulation of *FoxP2* disrupts vocal learning. Additionally, overexpression of Area X *FoxP2* in deafened adult birds hastens song degradation, which links *FoxP2* and auditory feedback processing. To further establish a connection between *FoxP2* and sensorimotor error correction, we used disruptive auditory feedback to evoke learning in adult finches. Briefly, a bird received a short burst of white noise when he performed a specific syllable in his song above (or below) a specified pitch threshold. All birds were able to learn to modify their song to avoid punishment. Following three days of incremental learning and one day of rapid learning, a subset of birds was injected with a herpes simplex virus (HSV) to drive overexpression of *FoxP2* in Area X. We compared learning before and after HSV injection to test whether *FoxP2* overexpression interferes with sensory-guided vocal output. Data from five birds suggests that adaptive song modification is impaired following *FoxP2* overexpression, which prevented them from effectively avoiding negative reinforcement. Our results implicate *FoxP2* in song evaluation, establishing a molecular basis for auditory processing that guides reinforcement-based learning.

Disclosures: N.F. Day: None. **S.N. Freda:** None. **S.A. White:** None.

Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

Location: SDCC Halls B-H

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Title: A songbird model system for understanding the biological evolution of human language

Authors: *M. FARIAS-VIRGENS[1], P. INGLE[1], T. DEACON[2], K. OKANOYA[3,4], S. A. WHITE[1], E. HUERTA-SANCHEZ[5]
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Abstract: Recent discoveries demonstrate striking analogies between birdsong and human speech. These comprise similarities at several levels, including structural (concerning the rule-dependent sequencing of vocal units), developmental (the social and asocial aspects of vocal production learning), neural (the overall architecture of engaged brain areas), and molecular (the underlying genetic regulatory networks). These striking parallels motivate the additional search for evolutionary analogies (parallel evolutionary trajectories) between vocal production learning in humans and songbirds. Our research empirically identifies evolutionary processes leading to increased complexity of vocal production learning in a songbird model system that has long been proposed to parallel key aspects of human speech evolution. Among songbird model species, the Bengalese finch (BF) (*Lonchura striata domestica*) draws special attention, due to its remarkably complex song, in which transitions between vocal units (i.e., syllables) are not firmly fixed, introducing variability in song sequencing. This vocal complexity evolved during BF's domestication from the white-backed munia (WBM) (*Lonchura striata*), and it is greater than that exhibited by more popular songbird model species, such as the zebra finch (*Taeniopygia guttata*). Moreover, BFs have evolved the ability to learn multiple tutor's songs, thus generating a composite song. Different hypotheses have been proposed to explain how BF evolved a more complex vocal behavior than its wild ancestor. One such hypothesis argues for the major role of positive selection (e.g. female choice for more complex songs), while an alternative hypothesis argues for a major role for relaxation of evolutionary constraints due to domestication. Sources of evolutionary constraints commonly found in the wild, but absent in the domesticated setting, would include pressures to avoid confusion with other cohabitating finch species and female attraction. Our study investigates the genetic signatures left by the evolutionary forces that led to BF's increased song complexity relative to WBM. For this, we have sequenced whole genomes of individuals within the two bird strains (12 BFs and 11 WBMs), which we are scanning for signatures of positive selection or relaxation of evolutionary constraints, thus allowing us to identify candidate genes modified in this evolutionary transition. Preliminary analysis of this data signals several genomic areas with high differentiation between the BFs and WBMs, as evidenced by the Fixation Index, and relatively reduced levels of genetic variability in BF, as evidenced by measures of heterozygosity.

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Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

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Topic: F.01. Neuroethology

Support: Leopoldina German National Academy of Sciences
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Title: Bengalese finches can use learned sensory cues to flexibly shift between opposing song modifications

Authors: *L. VEIT[1], L. Y. TIAN[1], C. J. M. HERNANDEZ[2], M. S. BRAINARD[1]
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Abstract: Bengalese finch (*Lonchura striata*) song is a complex learned motor skill with variable sequencing of discrete song elements, or syllables. In addition to initial song learning during development, adult Bengalese finches can be trained to modify the pitch or sequencing of individual song elements through reinforcement learning. These modifications typically occur over the timescale of hours or days, presumably reflecting the gradual updating of song control parameters in response to the recent history of performance-related feedback (Tumer & Brainard 2007, Warren et.al. 2012). However, a key feature of motor sequencing in humans is its flexible control by cognitive or sensory contextual variables, such as internal goals or task demands. It is unknown whether birdsong can be flexibly modified by contextual cues, and how sensory information may guide the flexible deployment of different actions by the song motor circuit. Here, we show that adult Bengalese finches have the capacity to flexibly and rapidly switch between learned changes to their song if they are provided with contextual cues indicating that different song modifications are adaptive in different contexts. In a pitch learning experiment, we paired opposite directions of pitch reinforcement for the same song syllable with different colors of cage illumination, e.g., reinforcing upward pitch shifts in orange light and downward pitch shifts in green light. In a sequence learning experiment, different light colors were paired with aversive feedback delivered to either of two alternate syllable sequences at a point in the song with naturally variable syllable sequencing, e.g. punishing syllable sequence a-b-c in orange light and a-b-d in green light. After training birds on these protocols, light switches elicited immediate adaptive changes to the song that minimized the aversive feedback in each context. These changes were apparent in the first song bout after light switches, as well as in probe contexts without reinforcing feedback. Therefore, the light cue alone had become sufficient for eliciting the song changes after training. These results indicate that Bengalese finches can learn to associate arbitrary contextual cues with specific changes to both the pitch of individual syllables

and to syllable sequencing. This suggests that the song system could be an excellent model for investigating how neural circuits enable flexible and adaptive reconfiguration of motor output in response to different contextual demands.

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Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

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Topic: F.01. Neuroethology

Support: NIH 5R01DC002524-20

Title: Testing whether LMAN acoustically biases juvenile zebra finch song

Authors: *S. N. BRUDNER[1], R. MOONEY[2]
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Abstract: Juveniles acquire certain crucial motor skills by imitating the behavior of highly skilled adults. An influential idea is that to imitate an adult target, juveniles must: (1) produce sufficiently variable behavior to sample both target-similar and target-dissimilar actions, and (2) bias the distribution of their behavior towards increasingly target-similar actions. We do not understand how the juvenile brain implements and coordinates these processes. Zebra finch song learning, a primary model for studying juvenile imitative learning, requires a song-dedicated, premotor cortical region called LMAN that increases the acoustic variability of juvenile song. Although LMAN-dependent song variability may be essential for learning, adult conditioning experiments also reveal that LMAN can alter song acoustic distributions to avoid pitch-contingent punishment. Repeated, transient LMAN inactivations over multiple days of conditioning show that large song alterations can arise from the downstream integration of small, daily LMAN-dependent biases. Here, we test the hypothesis that LMAN biases juvenile song acoustic distributions towards more target-similar acoustics. We repeatedly, transiently inactivate LMAN in juveniles learning to copy an adult while recording acoustic parameters of their song output across multiple days. By modeling the acute contribution of LMAN to song and changes in juvenile song acoustics over time, we assess whether LMAN induces an acute bias in modal song characteristics, and whether such a bias predicts the trajectory the juvenile will take towards his target song. Because LMAN activity is the exclusive premotor output of a song-dedicated cortico-basal ganglia circuit in zebra finches, this experiment may have implications for the premotor function of cortico-basal ganglia circuits in juvenile imitative learning more generally.

Disclosures: S.N. Brudner: None. **R. Mooney:** None.

Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

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Support: MEXT JP17H05932
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Centre for Research in Brain, Language and Music

Title: Motor contributions to vocal sequence learning biases

Authors: *L. S. JAMES[1,4], R. DAVIES, Jr[2], C. MORI[5], K. WADA[5], J. T. SAKATA[3,4,6]
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Abstract: Vocal learning is sculpted by biological predispositions. Revealing the nature of these predispositions requires controlled experiments and computational approaches. We recently discovered biological predispositions in vocal sequence learning in the zebra finch (James and Sakata, Current Biology, 2017). In particular, following tutoring with random sequences of species-typical syllables, zebra finches consistently produced specific types of syllables at particular positions in their song motif. Zebra finches also differentially produced syllables with particular acoustic features (duration, pitch, amplitude, and measures of entropy) at different positions within the motif; for example, they tended to produce longer syllables at the end of the motif. To further understand the importance of each acoustic feature to syllable sequencing, we employed machine learning algorithms to reveal which features best differentiated syllables across motif positions. Using random forest algorithms, we found that syllable duration provided the most predictive information about position in song, with pitch, amplitude and various measures of entropy providing secondary information about motif position.
We then used this computational framework to assess how motor biases contribute to vocal learning biases. We investigated acoustic patterning within the songs of zebra finches developmentally deprived of auditory experience and the extent to which acoustic information similarly predicted syllable position within the songs of these birds. We specifically analyzed the songs of birds that were not exposed to song throughout the sensitive period for song learning ("isolate birds") and of birds that were deafened prior to the sensitive period for song learning ("deafened birds"). Isolate and deafened birds produced songs with acoustic patterns that resembled those observed in experimentally tutored birds; for example, they also produced

longer syllables at the end of their motifs. Random forests revealed that duration similarly provided the most predictive information about motif position. Because these birds were not able to hear either a tutor song or themselves sing, these data suggest that motor biases contribute to some of the acoustic patterning observed in tutored birds and, thus, to vocal learning biases. In addition, these data suggest that the vocal motor system could be predisposed to organize sequences of sounds based on syllable durations.

Disclosures: L.S. James: None. **R. Davies:** None. **C. Mori:** None. **K. Wada:** None. **J.T. Sakata:** None.

Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

Location: SDCC Halls B-H

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Program #/Poster #: 588.13/SS13

Topic: F.01. Neuroethology

Title: Visual reinforcement of vocal pitch in deaf songbirds

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Abstract: Auditory feedback is important for vocal learning and maintenance in both songbirds and humans. Lacking auditory feedback in young subjects leads to vocal learning deficits (Goldin-Meadow 1989, Konishi 1965) and in adults it leads to vocal degradation (Waldstein 1990, Nordeen and Nordeen 1992). However, is auditory feedback also necessary for trial-and-error processes of vocal learning and to what extent can auditory feedback be replaced by feedback from another sensory modality?
We test an alter-modal approach to vocal learning using visual feedback as a substitute for auditory feedback. We adapted a widely used reinforcement learning paradigm. Instead of delivering auditory reinforcement in form of a loud white-noise burst or a song syllable, we briefly switched off the light in the sound-isolation chamber whenever the pitch of a particular song syllable was below (or above) a manually set threshold. We find that deaf zebra finches reliably adapt pitch in response to the visual feedback. Our results show that auditory feedback is not necessary for adaptive changes of song.
Furthermore, we tested the involvement of the basal ganglia in visual reinforcement of pitch. Bilateral lesion of Area X, homologous to the mammalian basal ganglia, prevented visual reinforcement learning of pitch in deaf birds, showing that the basal ganglia do not require vocal performance signals to mediate reinforcement learning.
Hearing birds can also adapt their pitch in response to visual feedback, suggesting that birds are born with the ability to use reinforcement signals from various sensory modalities for vocal

learning. However, deaf birds not only adapted pitch faster than hearing birds, they also adapted pitch to increase the rate of light-off events, whereas hearing birds adapted pitch to decrease that rate. This demonstrates that the presence or absence of one sensory modality can change the reinforcement valence of another sensory modality. Thus, songbirds are flexible learners that are not limited by their ability to evaluate motor performance; they can take advantage of multimodal correlations between their singing and their sensory surrounding.

Disclosures: A.T. Zai: None. **S. Cavé-Lopez:** None. **N. Giret:** None. **R.H. Hahnloser:** None.

Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

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Program #/Poster #: 588.14/SS14

Topic: F.01. Neuroethology

Support: NIH DC04722-19

Title: Is there synergy between song learning and vocal stimuli discrimination?

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Abstract: Song recognition and production are intimately linked in songbirds. The principal motor song nuclei are connected to auditory pathways and to reinforcement stimuli from the brainstem (Gadagkar et al, 2016). Auditory input and reinforcement via auditory feedback then shape vocal changes, driven by the anterior forebrain pathway (Sober & Brainard, 2009). There is, however, no direct behavioral evidence that specific auditory discrimination abilities are mechanistically coupled with vocal changes during song learning. Are juvenile birds with better auditory discrimination capacity more likely to imitate song accurately? Can the acquisition of auditory discrimination skills affect the outcome of specific song learning? In this exploratory study, we investigated these questions in juvenile zebra finch (*Taeniopygia guttata*) males. Birds were trained to imitate operant song playbacks (Tchernichovski, et al 2001). We tested if the accuracy of vocal imitation of specific song syllables relates to auditory discrimination between tutor's song syllables. Birds were trained to discriminate between syllables during early stages of song learning using a social reward (Tokarev & Tchernichovki, 2014, Tokarev et al, 2017); the tested bird was allowed to interact with an adult female via a small window. Song syllables were played sparsely, only during social interactions. Playbacks of one syllable type were followed by an aversive air puff, unless the bird escaped within 2 seconds (aversive syllable). Playbacks of the other syllable type allowed social interactions to continue (social syllable). We found that juvenile birds quickly learned to escape while hearing the aversive syllable, but not after

playbacks of the social syllable. This allowed us to rapidly assess auditory discrimination between any pairs of song syllables during vocal learning. Preliminary results suggest that birds that were trained with auditory discrimination show faster song learning than we usually observe. Follow-up experiments now test if this effect is replicable, and if the putative facilitation of vocal learning is associated with specific auditory discrimination tasks presented to the birds.

Disclosures: K. Watanabe: None. **K. Tokarev:** None. **O. Tchernichovski:** None.

Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

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Program #/Poster #: 588.15/TT1

Topic: F.01. Neuroethology

Title: Performance error computation in zebra finch song at sub-syllabic time scales

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Abstract: When learning a complex motor skill with many constituent parts, such as a song, a dance, a language, or a sports game, it is too daunting to treat the desired target behavior as a single goal. Rather, it may be more efficient to divide the learning task into sub-goals and use distinct subroutines to attain each. For example, young Zebra finches (*Taeniopygia guttata*) simplify the problem of imitating the complex song of an adult "tutor" by learning the syllable repertoire of the target song independently of the syllable order. Birds initially make the minimal adjustments necessary to match the acoustic structure of the syllables of the target song, even if this results in sequencing errors, which they correct later. Such a "greedy" strategy requires the ability to rearrange syllable positions within the song to correct sequencing errors. We therefore hypothesized that if smaller sub-syllabic units cannot be rearranged with respect to each other, a different error-correction algorithm would be employed to learn sub-syllabic structure. We tested strategies of sub-syllabic error correction in zebra finches using artificial tutoring with synthetic songs. We trained juvenile zebra finches to perform a song, in which one of the syllables was composed of two distinct sub-syllabic notes (N_1N_2). Once the birds learned the song, we introduced a second song, in which the order of the two notes within the syllable was reversed, and the pitch of one of the notes slightly shifted ($N_{2+}N_1$). We then tracked the vocal adjustments that birds made to match the altered target syllable. We found that most birds successfully matched the altered syllable by employing non-greedy strategies: they either shifted the pitch of both sub-syllabic notes within the context of the entire syllable ($N_1 \rightarrow N_{2+}$; and $N_2 \rightarrow N_1$), or generated a new syllable $N_{2+}N_1$ from scratch. In contrast, using a greedy strategy ($N_2 \rightarrow N_{2+}$, leading to the incorrect syllable N_1N_{2+}) occurred rarely, and lead to a developmental dead end

due to inability to correct the ordering of sub-syllabic notes. Our results indicate that zebra finches employ error evaluation strategies specific to the level of their song hierarchy: while the syllable repertoire of the song is learned independently of syllable ordering, the structure of sub-syllabic notes is learned with respect to neighboring notes within a syllable. This combination of strategies may be an adaptation to efficient learning of a complex motor skill within a relatively short developmental time window.

Disclosures: D. Lipkind: None. **O. Tchernichovski:** None. **R.H.R. Hahnloser:** None.

Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 588.16/TT2

Topic: F.01. Neuroethology

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Department of Science and Technology (EMR/2015/000829),(RR)
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Natural Sciences and Engineering Research Council (05016-2016),(JTS)

Title: Understanding the origin of introductory vocalizations in a song bird, the zebra finch

Authors: *S. KALRA[1], V. YAWATKAR[1], L. S. JAMES[2], J. T. SAKATA[3], R. RAJAN[1]
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Abstract: Many passerine birds initiate their song sequence with a repeated number of simple introductory vocalizations (Richards DG, Behavior, 1981). These vocalizations have been hypothesized to function as motor preparation for song sequence initiation (Rajan R, Doupe AJ, Current Biology, 2013) or as an alerting component for song (Richards DG, Behavior, 1981). It is widely known that the acoustic structure and sequencing of song elements ('syllables') are generally learned from a tutor, though some components of song are unlearned. However, the extent to which introductory vocalizations are learned or unlearned remains largely unknown.Here, we address this question using the zebra finch, a songbird that learns its vocalizations during development.Song bouts of adult male zebra finches begin with a variable number of introductory notes (INs). To determine the extent to which the number and acoustic structure of INs is learned, we first compared INs produced by sons ('pupils') and their fathers, who serve as their tutors. Number of INs produced by pupils was positively correlated with the number of INs produced by their fathers (n=54 birds; 16 nests). Further, INs of pupils showed high acoustic similarity with INs of their fathers. Next, we isolated juvenile zebra finches from

their father (starting at day 10 post-hatching) and experimentally tutored them using one of two different methods: (1) social tutoring with a male that produced a different number of INs from their father (n=5 birds; 3 nests) or (2) artificial tutoring with playbacks of their father's song without INs (n=8 birds; 3 nests). Our preliminary results demonstrate that the number and acoustic structure of INs of socially tutored birds were similar to those of their social tutor. Interestingly, birds tutored with playbacks of their father's song without INs still produced variable number of INs at the beginning of song bouts. IN numbers were similar to their father but the acoustic structure of INs was variable across birds and not similar to their father. Together, these data suggest that, like song, INs also appear to have innate and learned components.

Disclosures: S. Kalra: None. **V. Yawatkar:** None. **L.S. James:** None. **J.T. Sakata:** None. **R. Rajan:** None.

Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

Location: SDCC Halls B-H

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Department of Science and Technology (EMR/2015/000829) [RR]

Title: Analysing the role of sensory feedback in the initiation of zebra finch song

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Abstract: The song of the adult male zebra finch is a widely established model to study naturally learned motor sequences. However, how such movements are initiated by the brain remains poorly understood. Song bouts begin with a variable number of short introductory notes (INs). Sequences of such INs speed up and reach a consistent acoustic “ready” state just before the start of each song, suggesting a role for INs in motor preparation (Rajan and Doupe 2013). Here, we test a related hypothesis that INs represent a calibration process that uses peripheral sensory feedback to get the brain “ready” to produce song.
To analyse the role of feedback, we first characterised baseline changes in IN properties in adult male birds by recording the same bird multiple times within a 3 year period. We found that the mean number of INs showed very little day-to-day fluctuations (-0.0152 +/- 0.0032; mean +/- SEM; n=21 birds recorded twice within 5 days). Mean IN number increased by 0.3493 +/-

0.1105 (mean +/- SEM) after the first year and then remained mostly unchanged (-0.05 +/- 0.0151; mean +/- SEM). The timing and progression of INs remained consistent across all ages. At all ages, we found that the number of INs at the start of a bout was significantly lower if the first IN of the bout was preceded by non-song vocalizations (calls). This reduction in number of INs was strongest when calls preceded the first IN by < 250ms.
Next, we examined the influence of feedback by removing either (1) peripheral proprioceptive feedback by bilaterally severing the tracheosyringeal (ts) nerve or (2) peripheral auditory feedback through bilateral removal of cochlea. Mean IN number reduced immediately post ts nerve cut, but remained largely unchanged immediately post-deafening. Timing and acoustic progression of INs remained unaffected by both these manipulations. Interestingly, in both sets of birds, mean IN number still reduced when the first IN of a bout was preceded by calls. Together, these data hint at a role for sensory feedback and vocalisation history in determining the mean number of INs at the start of each song bout.

Disclosures: D. Rao: None. **A. Kumar:** None. **S. Kojima:** None. **R. Rajan:** None.

Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

Location: SDCC Halls B-H

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Program #/Poster #: 588.18/TT4

Topic: E.04. Voluntary Movements

Support: NIH Grant R01DC007603
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Title: Sensorimotor learning in children and adults who stutter

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Abstract: Stuttering is a neurodevelopmental speech disorder in which speech fluency is disrupted by repetitions and prolongations of articulatory and/or laryngeal movements. Studies have demonstrated evidence for sensorimotor deficits in stuttering individuals (e.g., slow movements even during fluent speech, fluency enhancement under altered auditory feedback). We also have shown that adults who stutter lack the pre-speech auditory modulation that is observed in fluent control subjects but that this difference disappears when speaking with delayed auditory feedback. In addition to the above evidence, other work suggests that stuttering may be associated with sensorimotor learning deficits. However, it remains unknown whether sensorimotor learning limitations (a) are already present in childhood, and (b) affect only speech or both speech and nonspeech effector systems. Here, we integrate the final data from a series of four experiments investigating not only speech auditory-motor learning but also limb visuo-

motor learning in both children and adults who stutter versus matched nonstuttering individuals. The speech experiments (one for children, one for adults) made use of an auditory-motor adaptation paradigm in which subjects produced monosyllabic words with real-time formant-shifted feedback. In separate conditions, the formant frequencies were shifted up or down, and this shift was implemented suddenly or gradually. We measured formant frequencies across trials to quantify auditory-motor adaptation. The nonspeech experiments (one for children, one for adults) made use of a visuo-motor rotation paradigm in which subjects made reaching movements toward targets while visual feedback was rotated counterclockwise around the center of the workspace. We measured the hand's initial reach direction across trials to quantify visuo-motor adaptation. Speech auditory-motor adaptation results indicate that both stuttering children and stuttering adults show absent or reduced speech sensorimotor learning relative to nonstuttering peers. Reach visuo-motor adaptation results from adults suggest a subtle difference between stuttering and nonstuttering subjects in the rate (but not extent) of limb sensorimotor learning. Reach visuo-motor adaptation from children are ambiguous: a between-group difference emerged only in the oldest age group where control subjects increased adaptation extent but stuttering subjects decreased adaptation extent (relative to the younger groups). Overall, findings suggest that stuttering is associated with substantial difficulties in auditory-motor learning and more subtle limitations in visuo-motor learning.

Disclosures: K.S. Kim: None. **L. Max:** None.

Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

Location: SDCC Halls B-H

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Topic: F.01. Neuroethology

Support: NIH Grant 1R21MH105811-01A1
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Title: Song preference predicts vocal development in juvenile zebra finches

Authors: *C. A. RODRÍGUEZ-SALTOS[1], G. RAMSAY[3], T. J. LIBECAP[2], D. MANEY[1]
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Abstract: In many species of vocal learners, strong social bonds develop between learners and their tutors. The zebra finch is a great model in which to study this phenomenon. In this species, juveniles learn to sing by imitating their male caregivers, which under natural conditions are their fathers. The quality of interactions between caregivers and juveniles has been shown to affect the quality of imitation of caregiver song. We hypothesize that early social interactions cause the juvenile to ascribe incentive salience to caregiver song. In this study, we tested whether

the incentive salience of a song predicts how well juvenile finches learn to sing it. We studied 10 male finches who were isolated throughout the sensorimotor phase of song learning (35-90 post-hatch day). Each day, we quantified the extent to which each juvenile preferred to hear one of two songs, one produced by the caregiver and the other by an adult neighbor that was present when the juvenile was reared. To quantify preference, we presented the juveniles with two keys that, upon being pressed, elicited playback of one of the two songs. The juveniles were trained to associate playback of each song with a particular key, allowing us to quantify preference for each song. At the same time, our operant schedule allowed us to balance the number times the bird heard each song. Despite equal exposure to each of the two songs, the juveniles ultimately learned to sing caregiver song only. We calculated the degree of similarity between the final songs of the juveniles and their respective caregivers, in other words a "similarity score", using the software Sound Analysis Pro. These similarity scores were positively correlated with the strength of the preference to hear caregiver song early during the sensorimotor phase, but not after the birds were 60 days old. Thus, the correlations were found before the age by which zebra finch song typically becomes stable. Our results suggest that being attracted to a song early in song learning may facilitate accurate imitation of that song. The fact that the birds in our study preferred to learn caregiver song further suggests that social interactions with an adult male before the sensorimotor phase are important to establish a preference to learn a given song.

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Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 588.20/TT6

Topic: E.04. Voluntary Movements

Title: The role of new neurons in the emergence of precisely timed bursts in songbird HVC

Authors: *Y. TUPIKOV, D. Z. JIN
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Abstract: The premotor nucleus HVC (proper name) is critical for song learning and production in songbirds. New neurons that project to the motor pathway are continuously added to HVC in juveniles, mostly before and during song learning [1]. The functional role of this addition is poorly understood. Here we propose that new neurons facilitate the emergence of temporal sequence in HVC that controls the song structure. With a computational model, we show that high spontaneous activity of new neurons makes them the prime targets for assembling a feed-forward network through a self-organized process via synaptic plasticity. High spontaneous activity of new neurons originates from their increased excitability, which is observed

universally in many brain regions [2-5]. Once recruited, the new neurons fire readily at precise times, and they become mature. The network is assembled neuron-by-neuron and such gradual formation is supported by experimental observations [6]. Our model incorporates spatial structure of HVC with realistic axonal delays between HVC neurons. The emergent forward network of the projection neurons has a characteristic that the burst timing is uniform and there are no silent gaps. This property arises due to the substantial axonal delays. This is in contrast to the synfire chain model of HVC, in which groups of projection neurons burst synchronously. In this case, synchronous bursting combined with axonal delays create gaps in burst timings between adjacent groups. Electrophysiological [7] and calcium fluorescent imaging [8] recordings support continuous time representation of song in HVC, which is inconsistent with the synfire chain model. The network emerged in our model, "polychronous chain", has no distinctive groups. The spatial distributions of the synapses between the projection neurons agree with recent experimental data [9]. Our model suggests that projection neurons in HVC are wired into a polychronous chain with continuous and uniform time coverage, and the addition of new neurons in juveniles is a critical component in this self-organized process.

[1] Wang et al., J. Neurosci. (2002). [2] Mongiat et al., PLOS ONE (2009). [3] Oswald et al., J. Neurophysiol. (2008). [4] Zhang et al., J. Neurophysiol. (2004). [5] Spigelman et al., J. Neurophysiol. (1992). [6] Okubo et al., Nature (2015). [7] Lynch et al., Neuron (2016). [8] Picardo et al., Neuron (2016). [9] Kornfeld et al., eLife (2017).

Disclosures: Y. Tupikov: None. **D.Z. Jin:** None.

Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

Location: SDCC Halls B-H

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Topic: E.04. Voluntary Movements

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Title: Cortical adaptations to enable enhanced vocalization

Authors: *C. M. CERKEVICH[1,2,3], P. L. STRICK[1,2,4,3]
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Abstract: Marmoset monkeys vocalize more often and exhibit more complex vocal motor behavior than macaque monkeys. To gain insight into the central control of vocalization, we used retrograde transneuronal transport of rabies virus from the cricothyroid (CT) muscle to identify cortical areas that influence this muscle in the two species.
This comparative approach revealed that five cortical motor areas in the frontal lobe of both species are involved in the descending control of this laryngeal muscle. Three of these areas are on the medial wall and near the midline of the hemisphere: the rostral cingulate motor area (CMAr), the ventral cingulate motor area (CMAv), and the supplementary motor area (SMA). Another two cortical areas are on the lateral surface of the hemisphere: the primary motor cortex (M1) and ventral area 6 (6V). All of these areas displayed patterns of labeling indicative of disynaptic projections to CT motoneurons.
Although the same five cortical areas influenced the CT muscle in both monkeys, two cortical areas in the marmoset contained many more output neurons than in the macaque. Area 6V of marmosets contained ~3.5 times more output neurons than area 6V of macaques. Similarly, the SMA of marmosets contained ~2.5 more output neurons than the SMA of macaques. These results suggest that the enhanced vocal skills of marmosets are the result of increases in output from both a lateral motor area, area 6V, and a medial motor area, the SMA.

Disclosures: C.M. Cerkevich: None. **P.L. Strick:** None.

Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 588.22/TT8

Topic: E.04. Voluntary Movements

Title: Cortical control of vocal interactions in a neotropical singing mouse

Authors: *A. BANERJEE[1,2], D. E. OKOBI, Jr[2], G. A. CASTELLUCCI[3], S. M. PHELPS[4], M. A. LONG[2]
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Abstract: How the brain enables flexible sensorimotor transformations is a central question in neuroscience. During conversation, for instance, we listen to the words of another person, interpret them and modify our speech accordingly. Carefully designed behavioral tasks with experimental control over both the stimuli and motor responses have advanced our understanding of neural mechanisms that support sensorimotor transformations. We hope to extend these paradigms to study neural mechanisms that govern more flexible and natural sensorimotor behaviors such as vocal interactions. Seeking a rodent model to fill this niche, we have begun to investigate the neural mechanisms of vocal communication in Alston's singing mouse

(*Scotinomys teguina*) - a highly vocal neotropical rodent native to the cloud forests of Central America. Acoustically isolated S. teguina males produce advertisement calls, and each individual vocalization is composed of a series of human audible syllables of increasing duration, that are highly stereotyped across renditions. We find that this vocal stereotypy is broken and songs become significantly more variable when exposed to other males in a social context. In fact, two or more males temporally coordinate their advertisement songs in a phenomenon known as countersinging. To test if motor cortical circuits underlie song execution and coordination, we used intracortical micro-stimulation (ICMS) to localize an anterolateral motor cortical hotspot that activated laryngeal muscles with short latency. Subsequently, bilateral electrical stimulation of motor cortex during singing temporarily paused song progression while focal cooling of motor cortex slowed down song progression without stretching individual syllables. These results are consistent with a hierarchical control of song timing, with motor cortex being functionally upstream of a song-generating circuit, and they suggest a potential role for motor cortex in mediating this vocal social interaction. We tested this idea by inactivating motor cortex with muscimol (GABAA agonist) and found that countersinging was severely compromised, highlighting the utility of motor cortex for integrating sensory information and generating a socially appropriate behavioral response. Going beyond these specific results, we believe that S. teguina is an excellent rodent model to investigate neural dynamics underlying an experimentally tractable, naturally occurring, vocal sensorimotor behavior.

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Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

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Title: Stable sequential activity underlying the maintenance of a precisely executed skilled behavior

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Abstract: A vast array of motor skills can be maintained throughout life. Do these behaviors require stability of individual neuron tuning or can the output of a given circuit remain constant despite fluctuations in single cells? This question is difficult to address due to the variability inherent in most motor actions studied in the laboratory. A notable exception, however, is the courtship song of the adult zebra finch, which is a learned, highly precise motor act mediated by orderly dynamics within premotor neurons of the forebrain. By longitudinally tracking the activity of excitatory projection neurons during singing using two-photon calcium imaging, we find that both the number and precise timing of song-related spiking events remain nearly identical over the span of several weeks to months. These findings demonstrate that learned, complex behaviors can be stabilized by maintaining precise and invariant tuning at the level of single neurons.

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Poster

588. Vocal and Oral Control Mechanisms From Song to Speech

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Program #/Poster #: 588.24/TT10

Topic: E.04. Voluntary Movements

Support: DFG EG 401/1-1
EMBO ALTF 348-2017

Title: Local network mechanisms for sequence generation underlying a complex learned behavior

Authors: *R. EGGER[1], Y. TUPIKOV[2], K. KATLOWITZ[1], S. E. BENEZRA[1], M. A. PICARDO[1], F. MOLL[1], J. KORNFELD[3], D. Z. JIN[2], M. A. LONG[1]
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Abstract: Sequential neuronal activity patterns form the network basis for a diverse range of neural processes across various brain regions. For instance, such sequences have been observed in the hippocampus during spatial exploration and mental replay, in the striatum during movement planning, and in the parietal cortex during decision-making. However, the cellular and network mechanisms underlying sequence generation in forebrain circuits are at present not

well understood.
The zebra finch has emerged as an excellent model system for studying sequential network dynamics underlying a complex learned behavior. During song production, HVC premotor neurons produce a sequence of bursting activity, with each individual premotor neuron active at only one point throughout the sequence. Previous perturbation studies are consistent with the notion that these dynamics are likely due to circuitry intrinsic to HVC and have prompted a range of network models capable of driving sequential activity. However, due to lack of information about the organization of premotor neuron circuits, the validity of these models has not been rigorously tested.
Here we describe our efforts to use newly collected functional and anatomical data to constrain hypotheses concerning the network architecture of HVC. To determine the spatiotemporal organization of HVC neurons, we used 2-photon calcium imaging during singing. Using single-cell labeling and reconstructions, we investigated the spatial organization of local premotor neuron axons. We found a structural basis for the observed spatiotemporal organization during singing, consistent with the hypothesis that local circuits in HVC drive sequential activity. At the subcellular level, we used *in vivo* electrophysiology and anatomical measurements to determine the precise conduction delays of premotor neuron axons. These experimental constraints were then used to construct several network models, with a range of network connectivity schemes, and we tested the validity of these models by comparing the output of each with experimental observations. Our simulations and observations support a *polychronous* network organization, which takes advantage of a variety of axonal conduction delays to generate a feedforward network capable of generating continuous neural sequences throughout song performance. These efforts provide a framework for formalizing the network structures of other neural circuits associated with the generation of sequential activity.

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Poster

589. Novel Electrode Designs, CNS, and Periphery

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 589.01/TT11

Topic: E.05. Brain-Machine Interface

Title: Improving usability of silicone-based neural electrodes by introducing color labels

Authors: M. ULLOA, *M. SCHUETTLER, R. PFEIFER, C. BIERBRAUER, S. BENSCH, C. HENLE
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Abstract: Background:
In the past years, we developed a method for semi-automated laser-based microfabrication of neural electrode arrays that allows fast prototype realization as well as series production. Devices built using this method have been extensively used acutely and chronically in animal research as well as acutely and sub-chronically in man.
Materials and Methods:
The electrodes typically consist of implantable grade silicone rubber as substrate material with embedded metal contacts, conduction lines and weld pads, typically made from platinum-iridium alloy.
Silicone was chosen as a substrate material since it has proven excellent bio-acceptance and biostability over the last decades it is used as implant material. The silicone we use is very soft (Young's Modulus in the same order of magnitude like that of neural tissue) minimizing trauma during implantation, and is optically transparent, which helps to e.g. spot air bubbles accidentally trapped underneath the electrode but also to be able to visually inspect the neural tissue underneath the electrode. In many application, such as brain mapping, direct association of electrode contact number to the corresponding biological tissues is desired. Consequently, visible labels are required to improve the usability of the electrode arrays.
Such labels shall not compromise the bio-compatibility of the device and should be of high optical contrast to the tissue and electrode contact material. In case of micro electrode labelling, the labelling process should allow very small feature sizes (range: 1mm font height) and need to be integrated in the existing microfabrication process.
Results:
We developed a process that meets these requirements by blending implantable grade pigments (blue, black, green, or white) into silicone adhesive resulting in a high-contrast ink. During our layer-by-layer build-up production process, electrodes are labelled by depositing this ink in an intermediate process step. Labels can use letters as small as 0.8 mm in height before blurring. Samples produced this way include micro-ECoG arrays with contact numbering on the back for easy identification, high-channel peripheral nerve cuff electrodes with contact numbers and spinal cord stimulation paddles with contact numbering and branding.

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Poster

589. Novel Electrode Designs, CNS, and Periphery

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Topic: E.05. Brain-Machine Interface

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Title: Stability of siloxane sensors *in vivo* for real-time, spatiotemporal mapping of oxygen in brain tissue

Authors: *L. DE MESQUITA TEIXEIRA, A. SRIDHARAN, B. MOGHADAS,, V. KODIBAGKAR,, J. MUTHUSWAMY
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Abstract: Measuring oxygenation levels with high spatiotemporal resolution can serve as a biomarker for interfacial tissue health for neural implants. Brain cells typically rely on aerobic respiration to obtain energy and consequently, proper oxygen tension (pO_2) is needed to maintain neuronal function. The main objective is to develop a PDMS (poly-dimethyl siloxane) based carrier for liquid siloxane contrast agent capable of sensing pO_2 around microelectrodes using a novel magnetic resonance MR-PISTOL (proton imaging of siloxane to map tissue oxygenation levels) imaging technique. Previous work demonstrating MR-PISTOL on cells and muscle tissue *in vivo* demonstrated that T1 relaxation rates of the siloxanes have a linear dependence to pO_2 levels. In this study, carbon fiber probes (50-100 microns width) were insulated with epoxylite™ and coated with either hard (elastic modulus, E~1-3 MPa) or soft (elastic modulus, E~3-5 kPa) silicone using an injection molding process. Subsequently, the coated fibers were loaded with siloxane (PDMSO - 410 g/mol) contrast agent by absorption. Nile red (100 µM) fluorescent dye that was solubilized in PDMSO was used as a marker for testing retention under simulated blood-brain-barrier (BBB) breach conditions in in vitro tests. Untreated or surface-passivated siloxane loaded probes were tested under accelerated conditions using 50 mg/ml albumin in artificial cerebrospinal fluid (aCSF) at 37°C for 3 days. Both ‘hard’- and ‘soft’-coated probes that were surface passivated were able to retain 65-78% of the fluorescent signal intensity. Without surface passivation, ‘hard’ coated probes lost >56% of signal intensity, while ‘soft’ coated probes lost >95% of signal intensity, suggesting surface passivation protects against biofouling. Probes placed in control solutions with no albumin had no change in signal intensity. For validation in vivo, ‘hard’ and ‘soft’ probes with siloxane sensors were implanted in the mouse brain and T1 relaxation values corresponding to pO_2 levels in brain tissue around the probes were measured over 4 weeks to create spatio-temporal maps of oxygen concentration. PO_2 values were more stable in brain tissue around ‘soft’ compared to ‘hard’ coated probes in vivo. Histology around the probes after 8 weeks of implantation showed significantly increased IgG (biomarker for BBB breakdown) for ‘hard’ compared to ‘soft’ probe. Future studies will correlate tissue response of the interface with electrophysiology and quantitative, high resolution spatial maps of pO_2 levels *in vivo* under chronic conditions.

Disclosures: L. De Mesquita Teixeira: None. **A. Sridharan:** None. **B. Moghadas,:** None. **V. Kodibagkar,:** None. **J. Muthuswamy:** None.

Poster

589. Novel Electrode Designs, CNS, and Periphery

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 589.03/TT13

Topic: E.05. Brain-Machine Interface

Support: NSF I/UCRC BRAIN center Grant 1650566

Title: Brain-like, soft, silicone scaffolds improve stability of enzyme based electrochemical sensors for chronic applications

Authors: J. SARBOLANDI[1], A. SRIDHARAN[2], *J. MUTHUSWAMY[1]
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Abstract: The detection of neurotransmitters (i.e dopamine) and other biochemical activity in specific neural circuits *in viv*o is vital for elucidating neurodegenerative mechanisms and pathways involved in many diseases (i.e Parkinson's, Schizophrenia , Alzheimer's etc.). Electrochemical sensors utilizing amperometry and voltage-based methods such as fast cyclic voltammetry have made significant strides in real-time characterization of synaptic function in deep brain neural circuits *in vivo* with fast response times and high sensitivity. However, these electrochemical methods have significant limitations for use *in vivo* due to challenges with selectivity, interference, biofouling, sensor degradation and instability under long-term implantation conditions. Our group previously developed a soft, brain-like, conductive silicone that has stable electrochemical impedances and is capable of recording single units over one year in a rodent model. In this work, we embed tyrosinase (a dopamine-sensitive enzyme) within the novel, soft silicone based scaffold to demonstrate enzyme-based amperometric detection capability and stability in benchtop experiments. Tyrosinase is physically adsorbed onto multi-walled carbon nanotubes and embedded in the conductive, silicone scaffold using a fast heat curing process onto bundled carbon fibers (~100 µm final thickness). Amperograms are obtained with L-tyrosine methyl ester as a substrate with concentrations of 0 - 20 mM (corresponding to physiological concentrations) in (a) artificial cerebrospinal fluid (aCSF) (b) aCSF with 0.2-50 mg/ml albumin (to simulate blood-brain-barrier breach), and (c) aCSF with 10 µm-10 mM hydrogen peroxide (to simulate oxidative stress). The sensitivities of the enzyme embedded electrodes in PBS, aCSF, aCSF-albumin (50 mg/ml), and aCSF-H_2O_2 mediums were 6.08, 18.58, 39.17, and 0.29 nA/mM, respectively. Subsequent cyclic voltammetry show that the functionality of the enzyme is preserved upon removal of the hydrogen peroxide from the medium. Cyclic voltammetry (CV) of tyrosinase embedded electrodes show characteristic reduction potentials between 0.4-0.6 V. Storage stability tests suggest that the enzyme remains functional in the silicone scaffold for at least 6 weeks. Operational stability tests using a 100 continuous cycles of CV (-1V to +1V at 100 mV/s scan rate) show significantly improved stability with enzyme

embedded silicone scaffolds experiencing only a 5.75% decrease in peak currents compared to controls with no scaffold (>25% decrease). Currently, sensor validation and performance *in vivo* are in progress.

Disclosures: J. Sarbolandi: None. **A. Sridharan:** None. **J. Muthuswamy:** None.

Poster

589. Novel Electrode Designs, CNS, and Periphery

Location: SDCC Halls B-H

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Program #/Poster #: 589.04/TT14

Topic: E.05. Brain-Machine Interface

Title: Single unit recordings from central and peripheral nervous system using metallized graphene electrodes

Authors: *M. GONZÁLEZ-GONZÁLEZ[1], A. KANNEGANTI[1], C. L. FREWIN[1], J. J. PANCRAZIO[1], R. A. JALILI[2], G. G. WALLACE[3], M. I. ROMERO-ORTEGA[1]
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Abstract: Interfacing the nervous system is an effective approach to decode the neuronal functions and modulate its activity. The emerging use of therapies based on electrical stimulation and the design of prosthesis requires of low impedance electrodes with high signal-to-noise ratio (SNR) that allow for sensitive recording of single unit activity, and high charge storage capacity (CSC) for effectively and safe neural stimulation. Microelectrodes are commonly fabricated in silicon with platinum (Pt), Pt/Iridium and Iridium oxide electrodes. However, the micromotion of the silicone shafts implanted into the soft nervous tissue exacerbates the foreign body response and contributes to the eventual failure of these devices. The alternative use of carbon nanotube coated microelectrodes has been promising due to their biocompatibility and high CSC (~372 mC/cm^2) and low impedance (~20 MΩ), however the stiffness of the metal shafts and delamination of the carbon nanotube coating limits the chronic use of these electrodes. We have recently advanced the production of graphene fibers form liquid crystalline dispersions of graphene oxide that demonstrated excellent electrochemical and mechanical characteristics. Here, we report the improvement of the electrochemical performance of these fibers by the incorporation of a metallic coating that drastically improved the electrochemical characteristics of 40 µm diameter graphene fibers (CSC ~947 mC/cm^2 and impedance of ~11 MΩ). Adult rats were used to characterize these electrodes in vivo both for cortical recordings and for peripheral neural interfacing. The electrodes recorded a lower baseline, ~40µV compared to conventional Pt electrodes (~60-65 µV) and effectively recorded single unit recordings, with a SNR of 9.2 in the motor cortex. We also recorded spontaneous neural activity in the sciatic and vagus nerves at a SNR of 4.3. We demonstrated that these metallized graphene electrodes are effective in

stimulating evoking a localized motor response from the sciatic nerve. Together, the data supports the use of metallized graphene fibers as intraneural electrodes for the neural interfacing of brain and nerve activity.

Disclosures: M. González-González: None. **A. Kanneganti:** None. **C.L. Frewin:** None. **J.J. Pancrazio:** None. **R.A. Jalili:** None. **G.G. Wallace:** None. **M.I. Romero-Ortega:** None.

Poster

589. Novel Electrode Designs, CNS, and Periphery

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 589.05/TT15

Topic: E.05. Brain-Machine Interface

Support: DARPA HAPTIX
NIH NINDS Fast-Track

Title: Implantable amplifiers for chronic *in vivo* research

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Abstract: Percutaneous connections in long-term chronic experiments are commonly the site of failure of these experiments. Infection and bone degradation can be major limitations of these technologies which complicate chronic experiments. We have developed an implantable system to record biopotentials and transmit signals to an external transceiver. In this presentation, we report design considerations for system integration and results from *in vitro* performance of the system and an *in vivo* trial to validate device function in an animal model. The implants are constructed on a ceramic circuit board with a bioamplifier ASICs and additional discrete components. Theses implants are inductively powered by an external transceiver, and digitized signal data are sent from the implants by infrared data transmission. Recording implants have 32 single-reference channels that can be implanted independently from the hermetic electronics/telemetry module. The recording implant has been validated in a GLP study in canine subjects in a six-month trial. The 32-channel implant was surgically inserted in the forelimb with intramuscular electrodes implanted in deltoideous and lateral head of triceps. Following implantation, each animal was fitted with a backpack carrying an external transceiver coil and a battery-powered data acquisition system, and the dogs were allowed to freely walk down a hallway. EMG recorded from each animal as it walked down a hallway had very low noise and, in conjunction with recorded video, clearly indicated swing/stance phases of gait. Devices have also been implanted in non-human primates for chronic recording experiments. This technology will support clinical trials at multiple institutions for year-long take-home trials as part of the DARPA HAPTIX program. We will also provide implantable and wearable technologies to other

neuroscience investigators to provide a research platform for other large animal and human subject studies. This work was supported by the DARPA HAPTIX project and SBIR grants from DARPA and NIH.

Disclosures: D. McDonnall: A. Employment/Salary (full or part-time):; Ripple LLC. **I. Myers:** A. Employment/Salary (full or part-time):; Ripple LLC. **B. Crofts:** A. Employment/Salary (full or part-time):; Ripple LLC. **A.M. Wilder:** A. Employment/Salary (full or part-time):; Ripple LLC. **S. Hiatt:** A. Employment/Salary (full or part-time):; Ripple LLC.

Poster

589. Novel Electrode Designs, CNS, and Periphery

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 589.06/TT16

Topic: E.05. Brain-Machine Interface

Title: The biocompatibility of diamond ultramicroelectrode materials for neural sensing applications

Authors: M. B. SETIEN[1], S. DANIELS[1], C. RUSINEK[2], Y. GUO[1], R. RECHENBERG[2], M. BECKER[2], W. LI[1], *E. K. PURCELL[1]
[1]Michigan State Univ., East Lansing, MI; [2]Fraunhofer USA, Inc. - CCD, East Lansing, MI

Abstract: The drive to better understand normal brain function and pathological states has intensified demand for new technologies which can interrogate the nervous system with enhanced spatiotemporal resolution. Our team is developing all-diamond implantable ultramicroelectrode arrays to deliver long-term, stable recordings of extracellular bipotentials and neurochemical signals via cellular-scale site sizes (<50 μm^2). Here, we report the results of an initial characterization of the biocompatibility of the novel diamond-based materials used in the array, including conductive boron-doped polycrystalline diamond (BDD) and insulating polycrystalline diamond (PCD). BDD is an attractive electrode material based on its high corrosion resistance, minimal background current, and long-term stability for neurotransmitter detection. Indium tin oxide (ITO), which is an electrically conductive material previously shown to enhance the electrophysiological responses and network formation of attached neurons, is included as a reference control material in addition to cell culture-treated plastic. Primary cultures of rat embryonic cortical neurons (E18) are seeded onto the different materials and maintained for 21 days, where patch-clamp electrophysiology and immunohistochemistry is performed at 7, 14, and 21-day time points. Our ongoing analysis compares the substrate materials on the basis of four criteria: (1) the degree of neuronal attachment and viability (as assessed by caspase-staining), (2) the excitability of neurons on each material assessed with patch clamp electrophysiology (based on the maximum number of action potentials in response to injected current, the action potential amplitude, and the maturity of passive membrane

characteristics), (3) the associated expression of markers of excitability identified with immunohistochemistry (synaptic transporters and ion channels), and (4) the degree of neurite outgrowth from neurons on each material as assessed with Sholl analysis. The results presented will inform the transfer of the novel diamond substrate materials to sensing applications in the *in vivo* environment, where we expect to leverage the positive performance characteristics of the diamond materials displayed *in vitro*.

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Poster

589. Novel Electrode Designs, CNS, and Periphery

Location: SDCC Halls B-H

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Program #/Poster #: 589.07/TT17

Topic: E.05. Brain-Machine Interface

Title: Neurotransmitter analysis with all-diamond microfiber electrodes using fast scan cyclic voltammetry

Authors: *C. RUSINEK[1], J. GOPINATH[1], M. BECKER[2], Y. GUO[3], R. RECHENBERG[2], M. SETIEN[3], S. DANIELS[3], E. K. PURCELL[3], C. MCKINNEY[4], W. LI[3]
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Abstract: Developments in microelectrode technology has enabled deeper understanding of brain and nervous system function. The small size and low capacitance allow microelectrodes to sense neurotransmitters (NTs) at rapid rates on the sub-second time scale. These measurements have traditionally been executed using fast scan cyclic voltammetry (FSCV) with carbon-fiber microelectrodes (CFMs). While CFMs have exhibited the properties needed for *in vivo* neurochemical sensing, the need for a stable, batch-fabricated, and up-scalable microelectrode remains. Diamond is a material which exhibits excellent fabrication flexibility in conjunction with many other advantageous electrochemical properties such as good biocompatibility, low background current, and wide potential window. In this work we demonstrate the analytical capability of a novel, all-diamond microfiber (υ-fiber) electrode for neurochemical sensing. The diamond υ-fibers consist of a conductive boron-doped diamond (BDD) core encapsulated by insulating layers of un-doped polycrystalline diamond (PCD). Analysis by scanning electron microscopy (SEM) revealed overall dimensions of 6 υm high x 25 υm wide and ~2 mm in length with an electroactive surface area of roughly 70 υm^2. The diamond υ-fibers were electrochemically characterized using model redox analytes such as ferri/ferrocyanide ($Fe(CN)_6^{3-/4-}$), ruthenium hexaamine ($Ru(NH_3)_6^{2+/3+}$), and hydroquinone; excellent steady state response

was observed for each analyte using cyclic voltammetry (CV). The diamond υ-fibers were then assessed for their ability to several detect NTs- dopamine (DA), serotonin (SA), epinephrine (EPI), nor-epinephrine (NE), and 3,4 dihydroxyphenylacetic acid (DOPAC) using FSCV. Several FSCV parameters were investigated such as waveform, scan rate, and potential range. These were completed using the High Definition Cyclic Voltammetry (HDCV) interface developed at the University of North Carolina Chapel Hill. For further characterization and assessment, the FSCV performance diamond υ-fibers were thoroughly compared with commercially available CFMs. These novel all-diamond υ-fiber electrodes have commercial-scale potential, generating a powerful tool for neurochemical analysis.

Disclosures: C. Rusinek: None. **M. Becker:** None. **Y. Guo:** None. **R. Rechenberg:** None. **M. Setien:** None. **S. Daniels:** None. **E.K. Purcell:** None. **C. McKinney:** None. **W. Li:** None.

Poster

589. Novel Electrode Designs, CNS, and Periphery

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Program #/Poster #: 589.08/TT18

Topic: E.05. Brain-Machine Interface

Support: Michigan State University
Fraunhofer USA, Center for Coatings and Diamond Technologies

Title: Mechanical characteristics of microfabricated diamond ultramicroelectrode fibers for neural sensing applications

Authors: *W. LI[1], Y. GUO[1], R. RECHENBERG[2], C. A. RUSINEK[2], M. SETIEN[1], S. DANIELS[1], M. F. BECKER[2], E. K. PURCELL[1]
[1]Michigan State Univ., East Lansing, MI; [2]Fraunhofer USA, Ctr. for Coatings and Diamond Technologies, East Lansing, MI

Abstract: One of the greatest scientific challenges nowadays is to unveil the brain circuitry, and sensing for electrical and chemical signals of single neurons is a critical step to achieve this grand goal. Microelectrode implantation, as a practical approach, has shown promising feasibility for neuronal recording. In the effort of our group, a microfiber electrode, constructed with microcrystalline diamond (MCD) as an encapsulation and boron-doped polycrystalline diamond (BDD) as a conducting core, has been successfully developed. To fabricate such microfiber electrodes, a thin BDD layer was grown on top of an MCD layer on a silicon wafer; both layers were grown using microwave plasma assisted chemical vapor deposition and plasma etched using a Cu mask. A sealing layer of MCD was selectively grown on the patterned fiber, except for the contact pads where a Ti/Cu mask was applied to inhibit diamond growth. After being released from the Si substrate, the fiber was cleaved off from an anchor, exposing pristine

BDD at the tip for neural sensing. Then the fiber was mounted onto a custom-made PCB for subsequent mechanical testing. In this work, the mechanical flexibility and bulking force of the microfiber were studied using analytical calculation, finite element analysis, and experimental measurements. Analytically, the Euler's equation was used to estimate the buckling force of the microfibers with different dimensions. Furthermore, the devices were simulated using Solid Mechanics Module in COMSOL to investigate the stress distribution on the fiber shank and critical buckling load during perpendicular insertion. To measure the buckling force experimentally, the fiber was pushed against a solid substrate at a constant speed, and the corresponding force was monitored as a function of the tip displacement. The maximum force measured immediately before a reduction was seen as the critical force required for fiber failure due to buckling and compared with the theoretical value. For further development of device implantation strategy, experiments were conducted by pushing the fiber into a tissue-mimicking gelatin phantom to test the feasibility of fiber insertion into the brain tissue.

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Poster

589. Novel Electrode Designs, CNS, and Periphery

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Program #/Poster #: 589.09/TT19

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: CIHR
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CQDM

Title: Multi-neuromodulator measurements in the behaving macaque cortex and basal ganglia using solid-phase micro-extraction fibres

Authors: *S.-A. HASSANI[1,2], S. LENDOR[3], E. BOYACI[3], V. SINGH[3], J. PAWLISZYN[3], T. WOMELSDORF[1,2]
[1]Psychology, Vanderbilt Univ., Nashville, TN; [2]Biol., York Univ., Toronto, ON, Canada; [3]Chem., Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Different neuromodulators rarely act independent from each other to modify neural processes, but are co-released, gated, or modulated in yet unknown ways. To better understand this interdependence of neuromodulators and their collective influence on local circuits during different behaviors, it is necessary to reliably extract the local concentrations of multiple neuromodulators in vivo.

Here we describe the results from a versatile extraction method, Solid Phase Micro-Extraction (SPME), and illustrate sensitive, multi-neuromodulator measurements from micro-fibres. These biocompatible micro-fibres can be made with wires of arbitrary length and are coated with a matrix compatible polymer containing sorbent particles capable of retaining neurochemicals of small molecular size by chemical interactions after they diffuse into the coating. Given the small size, arbitrary length and ease of handling of the SPME fibres, multiple samples were taken from two cortical and one subcortical brain regions simultaneously: prefrontal cortex, premotor cortex and the head of the caudate nucleus. Data was taken from two adult male rhesus macaques while performing goal directed behavior in order to acquire liquid reward.
We obtained reliable measurements of Glutamate, Dopamine and Acetylcholine simultaneously within sampled brain regions in both macaque monkeys during goal-directed behavior. We find glutamate concentrations several orders of magnitude higher than acetylcholine and dopamine in all brain regions. Dopamine was reliably detected in the striatum at tenfold higher concentrations than Acetylcholine. Acetylcholine concentrations were detected with high consistency within monkeys, between monkeys, and across brain areas.
To our knowledge no prior dataset exists with simultaneous measurements of multiple neuromodulators across the fronto-striatal network in the behaving macaque. We demonstrate that glutamate, dopamine and acetylcholine exist in different concentrations within sampled brain regions, different animals have comparable concentrations of each neuromodulator in the same brain regions, and that dopamine is present at much higher concentrations in the caudate than in either cortical region sampled. These findings provide an important starting point for characterizing the neurochemical profiles of brain circuits underlying cognition during goal – directed behavior.

Disclosures: S. Hassani: None. **S. Lendor:** None. **E. Boyaci:** None. **V. Singh:** None. **J. Pawliszyn:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Owner of the intellectual rights (IP) of SPME technology. **T. Womelsdorf:** None.

Poster

589. Novel Electrode Designs, CNS, and Periphery

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Program #/Poster #: 589.10/TT20

Topic: E.05. Brain-Machine Interface

Support: NIH Grant 1U0 1NS094248

Title: CMOS technology for three dimensional neural recording using microwire arrays

Authors: *N. MELOSH, A. OBAID
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Abstract: Mammalian brains consist of billions of neurons operating at millisecond time scales, which current recording techniques only capture a tiny fraction. Recent advances in CMOS device design have led to high-recording quality planar probes, with diminishing sizes to ameliorate the extent of tissue damage. Matching these powerful silicon electronics to the inherently three dimensional architecture of the brain has remained challenging however, as devices are constrained to the planar two dimensional surfaces required for silicon processing. Here we show a new strategy to take advantage of the scalability and electronic processing power of CMOS-based devices with a low-tissue damage, three dimensional neural interface. The core concept is using a bundle of insulated microwires mated to a large-scale CMOS microelectrode array, such as found in modern camera chips or displays. Microwires are known to have low insertion damage and good electrical recording performance, yet required individual mounting and connectorization. Arranging them into bundles controls of the spatial arrangement and three dimensional structure of the distal (neuronal) end, while providing a robust parallel contact plane on the proximal side which is interfaced to a planar pixel array. The modular nature of the design enables a wide array of microwire types and size to be mated to a variety of different CMOS chips, making the same fundamental platform scalable from a few hundred electrodes to tens of thousands. We thus link the rapid progress and power of commercial multiplexing, digitisation and data acquisition hardware together with a bio-compatible, flexible and sensitive neural interface array. We present recent massively parallel recording using mouse and rat models, showing both spiking activity from single neurons and local field potentials. Immunohistology of microwire bundles was also done, demonstrating minimal to no observable damage post implantation.

Disclosures: N. Melosh: None. **A. Obaid:** None.

Poster

589. Novel Electrode Designs, CNS, and Periphery

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Program #/Poster #: 589.11/TT21

Topic: E.05. Brain-Machine Interface

Support: Schmidt Family Foundation
The Israeli Council for Higher Education (CHE)

Title: High-density microwires array for neurochemical monitoring

Authors: *N. HEMED[1], A. OBAID[2], P. WANG[2], N. MELOSH[2]
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Abstract: Various debilitating neuropsychiatric and neurodegenerative disorders are characterized by abnormal levels of neurotransmission in the brain. Detecting subsecond neurotransmission variation accurately and for extended, clinically relevant timescales is a critical unmet need. Chronic measurements of neurotransmission would enable the identification of specific neurotransmitors that contribute to complex behaviors being degraded as a result of disorders, and aid in testing the clinical feasibility of treatments. Fast scan cyclic voltammetry (FSCV) has been used over the last 20 years to study neurotransmission in brain tissue *in vivo* and *ex vivo* at the single cell level. This technique exhibits the best overall performance for measuring neurotransmission at the required timescales, chemical selectivity, and sensitivity. This well-established method, however, has only utilized for accurate monitoring of neurotransmitters in primates with acutely (few hours) implanted sensors. Despite recent advances in material science and engineering, this technique has not yet been integrated with microfabricated microelectrode arrays (MEAs) due to materials limitation. Progress towards a highly scalable, dense electrode arrays, will also enable observation of how different neurotransmitters and neuroanatomical areas function together and provide quantitative insight into the complicate nature of the brain. Currently, most studied are using implantable carbon-fiber microelectrodes (CFMs) with small diameter fused-silica. These electrodes can be affixed in the brain with minimal tissue response, enabling neurotransmission sensing in single recording locations during behavior. In contrast, those electrodes have been restricted to measurements at a single electrode. This research will focus on a new methodology whereby we perform heterogeneous integration of a bundle of microwires (BMWs): tens of thousands of functionalized (using PEDOT:PSS) metal-in-glass wires of less than 30µm in diameter, to a CMOS microchip. This architecture allows each wire to be independently addressable for sensing purposes, enabling different spatial and temporal sensing patterns, ameliorating issues of scalability. In addition, such large high-density microelectrode arrays could be integrated with stimulation and recordings, providing a broad neuroscience impact.

Disclosures: N. Hemed: None. **A. Obaid:** None. **P. Wang:** None. **N. Melosh:** None.

Poster

589. Novel Electrode Designs, CNS, and Periphery

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Program #/Poster #: 589.12/TT22

Topic: E.05. Brain-Machine Interface

Support: NIH Grant EY026365

Title: Neuroroots, an ultra-low damage scalable neural interface

Authors: *M. D. FERRO[1], A. GONZALEZ[2], E. ZHAO[1], L. M. GIOCOMO[2], N. MELOSH[1]
[1]Materials Sci., [2]Neurobio., Stanford Univ., Stanford, CA

Abstract: Communication between living brain tissue and engineered devices is the key link to understand the brain fundamental function and to clinically restore neurological deficits.
The tools that are currently broadly available for this interface exhibit some limitations such as invasiveness, low channel count, complicated implantation strategies and bulky connectorization, which hinder these devices from optimal performance and widely accepted clinical solutions.
Enabled by new materials and device designs, a new generation of brain interface technologies is replacing bulkier, non-compliant systems with the aim of seamless electronic-biological interfaces with lower tissue damage, reduced immunogenicity, high-density, tunable spatial distribution, and long-term stability. Recent successful examples leveraging mechanically compliant materials have demonstrated major breakthrough in brain research using ultra-flexible systems for ECoGs recordings, and for depth electrodes. Yet, surgical implantation damage, scalable channel count and the number of devices implanted simultaneously are still significant challenges.
Neuroroots is a new platform enabling facile implantation of ultra-low damage and scalable channel-count penetrating electrodes for chronical brain recording and stimulation. The platform consists of dangling 'root' electrodes only 5 um wide by 1µm thick, matching both cell-size dimensions and tissue mechanical properties, yet without interconnectivity between electrodes that can lead to tissue damage and block nutrient diffusion. We have developed a surgical apparatus based on the commonly used NeuraLynx Halo Microdrives to easily and precisely insert these ultraflexible arrays into the tissue target of interest. The microwires are too flexible to insert on their own, thus we developed an ultra slim, 35µm diameter microwire as a temporarily shuttle onto which numerous individual electrodes self-align. Once inserted, these wires delaminate, and the shuttle is removed.
This strategy enables implantation of a number of electrodes into various brain regions at once. Initial chronic implantation of an array of 32 electrodes into the hippocampus of freely moving trained rats exhibit recordings of single unit potentials a few minutes after electrode implantation, as well as a minimal damage both acute and chronically. The open structure of the mesh is believed to minimally perturb the ecosystem and nutriment diffusion. Subsequent implantation into the medial entorhinal cortex of trained rats has demonstrate the ability of this platform be implanted into region which are difficult to access for traditional probes.

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Poster

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Program #/Poster #: 589.13/TT23

Topic: E.05. Brain-Machine Interface

Support: Center for Sensorimotor Neural Engineering, National Science Foundation Engineering Research Center [EEC-1028725]

Title: Simultaneous detection of dopamine and 5-hydroxytryptamine through fast scan cyclic voltammetry using glassy carbon microelectrode arrays

Authors: *E. CASTAGNOLA[1,2], S. NIMBALKAR[1,2], B. CARIAPPA[1,2], C. CEA[1,2], A. GAUTAM[1,2], S. KASSEGNE[1,2]
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Abstract: Dopamine (DA) and 5-hydroxytryptamine (5-HT) are the two most important neurotransmitters in the brain that play a pivotal role in a large variety of neurophysiological functions. They often interact in their effect. Therefore, the simultaneous detection of DA and 5-HT concentrations represents a challenging neuroscience goal. A variety of electrochemical techniques have been mainly used to monitor neurotransmitter levels in *vivo*. Among these, Fast Scan Cyclic Voltammetry (FSCV) is preferred, ensuring a temporal resolution on the sub-second scale, compatible with the measurement of chemical fluctuations in the brain. One of the main concern in the optimization of FSCV co-detection of DA and 5-HT is the selectivity of the oxidation peaks of the two neurotransmitters, that are usually close to each other (in a range between 0.6 V to 0.75 V). Furthermore, in a physiological environment, ascorbic acid (AA) typically occurs in much higher concentration than that of DA and 5-HT (100-1000 times), interfering with their detection selectivity and sensitivity. In the present study, we use glassy carbon (GC) penetrating microelectrodes arrays (4 microelectrodes with 220µm vertical space and 2000 μm^2 area) to optimize a FSCV waveform capable of co-detecting 5-HT and DA *in vitro* in 0.01M phosphate-buffered saline solution (PBS). The sensing capability, in term of sensitivity and lower detection limits, is first evaluated separately for DA and 5-HT and, subsequently, in presence of 1mM of AA. GC microelectrodes detect 10 nM as lowest detection limit for both DA and 5-HT with a sensitivity of respectively 68±7 $pA/\mu m^2$ and 46±8 $pA/\mu m^2$ at 500nM concentration. In presence of 1mM AA, the lowest detection limit for both DA and 5-HT is 50 nM with a reduction of the selectivity of the 27% with respect to the one in PBS solution. In all the cases, we measured a linear range of neurotransmitter concentration from 10 nM to 1 µM. Finally, GC microelectrodes can simultaneously discriminate the reduction and oxidation peak of low concentration (10-50nM) of DA (-0,2; 0.62V) and 5-HT (0; 0.75V) in PBS solution. In conclusion, we demonstrated that our GC microelectrodes present promising neurotransmitters detection ability in term of sensitivity and selectivity. These results represent an important step towards the optimization of a sensor that will allow the understanding of the basic neurotransmission mechanisms in the brain.

Disclosures: E. Castagnola: None. **S. Nimbalkar:** None. **B. Cariappa:** None. **C. Cea:** None. **A. Gautam:** None. **S. Kassegne:** None.

Poster

589. Novel Electrode Designs, CNS, and Periphery

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 589.14/TT24

Topic: C.03. Parkinson's Disease

Title: Cyclic voltammetry and impedance characterization of modified electrodes in physiological levels of dopamine

Authors: *R. KEITH[1], N. PEIXOTO[2]
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Abstract: Many neurodegenerative diseases involve the alteration of key neurotransmitters in the brain, including Parkinson's Disease, Huntington's Disease, and Alzheimer's Disease. Real-time assessment of these neurotransmitters and their metabolites will undoubtedly become a necessary part of treatment monitoring in neurodegenerative patients. In Parkinson's Disease, dopamine-signaling neurons in the substantia-nigra and striatum are the first to degenerate, lowering physiological dopamine levels. This degeneration is thought to preclude clinical symptoms. Monitoring changes in dopamine levels over time could facilitate more precise treatment. In this study, we assess the longevity and stability of four types of electrodes: stainless steel wire, multi-walled carbon nanotubes (MW-CNTs), screen-printed electrodes, and gold coupons. Screen-printed electrodes were obtained commercially, and are coated with carbon-black (counter and working electrodes) and silver/silver-chloride (reference electrode) and an electrically insulating polymeric film. MW-CNT electrodes were fabricated by our team. These electrodes are stainless steel wire stripped for 3 mm at the tip and coated with research-grade MW-CNTs (2.55 mm^2 area). Gold coupons are glass substrates coated with a Chromium adherence layer and 1000 A Gold in a cleanroom environment (area of 5 mm^2). Stainless steel wires were used as control electrodes, with an area matched to the MW-CNT electrodes. All electrodes were examined for longevity in phosphate buffered saline for 0 hours (baseline), 24 hours, 48 hours, and 72 hours at room temperature. Before these longevity tests, the electrodes were tested in dopamine solution then subsequently tested following each 24-hour cycle to evaluate potential degradation of the analyte signal over time. Electrochemical characterization was performed using a 16-channel multiplexer attached to a CHI660D potentiostat. Cyclic voltammetry was performed, as well as electrochemical impedance spectroscopy. Based on preliminary data, the gold-coupon electrodes and the MW-CNT electrodes detected dopamine the most reliably at lower concentrations (50 to 100nM). Additionally, MW-CNT electrodes demonstrated greater longevity and stability compared to the other electrode types.

Disclosures: R. Keith: None. **N. Peixoto:** None.

Poster

589. Novel Electrode Designs, CNS, and Periphery

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 589.15/UU1

Topic: E.05. Brain-Machine Interface

Support: Center for Sensorimotor Neural Engineering (CSNE), a National Science Foundation Engineering Research Center (EEC-1028725)
Washington State Spinal Cord Injury Consortium (WASCIC)
Christopher and Dana Reeve Foundation (CDRF) International Consortium on Spinal Cord Injury Repair

Title: Development of a multi-functional glassy-carbon electrode for simultaneous stimulation and measurement of neurotransmitter response in the spinal cord

Authors: *S. THONGPANG[1,2,8], M. HIRABAYASHI[9], E. CASTAGNOLA[9], S. NIMBALKAR[9], B. CARIAPPA[9], C. CEA[9], A. FISCHEDICK[2], P. E. PHILLIPS[3,8,4,5], S. KASSEGNE[9,8], C. T. MORITZ[2,6,7,10,11]
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Abstract: Current technology does not allow measurement of neurotransmitters within the spinal cord with sufficient temporal and spatial resolution to understand the time-course of injury and recovery. Therefore we are developing a neurotransmitter sensing device that can be implanted in a rat model of spinal cord injury. Our goal is to develop microfabricated glassy carbon (GC) electrode (Fig. 1a) for neurotransmitter recording using Fast Scan Cyclic Voltammetry (FSCV) in vitro and in vivo. Our results show that GC electrodes can be successfully fabricated with high flexibility and with sensitivity to 10 nM serotonin (Fig. 1b) and dopamine detection in-vitro. The fabrication method consists of a modular transfer lithographic process that allows electrical and voltammetry microelectrodes in a common polymeric substrate. We focus on detecting serotonin since it is known to be dysregulated following injury. Based on both previous studies and our work, FSCV with N-shape waveform and Nafion electrode coating were used to increase serotonin selectivity. Our results confirm that greater selectivity of serotonin was obtained in vitro with N-shape applied voltage and Nafion coating electrodes compared to bare carbon-fiber electrodes. Finally, acute in-vivo FSCV recording were obtained from Long-Evan rat model

recording in lamina 8 of spinal segment C4-C5 in the cervical spinal cord. Our results demonstrate a promising neurotransmitter recording ability of glassy-carbon electrodes in-vivo.

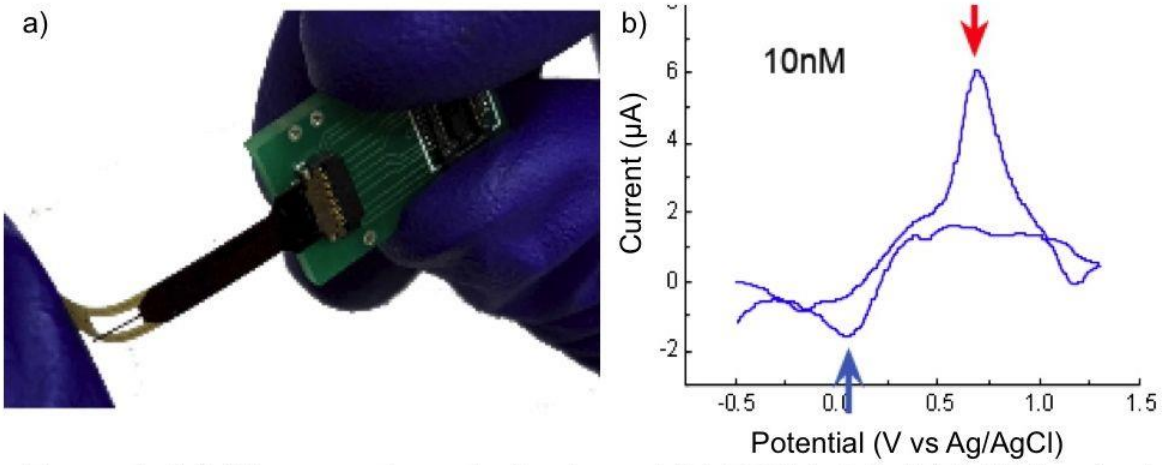

Figure 1: (a) Glassy carbon electrode and (b) FSCV plot of 10nM Serotonin concentration recording in vitro

Disclosures: S. Thongpang: None. **M. Hirabayashi:** None. **E. Castagnola:** None. **S. Nimbalkar:** None. **B. Cariappa:** None. **C. Cea:** None. **A. Fischedick:** None. **P.E. Phillips:** None. **S. Kassegne:** None. **C.T. Moritz:** None.

Poster

589. Novel Electrode Designs, CNS, and Periphery

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 589.16/UU2

Topic: E.05. Brain-Machine Interface

Support: DARPA HAPTIX contract No. HR0011-15-2-0008

Title: A novel osseointegrated neural interface (ONI) with percutaneous connections for chronic electrophysiology in the rabbit

Authors: *A. M. DINGLE[1], J. P. NESS[2], J. NOVELLO[2], W. ZENG[1], B. NEMKE[3], Y. LU[3], M. D. MARKEL[3], A. J. SUMINSKI[4], J. C. WILLIAMS[2], S. O. POORE[5]
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Abstract: Background: Today's advanced prosthesis hold great potential for restoring function and improving quality of life for amputees. The patient's ability to control these devices with ease and precision is constantly improving; however, seamless control with sensory feedback

remain futuristic goals. We have previously demonstrated proof of principal for interfacing with nerves transposed to the medullary canal of long bones to create an Osseointegrated Neural Interface (ONI). This method builds on the clinical translocation of nerves into bone to treat symptomatic amputation neuromas. **The objective** of our current research is to create a novel ONI, complete with percutaneous osseointegrated abutment for chronic bi-directional electrophysiology in rabbits. **Methods:** Above knee amputation was performed in male and female New Zealand white rabbits. Briefly, the sciatic nerve was isolated and severed above the point of trifurcation. The femur was amputated at the midpoint and the nerve passed through a corticotomy. The terminal end of the nerve was sutured into a bipolar cuff electrode, and pressed back into the medullary canal. A second bi-polar cuff electrode was secured proximal to the corticotomy in order to stimulate and record efferent and afferent signals between the proximal and distal electrodes respectively. Both electrodes were connected to independent printed circuit boards (PCBs), which were intern secured to a stainless steel screw. The stainless steel screw served as both the osseointegrated and percutaneous portion of the ONI device. The muscle and skin were closed over the femur. Animals underwent electrophysiological recordings of compound nerve action potentials (CNAPs) at weeks 3, 5, 8 and 12 weeks under anesthesia, as well as terminal recordings of somatosensory evoked potentials (SSEPs) at week 12. **Results:** Efferent signals can be generated from the proximal electrode and recorded from by the distal electrode from week 3 through to week 12. Moreover, efferent signals improve over the 12 week period, indicated by higher peak amplitudes achieved from lower stimulation over time. Afferent signals generated within the bone and recorded proximal to the corticotomy are not achieved prior to week 8, and improve at week 12. The writing of sensory information via an ONI is demonstrated by the ability to record SSEPs. **Conclusions:** Chronic implantation of an ONI is entirely achievable and repeatable. Furthermore, physiological function of nerves transposed into bone improve over a 12 week period, including the ability to generate sensory signals to the cortex via an ONI.

Disclosures: A.M. Dingle: None. **J.P. Ness:** None. **J. Novello:** None. **W. Zeng:** None. **B. Nemke:** None. **Y. Lu:** None. **M.D. Markel:** None. **A.J. Suminski:** None. **J.C. Williams:** None. **S.O. Poore:** None.

Poster

589. Novel Electrode Designs, CNS, and Periphery

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 589.17/UU3

Topic: E.05. Brain-Machine Interface

Support: MOTU-PPR-AI 1/2

Title: Development and validation of HYPE, a novel floating array for intrafascicular peripheral neural interfacing

Authors: *I. STRAUSS[1,2], F. M. PETRINI[2], A. CUTRONE[1], F. BERNINI[3], K. GABISONIA[3], L. CARLUCCI[3], S. RASPOPOVIC[4], F. RECCHIA[3], S. MICERA[1,2]
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Abstract: Intraneural electrodes showed in amputees to be able to successfully restore sensory feedback. Unfortunately, the implantation of such devices is very time consuming, increasing the physical exhaustion of the patient, and the risk of complications during the surgery. Furthermore, surgeons need to be trained well for the execution of the implantation making the process more complicated.
Here we developed a novel system, consisting of a 3D printed insertion device and a multifunctional peripheral neural interface, which can drastically reduce the surgery time, whilst maintaining a high spatial stimulation selectivity of nerve fascicles.
The electrode has a total of four epineural and eighteen intraneural active stimulation sites (ASs) which are manually implanted into the nerve using a purposely designed insertion device as a guidance. The epineural portion is placed around the nerve, while in a second step, nine needles are inserted into the nerve, leaving behind eighteen microwires, being in contact with the nerve fascicles.
Over a period of at least one month, the left posterior leg sciatic nerves of six farm pigs were implanted with two electrodes each. In addition, eight muscles innervated by the sciatic nerve, were implanted with monopolar, intramuscular EMG wire electrodes to verify the spatial selectivity of the implants.
Using a custom made graphical user interface, a neurostimulator and a recording unit were weekly used to provide bi-phasic, cathodic first, trains of current pulses amplitude modulated while recording the EMG response of the muscles being innervated by the sciatic nerve. Impedances, EMG recruitment curves and selectivity index were obtained to verify the spatial selectivity of the electrodes.The electrodes were successfully implanted in less than 20 minutes each. Furthermore, we could show that the implant had chronical mechanical and electrical stability, and can provide spatial selectivity over at least five months. Histological analysis showed no damage to the nerve. No infections occurred over the implantation period.

Disclosures: I. Strauss: None. **F.M. Petrini:** None. **A. Cutrone:** None. **F. Bernini:** None. **K. Gabisonia:** None. **L. Carlucci:** None. **S. Raspopovic:** None. **F. Recchia:** None. **S. Micera:** None.

Poster

589. Novel Electrode Designs, CNS, and Periphery

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 589.18/UU4

Topic: E.05. Brain-Machine Interface

Support: HR0011-15-2-0030

Title: Tissue-engineered electronic nerve interfaces (teeni): Functional and histological evaluation

Authors: *E. ATKINSON[1], E. A. NUNAMAKER[2], A. GORMALEY[3], A. BRAKE[3], M. YUSUFALI[3], B. SPEARMAN[3], C. KULIASHA[4], A. FURNITUREWALA[5], P. RUSTOGI[5], S. MOBINI[3], C. SCHMIDT[3], J. W. JUDY[4], K. J. OTTO[3,5,6,7,1]
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Abstract: Achieving a high-bandwidth recording and stimulation interface with the PNS has become increasingly important for potential therapeutic benefit as the significance of neural innervation of peripheral systems becomes better understood. Achieving a higher information resolution with the peripheral nervous system would allow for isolated nerve fiber interaction and a finer control over the communication taking place. In the case of prosthetic limbs for amputees, high-bandwidth PNS implants would be necessary to more ideally support sophisticated, modern prosthetics with multiple degrees of freedom for both sensory information and motor control. PNS implants require chronic compatibility with the tissue and the robustness to survive local environmental stresses. Our approach to a PNS interface takes advantage of the regenerative ability of the PNS and the common treatment of neuromas that can occur in amputees. Doctors excise neuromas from the nerve and approximate the nerve to a nearby muscle where it can innervate, leading to reduced recurrence of neuroma formation. In our device, a peripheral nerve of an amputee that no longer innervates the original targets would be cut and sutured to the device ends. The nerve then regenerates into the device via an engineered hydrogel where it would be in proximity with a high-density polyimide electrode array. Our implant, referred to as a Tissue Engineered Electrical Nerve Interface (TEENI), is an attempt to develop a reliable, chronic PNS interface with a sufficient input-output bandwidth to support next-generation prosthetic devices. This study demonstrates the functional and histological evaluation of TEENIs in regenerated rat sciatic nerves using a multimodal analysis approach including: electrochemical impedance spectroscopy (EIS), electrophysiological recordings, and immunohistological techniques. Lewis rats were anesthetized and a segment of the right hind-limb sciatic nerve was removed. TEENIs were then implanted into the gap by suturing the

proximal and distal nerve stumps into the respective ends of the device. A connection to the device was then run sub-dermally up to the head where it was secured using bone screws and dental cement. Impedance and electrophysiology were recorded daily and histology was conducted on the implant after 6 weeks of regeneration. The results presented here represent a preliminary analysis of a cohort of implanted rats. This work was sponsored by the Defense Advanced Research Projects Agency (DARPA) Biological Technologies Office (BTO) HAPTIX program under the auspices of Drs. Doug Weber and Eric Van Gieson through the Pacific Cooperative Agreement: No. HR0011-15-2-0030

Disclosures: E.A. Nunamaker: None. **A. Gormaley:** None. **A. Brake:** None. **M. Yusufali:** None. **B. Spearman:** None. **C. Kuliasha:** None. **A. Furniturewala:** None. **P. Rustogi:** None. **S. Mobini:** None. **C. Schmidt:** None. **J.W. Judy:** None. **K.J. Otto:** None.

Poster

589. Novel Electrode Designs, CNS, and Periphery

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 589.19/UU5

Topic: E.05. Brain-Machine Interface

Support: HR0011-15-2-0030

Title: Tissue-engineered electronic nerve interfaces (TEENI): Foreign body response in the peripheral nervous system

Authors: *A. K. GORMALEY[1], E. ATKINSON[2], E. NUNAMAKER[3], J. GRAHAM[2], A. M. BRAKE[1], M. YUSUFALI[1], B. SPEARMAN[1], C. KULIASHA[4], A. FURNITUREWALLA[5], P. RUSTOGI[5], S. MOBINI[1], C. SCHMIDT[1], J. W. JUDY[4], K. J. OTTO[1]
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Abstract: Neural interfaces have the potential to impact aspects of the human experience ranging from medical treatment to leisure activities. A limiting factor to reliable, chronic implants involves both biotic and abiotic failure mechanisms. While significant work has been accomplished characterizing abiotic failure mechanisms, work is still necessary to understand the biotic tissue response to implanted devices. Characterizing the mechanisms of the foreign body response (FBR) to implanted devices in the central nervous system (CNS) has proven to be a difficult task with electrodes in the same implanted array showing different responses. The variation in the response has made the task of investigating the exact biological mechanisms of FBR to implanted recording and stimulating devices difficult. In this study, we investigate the peripheral nervous system (PNS) as a potential model system to investigate biotic failure mechanisms to implanted neural devices. The device used for this investigation is a Tissue-

Engineered Electronic Neural Interface (TEENI) which allows for high bandwidth bidirectional communication. The purpose of this device is to provide dense sensory and motor communication for cognitive control of and sensory feedback from prosthetic limbs. The device consists of flexible polyimide threads surrounded by a hydrogel and encased in a small intestine submucosa (SIS) wrap. A segment of a rat sciatic nerve is removed and as the nerve regenerates, the hydrogel degrades, and the regenerating axons come into proximity of recording and stimulating sites on the TEENI. Over time, the regeneration matures and encapsulating tissue forms around the device which is remarkably uniform in its appearance compared to tissue encapsulation seen in the CNS. Fourteen male Lewis Rats were implanted with TEENIs by transecting the sciatic nerve in the right hind legs and grafting the device onto the proximal and distal stump. After 6 weeks the rats were euthanized, and the device was explanted. The samples were cryosectioned at 20μm and labeled with primary and secondary antibodies. Using ImageJ eight measurements were taken along each side (left, right, top, bottom) of each thread (1-9) to the edge of the FBR. Statistical analysis was performed on the collected data to compare. The preliminary results of our image analysis support the hypothesis that the FBR to the device is not statistically different in magnitude across threads. Qualitative observation support that the FBR is very similar in shape, but further analysis will be done to verify uniformity of shape and magnitude across the remainder of the threaded samples.

Disclosures: A.K. Gormaley: None. **E. Atkinson:** None. **E. Nunamaker:** None. **J. Graham:** None. **A.M. Brake:** None. **M. Yusufali:** None. **B. Spearman:** None. **C. Kuliasha:** None. **A. Furniturewalla:** None. **P. Rustogi:** None. **S. Mobini:** None. **C. Schmidt:** None. **J.W. Judy:** None. **K.J. Otto:** None.

Poster

589. Novel Electrode Designs, CNS, and Periphery

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 589.20/UU6

Topic: E.05. Brain-Machine Interface

Support: DARPA HR0011-15-2-0030

Title: Tissue-engineered electronic nerve interfaces (TEENI): Design, fabrication, and reliability testing

Authors: *J. W. JUDY[1], C. A. KULIASHA[1], P. RUSTOGI[1], A. S. FURNITUREWALLA[1], B. S. SPEARMAN[2], E. W. ATKINSON[3], E. A. NUNAMAKER[4], K. J. OTTO[2], C. E. SCHMIDT[2]
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Abstract: Neural interfaces for amputees should reliably capture the activity of motor neurons and stimulate activity in sensory neurons. Targeting peripheral nerves instead of the brain can minimize risk while still providing good prosthesis performance. The primary signal sources and stimulation targets in nerves are the Nodes of Ranvier, which are not spatially correlated between the thousands of neighboring fibers. As a result, the nodes are arranged in an approximately repeating 3-D "cloud" of targets for recording movement intent and stimulating sensory feedback. To comprehensively engage with the nerve and maximize the number of independent motor and sensory channels, neural interfaces for nerves should also be 3-D in nature and scalable.

To date, nerve interfaces have been 1-D (LIFE: longitudinal inter-fascicular electrode, TIME: transverse intrafascicular multichannel electrode, etc.) or 2-D (USEA: Utah slant electrode array, sieve electrodes, etc.) in nature, which means that they tremendously under sample the nerve fibers. Another challenge is the mismatch between the elastic properties of native peripheral-nerve tissue and the mechanical stiffness of interfaces. A significant mismatch is hypothesized to trigger an exaggerated foreign-body response that can negatively affect the functional longevity of neural interfaces.

Our novel multidisciplinary approach is to overcome these barriers by creating mechanically compliant, scalable, and high-performance nerve interfaces through the combination of microfabricated neural-electronic interfaces with tissue engineering and nerve regeneration. Specifically, we developed a hybrid tissue-engineered electronic nerve interface (TEENI), which consists of multi-electrode polyimide-based "threads" embedded into a biodegradable hydrogel composite scaffold that is wrapped in a bioresorbable small intestinal submucosa and sutured to the ends of a transected nerve. Multiple thread sets can be stacked and incorporated in the hydrogel to enable the TEENI device to be scaled up and functionally engage with the 3-D nerve target. Aggressive reactive-accelerated-aging (RAA) soak tests were used to facilitate rapid fabrication process improvements that ultimately have yielded TEENI that can survive RAA equivalent to a 6-month implant with less than 15% change in impedance and charge-storage capacity.

This work was sponsored by the Defense Advanced Research Projects Agency (DARPA) Biological Technologies Office (BTO) HAPTIX program under the auspices of Drs. Doug Weber and Eric Van Gieson through the DARPA Contracts Management Office, Pacific Cooperative Agreement: No. HR0011-15-2-0030.

Disclosures: J.W. Judy: None. **C.A. Kuliasha:** None. **P. Rustogi:** None. **A.S. Furniturewalla:** None. **B.S. Spearman:** None. **E.W. Atkinson:** None. **E.A. Nunamaker:** None. **K.J. Otto:** None. **C.E. Schmidt:** None.

Poster

589. Novel Electrode Designs, CNS, and Periphery

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 589.21/UU7

Topic: E.05. Brain-Machine Interface

Support: DARPA

Title: An experimental model for assessing long-term safety and efficacy of vagus nerve stimulation

Authors: *F. YAGHOUBY[1], B. SHAFER[2], S. ASGARI[2], S. VASUDEVAN[3]
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Abstract: Therapeutic findings of Vagus Nerve Stimulation (VNS) in several clinical disorders have benefited from investigations on experimental models. However, majority of research on VNS has been limited to acute timelines in such models and long-term effects have been overlooked. In this study, we propose a rat model for longitudinal assessment of safety and efficacy of VNS and validate the feasibility of the model by exploring VNS effects on cardiovascular and immune systems. Under approval by Institutional Animal Care and Use Committee (IACUC) at the U.S. Food and Drug Administration, female Lewis rats (n = 4) were surgically implanted with a telemetry device (EMKA Technologies) for continuous recording of Electrocardiogram (EKG), temperature and activity. Four weeks post-implantation, a custom nerve cuff electrode (Microprobes for Life Science) was surgically implanted around the left cervical vagus nerve in each rat. Cuff electrode leads were enclosed inside a connector mount secured to the rat's lumbar fascia. The transcutaneous mount interfaces with a magnetic connector and provides seamless plug and play connection for applying stimulation or measuring impedance in awake behaving rats. Physiological variables were continuously monitored for another 2-3 weeks post-implantation. After complete recovery, an isolated pulse stimulator was used to deliver a 30s biphasic pulse train of electrical stimulation to rats twice a week. VNS parameters used as 1 mA charge-balanced pulses with 100 μs pulse width at 30 Hz. The stimulation protocol continued for three months and physiological variables and electrode impedance were continuously monitored and analyzed. The robustness of the VNS implant for the proposed model was validated using electrode impedance measurements from a pilot cohort of three rats. Results showed relatively low impedance values (<15kΩ) indicating durability of the implanted electrodes. Physiological variables and particularly EKG were used to assess VNS target engagement in this rat model. Instantaneous bradycardia was observed during VNS in some rats and heart rate variability analysis was performed to investigate detailed changes in cardiovascular autoregulation. Preliminary results from the proposed model demonstrated easy-

to-use electrical stimulation as well as long-term monitoring of electrode impedance and physiological variables in awake behaving rats. In addition to impedance measurements and heart rate variability analysis, ongoing experiments involve cytokine analysis of blood samples for immune system response. This will supplement the preliminary results and validate the model in a long term experimental setup.

Disclosures: F. Yaghouby: None. **B. Shafer:** None. **S. Asgari:** None. **S. Vasudevan:** None.

Poster

590. Brain-Machine: Speech and Other Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 590.01/UU8

Topic: E.05. Brain-Machine Interface

Support: LBNL-internal LDRD "Neuromorphic Kalman Filters"
LBNL-internal LDRD "Deep Learning for Science"
LBNL-internal LDRD "Neuro/Nano- Technology for BRAIN"
NIH R00-NS065120
NIH DP2-OD00862
NIH R01-DC012379

Title: Deep learning for neural data: Speech classification and cross-frequency coupling in human cortex

Authors: *J. LIVEZEY, K. E. BOUCHARD[1], E. F. CHANG[2]
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Abstract: A fundamental challenge in neuroscience is to understand what structure in the world is represented in spatially distributed patterns of neural activity from multiple single-trial measurements. This is often accomplished by learning linear transformations between neural features and features of the sensory stimuli or motor task. While successful in some early sensory processing areas, linear mappings are unlikely to be ideal tools for elucidating nonlinear, hierarchical representations of higher-order brain areas during complex tasks, such as the production of speech by humans. Here, we apply deep networks (DNs) to predict produced speech syllables from cortical surface electric potentials (CSEPs) recorded from human sensorimotor cortex in 4 subjects and then analyze the DNs to understand what structure they are learning from the neural data.First, we show that DNs achieve superior classification accuracy compared to linear models, with increased gains for increasing task complexity, and improved efficiency as a function of dataset size. We then 'opened the black box' and used the DN confusions to reveal the latent structure learned from single trials, which revealed a rich,

hierarchical organization of linguistic features that recapitulated vocal tract configurations.Since DNs classified speech production from high gamma (HG) activity with higher accuracy that other methods, they are also candidates for comparing the relative information content across neural signals. We explored the cross-frequency amplitude-amplitude structure in the CSEPs and discovered a novel signature of motor coordination in beta-HG coupling. Using deep networks, we then show that although there is information relevant to speech production in the lower frequency bands, it is small compared to the amount in HG. Furthermore, the amplitude-amplitude correlations are not clearly related to overall information content and improvements in accuracy.Together, these results demonstrate the utilization of deep networks not only as an optimal black-box predictor with application to brain-computer interfaces, but as a powerful data analytics tool to reveal the latent structure of neural representations, and understanding the information content of different neural signals.

Disclosures: K.E. Bouchard: None. **E.F. Chang:** None.

Poster

590. Brain-Machine: Speech and Other Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 590.02/UU9

Topic: E.05. Brain-Machine Interface

Support: NIH DP2OD008627
NIH U01NS098971-01

Title: Synthesizing speech from the human sensorimotor cortex

Authors: *G. K. ANUMANCHIPALLI[1,2], J. CHARTIER[3], E. F. CHANG[2]
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Abstract: The ventral sensorimotor cortex (vSMC) encodes coordinated, multi-articulator kinematic movements of the vocal tract that accomplish specific articulatory goals needed to produce natural continuous speech. Our goal here was to decode audible speech only from the associated neural activity during speaking. Two approaches include direct decoding of speech spectrum or decoding articulatory kinematics followed by articulatory synthesis. Between these, kinematics is the closest representational correlate to vSMC neural activity, has less latency, and generalizes well to arbitrary word sequences. Despite these advantages, modeling articulatory kinematics for neural-to-speech decoding has not been demonstrated given methodological constraints on estimating articulatory movements. Here, we developed a model-based approach with two components, i) a neural decoder that converts neural activity into articulator kinematics, and ii) an articulatory synthesizer, that converts articulatory trajectories into audible speech. Both

components were computationally implemented using deep recurrent neural networks with LSTM units. Neural data were collected from five human participants (patients with medically refractory epilepsy), implanted with high-density subdural ECoG arrays, as they spoke fluent sentences. An optimal set of 460 sentences (MOCHA-TIMIT) was used as the speaking material. A previously developed statistical approach was employed for acoustic-to-articulatory inversion to estimate vocal tract kinematics from produced speech acoustics. Articulators were represented as 12 dimensional vectors coding displacements in x and y directions of three points on the tongue, jaw, upper and lower lips and the fundamental frequency coding the laryngeal function. Using this approach, we were able to successfully decode neural activity to synthesize intelligible speech. We found high degree of correlation between synthesized and original spectrograms of subjects' produced speech making this a viable path for future speech based brain-computer interfaces.

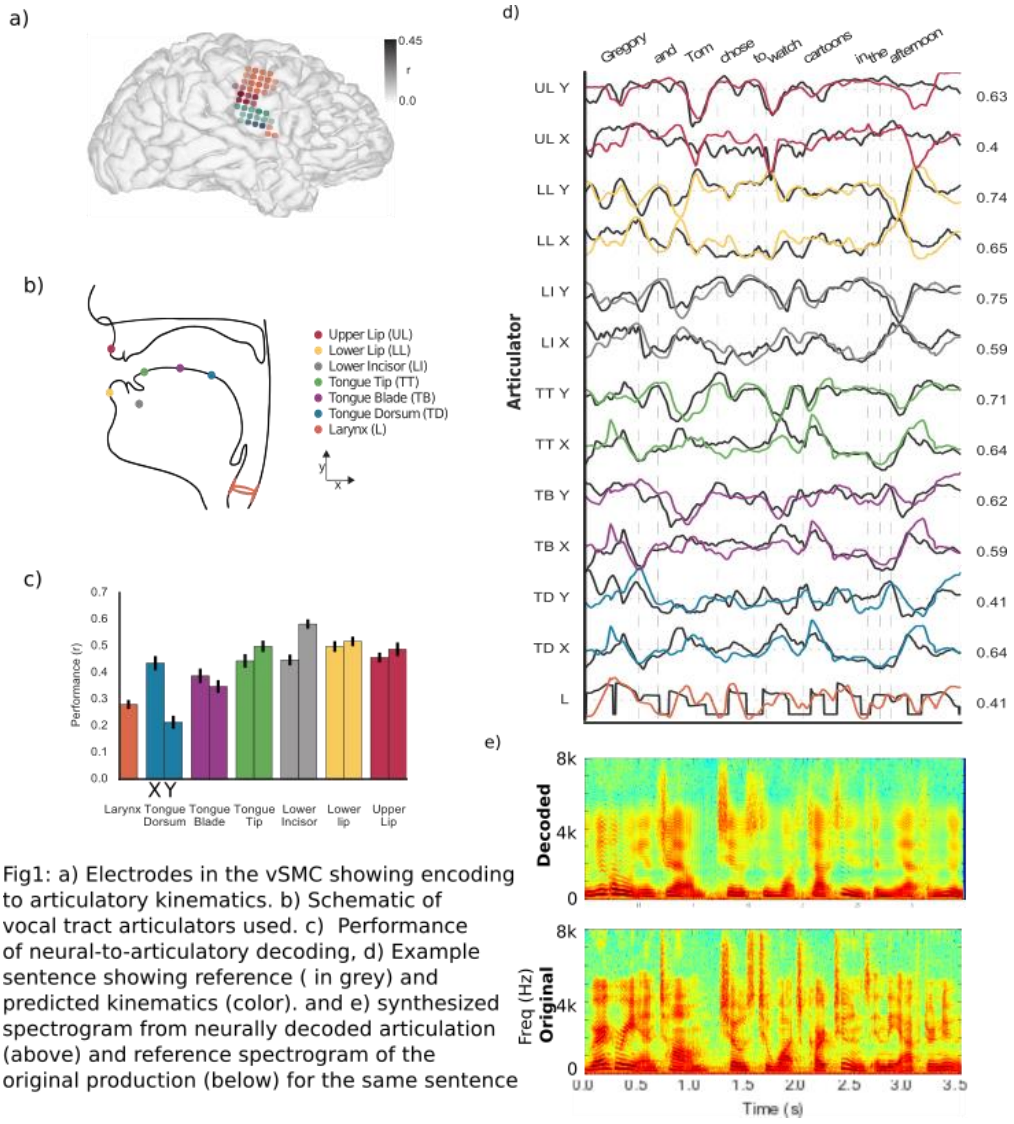

Fig1: a) Electrodes in the vSMC showing encoding to articulatory kinematics. b) Schematic of vocal tract articulators used. c) Performance of neural-to-articulatory decoding, d) Example sentence showing reference (in grey) and predicted kinematics (color). and e) synthesized spectrogram from neurally decoded articulation (above) and reference spectrogram of the original production (below) for the same sentence

Disclosures: G.K. Anumanchipalli: None. **J. Chartier:** None. **E.F. Chang:** None.

Poster

590. Brain-Machine: Speech and Other Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 590.03/UU10

Topic: E.05. Brain-Machine Interface

Title: Using single unit bursts to decode audible and silent speech recorded chronically from a speaking human

Authors: *P. R. KENNEDY
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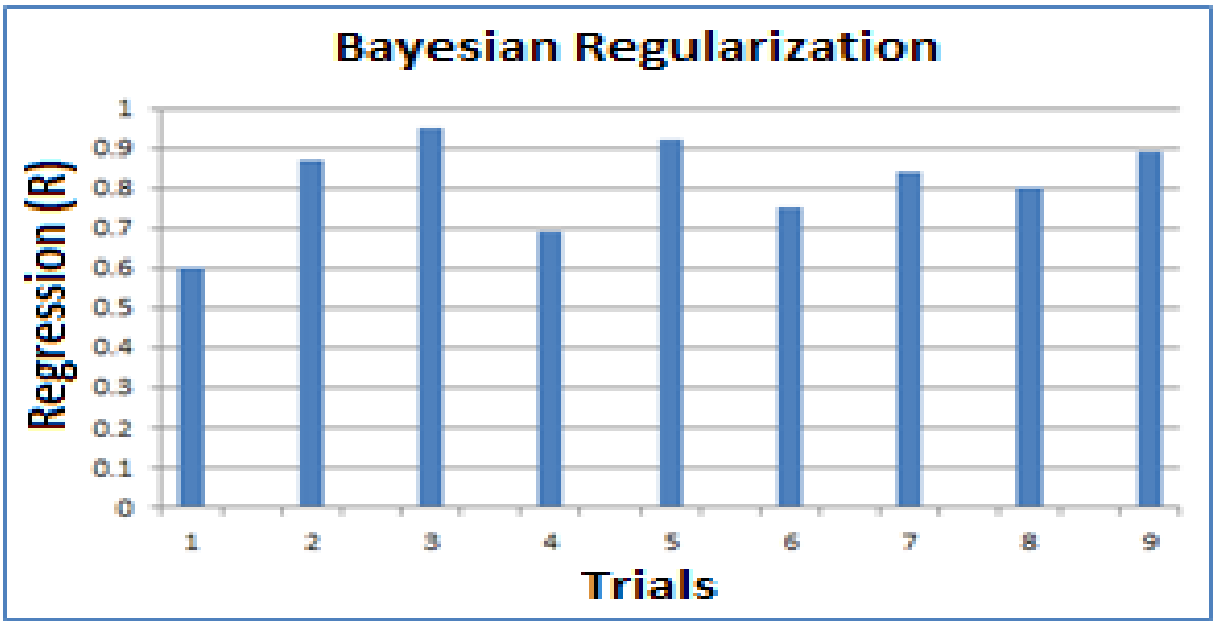
Abstract: A major issue with recording from a locked in subject (mute and paralyzed, but intelligent and awake) is that the investigator cannot be certain if the subject is actually speaking *silently*. The *timing* of speech onset as well as the *correctnes*s of the requested speech are also uncertain. For those reasons the articulatory motor area of a speaking human (PRK) was implanted with four Neurotrophic Electrodes on June 21st 2014. Amplifiers and transmitters, powered by inductive coils, were added in October 2014 and all were removed in January 2015 after several weeks of good quality recordings of 65 single units. Audible and silent speech epochs (plus silent control epochs) were analyzed during production of 39 phonemes, some of 290 short words and six phrases (containing all 39 phonemes). The pattern of single unit bursts was analyzed. During recording, an event marker (button push by subject) was used to determine the approximate onset of audible and silent speech. Actual speech, when available, was recorded on a separate channel. As previously reported (SFN abstracts 2015), the patterns of firing of single units were used to detect phonemes with an 80% detection rate using four phonemes that were associated with the most active single unit modulations. However, further analyses did not extend these results to many other phonemes. An alternative method was adopted that used patterns of single unit bursts to decode the speech using a Neural Net Fitting app from Matlab. This app requires a *target* set of values for each phoneme, word or phrase (e.g. Hello World) which target was then compared with an incoming set of values for each time the phrase is spoken audibly or silently.The example below using the phrase ‘Hello World’ *spoken silently*. Note that some of the regression values (R) are close to 0.9.
Conclusion: The present NN Fitting app has the disadvantage of requiring targets to which the incoming data streams are compared. However, deep learning computer paradigms will likely not have this restriction and will decode silent speech when hundreds of units are available.



Disclosures: P.R. Kennedy: None.

Poster

590. Brain-Machine: Speech and Other Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 590.04/UU11

Topic: E.05. Brain-Machine Interface

Support: Facebook's Sponsored Academic Research Agreement

Title: Real-time decoding of question-and-answer speech dialogue using human cortical activity

Authors: *D. A. MOSES[1], M. K. LEONARD[2], J. G. MAKIN[4], E. F. CHANG[3]
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Abstract: The development of an advanced speech prosthesis relies on real-time decoding of speech from high-resolution neural signals. Previous work has demonstrated that it is possible to decode perceived or produced speech with some success in relatively constrained contexts. However, to our knowledge, no work has utilized a naturalistic task where perceived and produced speech are integrated, which could have practical applications for patients who are unable to communicate. Here, we demonstrate real-time decoding of perceived and produced

speech from high-density electrocorticography (ECoG) activity in humans using a real-time neural speech recognition (rtNSR) software package that we developed (Moses *et al*., 2018). In our task, three human epilepsy patients implanted with ECoG arrays listened to questions (e.g., "When would you like me to check back on you?") and verbally produced answers (e.g., "Tomorrow"). The rtNSR system used the ECoG activity to reliably detect when subjects were listening or speaking and then performed phone-level Viterbi decoding to predict the identity of each speech utterance. We leveraged the fact that certain answers were only plausible responses to certain questions to dynamically update the prior probabilities of each answer using the preceding question likelihoods predicted from ECoG activity. Our system was able to reliably decode speech utterances for each subject, with accuracy rates as high as 75% for perceived questions and 61% for produced answers (chance rates were approximately 20% and 7%, respectively). Furthermore, using the decoded questions as context significantly improved answer decoding. We also demonstrated that high accuracy rates are achievable using only 15-20 minutes of training data, suggesting that this paradigm can be used practically in limited data settings. These results demonstrate that neural activity in speech perception and production regions can be used for real-time decoding of speech in natural, conversational settings.

Disclosures: D.A. Moses: None. **M.K. Leonard:** None. **J.G. Makin:** None. **E.F. Chang:** None.

Poster

590. Brain-Machine: Speech and Other Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 590.05/UU12

Topic: E.05. Brain-Machine Interface

Support: NSF GRFP

Title: LFP based classification of vocalizations in free-behaving zebra finch

Authors: *D. E. BROWN, JR[1], E. M. ARNEODO[5], S. CHEN[2], T. GENTNER[3], V. GILJA[4]
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Abstract: Songbirds, like humans, are one of the few species capable of learned vocal behavior, making them an attractive animal model for studying vocal learning. Understanding the neurobiological principles and mechanism that support vocal learning in songbirds, can yield useful insight into understanding human speech perception and production, and aid in the longstanding goal to develop a human speech prosthesis. With this goal in mind, we present a discrete neural decoder that predicts the vocalizations produced by an awake freely-behaving zebra finch, a species of songbird, based on local field potentials recorded in the sensorimotor telencephalic region HVC (used as a proper noun), shown in previous research to be involved

with the production and timing of song.
Using band power within multiple frequency bins of the local field potential (LFP) as a feature, and a linear discriminant analysis (LDA) classifier, we identified a separability in neural space for four distinct syllables and the introductory notes in a birds-own-song, and contemporaneous silence, recorded during free vocal activity. We computed syllable classification performance, using 4-fold cross-validation and a grid search over parameters of the LFP bins namely window length and window offset, achieving a peak syllable classification accuracy of 33±2% (mean +/- s.e.m; chance level is 16.7%). In general, classification performance increases as with window length shrinks. As we expected, better classification performance was observed when window onset close to the stimulus onset. We tested the classifier further in a series manner using a 4.5-second snippet of free vocal behavior.
Our results demonstrate that the syllables of a zebra finch's song can be "decoded" from local field potentials sampled in HVC. Notebaly, the model described here does not utilize the temporal structure present in either the bird's vocal behavior or the recorded neural activity. Future work will explore how consistent these dynamics are across subjects and how they correspond to volitional motor control.

Disclosures: D.E. Brown: None. **E.M. Arneodo:** None. **S. Chen:** None. **T. Gentner:** None. **V. Gilja:** None.

Poster

590. Brain-Machine: Speech and Other Motor Systems

Location: SDCC Halls B-H

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Program #/Poster #: 590.06/UU13

Topic: E.05. Brain-Machine Interface

Support: ONR N00014-13-1-0205
KIBM 2016004

Title: A brain-machine-interface to generate vocal communications

Authors: *S. CHEN[1], E. M. ARNEODO[2], D. E. BROWN, II[3], V. GILJA[3], T. Q. GENTNER[4]
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Abstract: Brain Machine Interfaces (BMIs) can restore impaired motor function and have been employed to understand the mapping between neural activity and motor control. State-of-the-art BMIs fall short, however, when it comes to decoding complex behaviors with high dimensionality, such as vocal communication. Using birdsong as a model for complex behavior similar to human speech, we previously created a BMI for birdsong in which spiking activity in the sensorimotor region HVC (used as a proper noun) can be fit to the parameters of a low-dimensional model of zebra finch syringeal dynamics that generates natural song, using a simple

feedforward neural network. The dimensionality reduction provided by the syringeal model is crucial to the performance of the feedforward network. Here we propose an innovative method that incorporates advances in machine learning, specifically a Long Short-Term Memory (LSTM) network, capable of temporal sequence mapping, to produce a BMI that directly translates HVC spiking activity into the frequency domain representation (mel spectrogram) of a bird's own song. The LSTM-based BMI yields synthetic bird's own songs that sound similar to natural songs using as little as 20% of a 70-song repertoire for training. Acoustic variability in the BMI synthesized songs (computed as the RMSE of the spectrograms) falls within the range of natural variation in the bird's own songs, and is significantly lower than the variability between songs from different conspecific birds. The LSTM-based BMI can also reconstruct novel vocalizations, not presented to the machine during training. For birdsong researchers, these results provide a platform where song output (and thus auditory feedback) can now be directly modulated in much more precise ways compared to previous methods. The BMI also provides a framework for a deeper quantitative investigation of the general intuition that transformation of HVC spiking patterns into a high-dimensional vocal motor behavior involves substantial non-linearities that are captured by the recurrent architecture of an LSTM. Comparing the capacities of various network architectures to generate song from the neural activity of RA and other well-studied song system nuclei can be used to isolate the source of different non-linear mappings and more specifically define processing functions throughout the song system. We suggest that once fully optimized, such a system will substantially advance our understanding of the physiological mechanism behind vocal communication, and benefit fully automated assistive technologies to regain a much wider range of lost motor function than currently available.

Disclosures: S. Chen: None. **E.M. Arneodo:** None. **D.E. Brown:** None. **V. Gilja:** None. **T.Q. Gentner:** None.

Poster

590. Brain-Machine: Speech and Other Motor Systems

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Program #/Poster #: 590.07/UU14

Topic: E.05. Brain-Machine Interface

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NINDS (T32NS100663)
NINDS (UH2NS095548)
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NICHD-NCMRR (R01HD077220)
NINDS (U01NS098968)
TATRC

Title: Tracking longitudinal changes in sleep features in an intracortical brain-computer interface user with tetraplegia

Authors: *D. J. THENGONE[1,2,3,4], T. HOSMAN[1,2], J. SAAB[1,2,4], J. D. SIMERAL[1,2,3,4], L. R. HOCHBERG[1,2,3,4,5]
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Abstract: A primary goal of intracortical brain-computer interfaces (iBCI) is to enable long-term neural control of assistive devices for individuals with severe motor disabilities. While continuous training and decoder calibration are known to influence the stability of iBCI control over time, relatively less is known about the neural circuit mechanisms and effective connectivity across cortical regions during extended iBCI usage in humans. We performed retrospective analyses to track the longitudinal changes in two canonical oscillations typical of NREM sleep: slow-wave activity (SWA) (0.5 - 2 Hz) and spindle frequency activity (SFA) (9 - 16 Hz oscillation lasting 0.5 - 2 sec). In both animal and human neurophysiological studies, SWA and SFA have not only been known to reflect the dynamics of cortical connectivity, but also have been directly linked to motor skill acquisition. Here we present data collected from research sessions carried out with a participant enrolled in the BrainGate2 clinical trial spanning 1 year of iBCI usage and track the sleep oscillations along the similar timecourse as the iBCI user performs neuroprosthetic control. Neural signals were collected using two 96-channels microelectrode arrays (BlackRock Microsystems) implanted in the precentral and middle-frontal gyri. Signals from each electrode were amplified and filtered to attain spike power and thresholded to yield spike rates. Prior to the identification of SWA and SFA, local field potentials (LFP) were low-pass filtered and downsampled to 1000 Hz. Retrospective identification of the NREM sleep epochs were performed using standard criteria: 1) eyes-closed state and 2) LFP periods dominated by high-amplitude, low-frequency oscillations. Using this methodology, a total of 3.8 hours (6 minute segment x 38 sessions) of sleep were selected from the relevant research sessions over the 1-year period. Multitaper power spectral analyses of the LFP revealed significant changes in the SWA and SFA across majority of the channels in both cortical regions over time. Specifically, increased power in the SFA was often accompanied by slight increases in the peak frequency of spindling activity as well. This was consistent across the recordings in both cortical arrays. Coherence analyses across the two regions during sleep revealed changes in local and long-range coherence in the SFA during the same timecourse. These initial observations support the use of iBCI to investigate dynamics of cortical oscillations and effective connectivity in humans.

Disclosures: D.J. Thengone: None. **T. Hosman:** None. **J. Saab:** None. **J.D. Simeral:** None. **L.R. Hochberg:** None.

Poster

590. Brain-Machine: Speech and Other Motor Systems

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Program #/Poster #: 590.08/UU15

Topic: E.05. Brain-Machine Interface

Support: Office of Research and Development, Rehabilitation R&D Services, Department of Veterans Affairs (N9288C, A2295R, B6453R, P1155R)
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NIDCD (R01DC009899)
NINDS (U01NS098968)
MGH-Deane Institute
The Executive Committee on Research (ECOR) of Massachusetts General Hospital
NIH (T32MH20068-17)

Title: Single unit activity in middle frontal gyrus of a person with tetraplegia reveals sensory specific modulation

Authors: *K. G. WILCOXEN[1,2], C. E. VARGAS-IRWIN[1,2], J. B. HYNES[1,2], T. HOSMAN[3,2], J. SAAB[3,4,2], B. FRANCO[5], J. KELEMAN[5], E. N. ESKANDAR[6], J. P. DONOGHUE[1,3,2,7], L. R. HOCHBERG[4,3,5,8,2]
[1]Dept. of Neurosci., [2]Carney Inst. for Brain Sci., [3]Sch. of Engin., Brown Univ., Providence, RI; [4]Dept. of VA Med. Ctr., VA RR&D Ctr. for Neurorestoration and Neurotechnology, Providence, RI; [5]Dept. of Neurol., [6]Neurosurg., Massachusetts Gen. Hosp., Boston, MA; [7]Wyss Ctr., Geneva, Switzerland; [8]Dept. of Neurol., Harvard Med. Sch., Boston, RI

Abstract: Intracortical brain computer interfaces (iBCIs) use neural activity to directly control external devices, bypassing damaged neural pathways with the aim of restoring communication and independence for people with impaired mobility due to stroke, spinal cord injury, or neurodegenerative disorders. Premotor cortex is a promising candidate for iBCI applications given its combination of prominent corticospinal projections coupled with strong links to frontal and parietal areas involved in sensory-motor transformations. Here, we compare single unit activity between the middle frontal gyrus (MFG) and precentral gyrus (PCG) in a person performing an instructed delay movement imagery game with both auditory and visual cues. Data was collected from participant T10, a 35 year-old right handed man with a spinal cord injury (C4 AIS-A), in the BrainGate2 pilot clinical trial*. T10 had two 96 microelectrode arrays (Blackrock Microsystem, Inc) implanted—one in the left MFG and one in the left PCG. T10 played a 2-part instructed delay game, in which four targets located at cardinal points of a monitor were cued using auditory and visual instructions. Auditory instructions indicated the color of the target (red or blue), while the visual instructions indicated the shape (circle or

square). Both types of information were required in order to identify the target unambiguously. We examined the effect of varying cue order (V-A vs. A-V) or presenting both cues simultaneously (A + V). We found that information related to target direction in MFG was highly dependent on the sensory modality used to instruct the movement. MFG, unlike PCG, was strongly biased towards encoding information presented as goal-relevant auditory cues, rather than visual cues. Information related to target position was evident in PCG only after both auditory and visual cues were presented (i.e. once the precise target location was known). By contrast, target-related information in MFG transiently peaked shortly after auditory cues were presented. Interestingly, MFG did not respond selectively for auditory information when the same auditory cues were presented outside the context of the task (i.e. passive listening). Our results suggest that MFG may be specifically involved in interpreting auditory cues for the purpose of movement guidance.
*The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health, or the Department of Veterans Affairs or the United States Government. CAUTION: Investigational Device. Limited by Federal Law to Investigational Use.

Disclosures: K.G. Wilcoxen: None. **C.E. Vargas-Irwin:** None. **J.B. Hynes:** None. **T. Hosman:** None. **J. Saab:** None. **B. Franco:** None. **J. Keleman:** None. **E.N. Eskandar:** None. **J.P. Donoghue:** None. **L.R. Hochberg:** None.

Poster

590. Brain-Machine: Speech and Other Motor Systems

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 590.09/UU16

Topic: E.05. Brain-Machine Interface

Support: NSF EEC-1028725
NIH Computational Neuroscience Training Grant 5T90DA032436-05

Title: The effect of default mode network disruption on reaction-timing and cortical activity in a modified Stroop task

Authors: *N. R. WILSON[1], K. WEAVER[2,5], J. WU[1,5], J. G. OJEMANN[3,5], R. P. N. RAO[4,5]
[1]Bioengineering, [2]Radiology, [3]Neurosurg., [4]Sch. of Computer Sci. & Engin., Univ. of Washington, Seattle, WA; [5]Ctr. for Sensorimotor Neural Engin., Seattle, WA

Abstract: Default mode network (DMN) activity increases with lowered attention and mind wandering during task performance. User performance with Brain-Computer Interfaces (BCI) tends to worsen with decreased attention. The monitored signals are typically modulated by attention and are likely influenced by DMN activity. Tracking DMN activity in real-time may

serve as a surrogate for attentional brain state, suggesting a strategy of disrupting the DMN in order to return users to their more alert state, thereby improving BCI performance. Here, we explore the effects of conscious and subconscious disruption of the DMN in humans via an audio beep or electrical stimulation of the cortex over DMN areas. Patients undergoing clinical seizure monitoring and implanted with subdural electrodes consented and volunteered to perform a modified Stroop reaction timing (RT) task. In each trial, subjects were presented with a color word, either congruent or incongruent in meaning and font color, and pressed one of two keys as quickly as possible to label the word as congruent or not. Between trials, a fixation dot was displayed during a variable interstimulus interval (ISI) randomly ranging from 1-10 seconds. In DMN disruption trials, conscious or subconscious disruption occurred 1 second prior to word presentation. Preliminary results suggest auditory disruption of the DMN may improve RT in trials with relatively long ISIs (Fig 1). In agreement with established DMN literature, longer ISIs increase the likelihood that subjects begin to lose focus and engage in mind wandering, leading to increased DMN activity. With cued disruption, subjects are reoriented to the task at hand. Ongoing efforts are implementing high-frequency direct cortical stimulation (DCS) to electrodes positioned over the DMN. Previous DCS research revealed electrical stimulation of the posterior parietal hub of the DMN does not yield a subjective conscious experience. Future analyses will compare the effects of conscious (cued auditory disruption) and subconscious (DCS) DMN disruption on task performance RT.

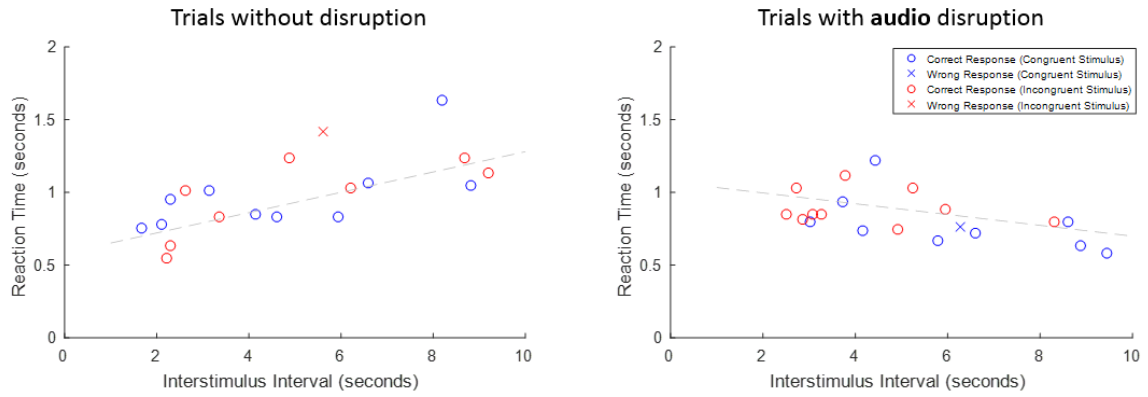

Figure 1. In at least one subject, reaction time appears to be positively correlated with interstimulus interval length in trials without any form of Default Mode Network disruption. This effect is eliminated when an audio beep is sounded one second prior to word presentation.

Disclosures: K. Weaver: None. **J. Wu:** None. **J.G. Ojemann:** None. **R.P.N. Rao:** None.

Poster

590. Brain-Machine: Speech and Other Motor Systems

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Program #/Poster #: 590.10/UU17

Topic: E.05. Brain-Machine Interface

Support: NSF Grant EEC-1028725

Title: A novel ischemic stroke model for non-human primate: Quantitative estimate of the scale of photochemically induced infarction in primate cortex

Authors: *Z. YAO, E. P. BURUNOVA[1], W. Y. HAN[1], W. K. S. OJEMANN[1], A. YAZDAN-SHAHMORAD[2,3]
[2]Bioengineering, [3]Electrical Engin., [1]Univ. of Washington, Seattle, WA

Abstract: Stroke is one of the global leading causes of disability. It has been challenging to translate rodent study-derived stroke therapies. On the other hand, non-human primate models are critical for preclinical stroke studies that may prelude effective medical translation. In light of scarce reports of such studies, we propose the use of photothrombosis in developing macaque stroke models.
Photothrombosis produces focal cerebral infarcts via the photodynamic effect of anionic xanthene dyes, e.g. Rose Bengal. Injected to the blood stream, it binds to the vascular endothelium, the platelets and other cells. Upon light exposure at interested cortical locations, the dye's photochemical reaction generates local oxidative stress, causing vascular endothelial damage and platelet aggregation, and resulting in ischemia and neuronal death.
Here, we present a computational model to predict the scale of photothrombotic lesions in the cortex. Based upon McLean's (1998) beam spread function method – assumeing photons reach locations in the media via various-lengthed paths, thus, resulting in time dispersion of intensity – we modeled the relative light intensity distribution in the cortical tissue of a collimated beam. We first calculated the time-resolved impulse response of a single photon energy packet using the beam spread function. It was then temporally integrated to generate the scattering profile of a continuous pencil beam. Such distribution was subsequently convolved in the transverse plain with the geometry of the beam to acquire the intensity distribution of a realistic beam.
We simulated the penetration and scattering profile of the 532 nm light – one of Rose Bengal's characteristic absorption wavelengths. Our model could predict the spatial extent of the radiation's effective region according to the width of the beam. It could also resolve the penetration depth as a function of beam intensity, which would be applicable in titrating the beam's power used to generate infarcts of ideal depth.
Our simulation demonstrated how the scale of photothrombotic infarction quantitatively depends on the intensity and the diameter of the beam. In addition to a multilayer model being developed to account for cortical tissue-optics heterogeneity, future effort will focus on the integration of computational results for in-vivo testing of the stroke model in macaques. We will then modify our computational model based on pathology/histology examinations.
Reference:
McLean, J.W., Freeman, J.D. and Walker, R.E., 1998. Beam spread function with time dispersion. *Applied optics, 37*(21), p.4701.

Disclosures: E.P. Burunova: None. **W.Y. Han:** None. **W.K.S. Ojemann:** None. **A. Yazdan-Shahmorad:** None.

Poster

590. Brain-Machine: Speech and Other Motor Systems

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Topic: E.05. Brain-Machine Interface

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Sloan Foundation
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Title: Virtual navigation via a closed-loop brain-machine interface

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Abstract: Brain-machine interfaces (BMIs) for reach control have enjoyed continued performance improvements, allowing remarkable 2D cursor control. Yet there remains significant clinical need for locomotor (e.g., wheelchair control) BMIs. Proof-of-concept locomotor BMIs were recently demonstrated, using decode methods derived from reaching BMI's. Here we adopt a different approach, and examine the viability of locomotor BMI's guided by rhythmic neural activity.

We exploit a behavioral task in which monkeys cycle a hand-held pedal, forward or backward, to advance along a virtual track and pause on targets to collect juice reward. This task does not involve natural locomotion - the patterns of muscle activity differ and the contribution of spinal pattern generators is likely very different. Instead, the task provides a view of the patterns of cortical activity during a learned, voluntary, rhythmic movement. Those patterns are robust and have been recently characterized, affording the opportunity to develop appropriate decode algorithms and test them in an online setting.

Neural activity was recorded from 96 electrodes acutely implanted in primary motor cortex. Based on data during hand-control, we estimated key parameters of the model, including the subspaces in which neural activity evolved during forward and backward cycling. Our decoder estimated the neural state of the neural population, the velocity of which used to control the direction and speed of virtual motion. A Hidden Markov Model estimated whether the monkey intended to move or remain stationary, and gated output accordingly. The decoder was almost

always successful in driving motion in the appropriate direction, forward or backward, yielding progress toward the juice target. A more challenging aspect of the task involved stopping on that target, which delivered maximal reward. Stopping successfully required a precision, relative to the traveled distance, of 14% - 50% (depending on target distance). In 5 online experiments over the course of two weeks, the monkey stopped successfully on 70.9±20.8% of targets. Performance increased with experience: on the 5th day the success rate was 94%, only slightly worse the native arm performance (98%). The target size was held constant over these experiments, resulting in an average Fitts' law throughput of 1.1±0.1 bits/s, compared with 1.6 bits/s in arm control, when using six distance conditions. Higher throughputs are possible using more distances, pending improvements in stopping precision. These data demonstrate the viability of decoding locomotor signals from rhythmic cortical activity.

Disclosures: K.E. Schroeder: None. **S.M. Perkins:** None. **Q. Wang:** None. **M.M. Churchland:** None.

Poster

590. Brain-Machine: Speech and Other Motor Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 590.12/UU19

Topic: E.05. Brain-Machine Interface

Support: Neilsen Senior Research Grant 340943

Title: Development of cortically controlled FES following spinal cord injury in the rat

Authors: F. BARROSO[1], B. YODER[1], J. WALLNER[1], D. TENTLER[1], P. TOSTADO[3], L. E. MILLER[1], *M. C. TRESCH[2]
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Abstract: Cortically controlled functional electrical stimulation (FES) is a promising approach for restoring motor function following spinal cord injury (SCI). In cortically controlled FES, intended movements (or patterns of muscle activity) of a paralyzed limb are estimated from cortical activity and those movements are produced by electrical muscle stimulation. In addition to its potential for restoring function, there is evidence that cortically controlled FES improves rehabilitation. In this application, repeated pairing of cortical activity with evoked movements is thought to strengthen residual descending connections through activity-dependent plasticity, thereby improving overall function.
To evaluate this application, we are developing cortically controlled FES in rats with SCI. We have previously shown that we are able to obtain good predictions of muscle activations and limb kinematics from cortical recordings in intact animals. In the experiments reported here, we

describe the use of cortical recordings to predict and restore function following SCI. We implanted electrode arrays in motor cortex and EMG electrodes in hindlimb muscles. We recorded cortical activity, EMGs, and kinematics while animals walked on a treadmill. We then transected the spinal cord at mid-thoracic levels so that the cord ipsilateral to the implanted hindlimb was fully transected, together with approximately one-third of the contralateral cord. We then recorded cortical activity, EMGs, and kinematics while animals attempted to walk on the treadmill over several weeks after SCI.
Immediately after SCI the hindlimb ipsilateral to the fully transected cord was paralyzed. Over several weeks, animals spontaneously recovered some degree of function. We are currently evaluating predictions of EMGs and limb kinematics from cortical activity following SCI. We are examining predictions immediately after SCI, evaluating whether cortical activity might be related to spontaneous functional recovery. We are also examining whether decoders used to predict EMGs and kinematics before SCI can be used to predict similar EMGs and kinematics immediately after SCI. Using these decoders we will then evaluate the efficacy of repeated training with cortically controlled FES for functional rehabilitation following SCI.

Disclosures: F. Barroso: None. **B. Yoder:** None. **J. Wallner:** None. **D. Tentler:** None. **P. Tostado:** None. **L.E. Miller:** None. **M.C. Tresch:** None.

Poster

590. Brain-Machine: Speech and Other Motor Systems

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Program #/Poster #: 590.13/UU20

Topic: E.05. Brain-Machine Interface

Support: R01NS088606

Title: Classification of flexion and extension of upper limb joints from the sensorimotor cortex using electrocorticography

Authors: *T. M. THOMAS[1], D. N. CANDREA[1], N. E. CRONE[2]
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Abstract: Because subdural electrocorticography (ECoG) can provide stable and comprehensive recordings of human sensorimotor cortex, it continues to be investigated as a potential brain-computer interface for restoration of communication and upper limb control. To date, these studies have only achieved rudimentary cursor control or simple reaching and grasping movements that involve the simultaneous control of multiple joints. To test whether movements at individual joints in the upper limb can be classified from ECoG recordings, we recorded from electrode arrays of varying spatial resolutions (1-cm, 5-mm, and 0.9-mm spacing) implanted in

three subjects. The subjects performed randomly cued flexions or extensions of the fingers, wrist, or elbow contralateral to the implanted electrodes. Electrodes that showed significant differences (ANOVA, $p < 0.05$) in high-gamma (HG, 70-110 Hz) responses during different movements were selected for decoding. We trained a linear model to classify the individual joint movements using averaged HG power modulations within a 328 ms sliding window at different latencies with respect to movement onset. We tested the model using 10-fold cross validation. Peak classification accuracies for patients S1, S2, and S3 were 54.4%, 57.5%, and 65.3%, respectively (chance 16.7%). When decoding from a longer (1.52-2.24 s) time window, bounded by where the temporal accuracy was significantly higher than chance, classification accuracies for patients S1, S2, and S3 were 62.4%, 67.5%, and 86.9%, respectively (chance as above). When comparing classification accuracies of movements at different joints, flexion and extension of the hand and wrist were classified more accurately than those of the elbow. We also found that the neural activity for different movements was more classifiable at the macro-ECoG scale than at micro-ECoG scale, but this could have been due to suboptimal placement of micro-ECoG arrays. The results of this exploratory study suggest that recording from a larger area of cortex may be useful for classifying the neural representations of individual degrees of freedom at different upper limb joints.

Disclosures: T.M. Thomas: None. **D.N. Candrea:** None. **N.E. Crone:** None.

Poster

590. Brain-Machine: Speech and Other Motor Systems

Location: SDCC Halls B-H

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Program #/Poster #: 590.14/UU21

Topic: E.05. Brain-Machine Interface

Support: 4R01NS072342-05

Title: Effects of dorsal root ganglia microstimulation in advance of postural perturbation on hindlimb motor output in behaving cats

Authors: *M. URBIN[1], E. C. BOTTORFF[1], R. A. GAUNT[1], L. E. FISHER[2], D. J. WEBER[3]
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Abstract: We previously presented findings to demonstrate that microstimulation of the dorsal root ganglia (DRG) can activate reflex pathways projecting onto hindlimb motor neurons in behaving cats. Here, we report on the effects of microstimulation in advance of postural perturbation on hindlimb motor output. Penetrating, 32-channel microelectrode arrays were implanted chronically in the left L6 and L7 DRG of four male cats. Microelectrodes with low thresholds for recruiting antidromic volleys in Group I afferents were selected for delivering 100-ms, 100-Hz pulse trains. Stimulus trains were delivered at 1.5x (low) and 2.0x (high)

threshold, 150 ms (early) and 50 ms (late) prior to forward or backward translation of a platform. EMG and ground reaction forces were recorded in a perturbation-only condition and in four stimulation+perturbation conditions in both translation directions. The initial behavioral response (ie, hindlimb unloading in backward condition; loading in forward condition) was quantified as the peak change in force pre- to post-perturbation. Two different epochs were used to quantify EMG power: A) a window in which a given muscle was active in the perturbation-only condition; B) a fixed window consisting of the first 150 ms after perturbation onset. Results indicated that peak force was increased during backward perturbation ($\chi^2(4)=18.33$, $p<0.001$) for late-low, early-high, and late-high stimulation relative to perturbation only. EMG power was reduced within the active epoch ($\chi^2(4)=14.64$, $p=0.006$) for early-low stimulation relative to perturbation only, but differences in the fixed window epoch failed to reach significance ($\chi^2(4)=8.72$, $p=.068$). In contrast to the backward direction, peak force was reduced during forward perturbation ($\chi^2(4)=22.76$, $p<0.001$) for all stimulation conditions relative to perturbation only. EMG power was reduced within the active epoch ($\chi^2(4)=21.52$, $p<0.001$) for all stimulation conditions relative to perturbation only. EMG power was also reduced within the fixed window epoch ($\chi^2(4)=10.24$, $p=0.037$) but only for early-low, late-low, and late-high stimulation relative to perturbation only. These findings demonstrate that DRG microstimulation drives hindlimb muscle activity, resulting in postural responses that either attenuate or magnify the natural response to surface perturbation. Consistent with our prior findings, activated reflex circuits recruited complex EMG patterns, the net effect of which was an unloading response irrespective of perturbation direction. Further work is needed to understand how other recruitment patterns might be engaged within the heterogeneous somatotopy of the DRG.

Disclosures: M. Urbin: None. **E.C. Bottorff:** None. **R.A. Gaunt:** None. **L.E. Fisher:** None. **D.J. Weber:** None.

Poster

590. Brain-Machine: Speech and Other Motor Systems

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Program #/Poster #: 590.15/UU22

Topic: E.05. Brain-Machine Interface

Support: NIH award NINDS R01 NS088184

Title: Characterizing neural responses and organization in the dorsal root

Authors: *D. SARMA[1], M. F. LIU[2], C. GOPINATH[3], L. E. FISHER[3], R. A. GAUNT[3], D. J. WEBER[2]
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Abstract: The dorsal root ganglion (DRG) is an ideal location to record somatosensory neural signals, which convey body-state information such as tactile and proprioceptive feedback from the limbs. Neural signals from mechanosensory neurons in just one or two DRG can be used to predict the joint angles and the position and velocity of a limb with high accuracy, in addition to providing cutaneous and force information. These signals are ideal for use as control signals in closed loop applications with somatosensory feedback. In a similar way, afferent signals from visceral organs such as the bladder may provide pressure or volume information that could be used for a bladder neuroprosthesis. Recent work in our lab and by other groups has shown the ability to record simultaneously from up to one hundred neurons with arrays of microelectrodes implanted in DRG. However, there are still remaining questions regarding the potential somatotopy of cell bodies and the actual organization of neurons across layers of the DRG. In this study, during intraoperative experiments in feline and macaque DRG, neural responses were characterized along the shanks of 3D microelectrode arrays (4x8-channel, 125 micron vertical spacing, N-form 3D arrays, Modular Bionics), implanted transversely, to examine commonalities in field potentials and spike waveforms across pairs of tightly spaced electrodes. In one experiment conducted in an isoflurane anesthetized male cat, after a laminectomy exposing the L5 to S4 spinal segments, we implanted these probes into the L7 DRG. Neural responses were recorded at each electrode during passive movement and manipulation of the hindlimbs. In most cases, electrodes were most highly correlated to the neighboring electrodes, with the deepest electrode the most independent. In one shank, however, the greatest coactivation was found at the lowest (e1/e2) and highest ends (e7/e8) with the largest amplitude response seen in the middle across all conditions. Further examination is still needed to fully understand the spatial relationships along as well as across the DRG layers. These studies help support the efficacy of recording and decoding neural activity from the DRG for somatosensory feedback and will drive future experiments involving longer-term (sub-chronic and chronic) evaluations of DRG recordings in animal and human subjects with optimized electrodes.

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Poster

590. Brain-Machine: Speech and Other Motor Systems

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Program #/Poster #: 590.16/VV1

Topic: E.05. Brain-Machine Interface

Support: NSF Grant IIS-1602337

Title: Patterns of cortical population activity during intentional control of single neurons

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Abstract: When learning to control an intra-cortical brain-computer interface (BCI), evidence suggests that subjects achieve behavioral goals by re-organizing a fixed repertoire of neural activity patterns. From a computational perspective, this is somewhat unsurprising: searching within a restricted pattern space represents a relatively low-dimensional strategy, in contrast to the high-dimensional search that neuron-by-neuron optimization would entail. However, this contrasts with previous reports suggesting that patterns of cortical population activity are rather labile, and are readily adapted to suit arbitrary behavioral demands. A fundamental difference between the evidence used to support these two conclusions lies in the behavioral task: the former results tend to be derived from population-based BCI experiments, whereas the latter tend to be derived from the single-neuron operant conditioning paradigm.
Here, we aim to characterize patterns of task-relevant population activity during performance of a single unit conditioning task. We recorded from populations (N > 20) of neurons in primary motor cortex as subjects were rewarded for systematically varying the firing rates of individual neurons between high and low frequencies. We found that a substantial percentage of nearby neurons tended to co-vary, even though behavioral goals (i.e., reward) depended only on the activity of single neurons. Further, the exact pattern of task-relevant covariation could change with the identity of the neuron targeted for conditioning. Across conditions, most of the variance in the population could be accounted for by a few principal components, indicating distinct patterns of activity, but also substantial overlap among these patterns. Our results seem to favor the possibility that subjects draw from a fixed repetoire, which is not optimized for the control of single neurons. We suggest that the flexibility of a cortical population is constrained by some intrinsic structure -- perhaps due to network connectivity patterns and/or prior experience.

Disclosures: A.S. Whitford: None. **S.M. Chase:** None. **A.B. Schwartz:** None.

Poster

590. Brain-Machine: Speech and Other Motor Systems

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Topic: E.05. Brain-Machine Interface

Support: DAPA Grant UD170030ID

Title: Brain-machine interface controlled by non-motor brain area

Authors: *Y. A. CHO[1], Y. LEE[2], J. LEE[2], D. YEO[5], K. KIM[5], S. JUN[3,4]
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and Electrical Engin., [4]Dept. of Brain and Cognitive Sci., Ewha Womans Univ., Seoul, Korea, Republic of; [5]Yonsei Univ., Wonju, Korea, Republic of

Abstract: Recently, research on invasive brain-computer interface (BCI) or brain-machine interface (BMI) is growing impressively for both experimental and clinical purposes. To date, most of BCI studies have targeted cortical neurons from motor-related cortex regions to extract neural signals. Based on the motor-related neuronal activities, various algorithms were proposed to control an external actuator such as robot arms, wheel chairs, and so forth. However, BMIs targeting motor-related cortex area are not always acceptable for patient who are suffering from disorders affecting motor-related brain areas including a stroke or a damage in a particular part of motor cortex. Therefore, another brain area to provide reliable neural signals for BMI is required. Previous study identified that prefrontal cortex (PFC) is a reasonable alternative. PFC is well known to be associated with behavioral flexibility, working memory, planning, spatial navigation, and goal-directed behavior. In the present study, 16-channel multi-electrode array is implanted in PFC of a rodent for *in vivo* neural signal recording. The animal was placed in a box with a wall equipped with a small cart containing pellet and controlled by two motors. In order to receive the food reward, the animal is required to control the cart position close to the open hole made on the transparent acrylic wall by modulating its brain activity in PFC.

Disclosures: Y.A. Cho: None. **Y. Lee:** None. **J. Lee:** None. **D. Yeo:** None. **K. Kim:** None. **S. Jun:** None.

Poster

590. Brain-Machine: Speech and Other Motor Systems

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 590.18/VV3

Topic: E.05. Brain-Machine Interface

Support: NIH Support

Title: Local network coordination supports neuroprosthetic control

Authors: *W. A. LIBERTI, III[1,2], L. XUE GONG[2], A. YOU[2], N. VENDRELL LLOPIS[2], T. ROSEBERRY[2], R. M. COSTA[3], J. M. CARMENA[2]
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Abstract: Several studies of neuroprosthetic learning have shown that after an initial phase of exploration, particular spatiotemporal activity patterns which lead to desired outcomes are selected and consolidated. Neurons that do not directly control a neuroprosthetic effector, termed ‘indirect’ neurons, show a suppression of modulation depth across learning, but can also

demonstrate tuning relative to the task. This suggests that these neurons become incorporated into functional neuronal assemblies that help coordinate neurons that directly drive an effector- but why this particular subset of neurons becomes involved, and how they act to stabilize and reinforce output- is not known. We used chronic single and multi-photon calcium imaging to create a Brain-Machine Interface (BMI) paradigm in which mice learned to perform a neuroprosthetic task using the coordinated activity of a small ensemble of neurons in layer 2/3 of somatosensory or motor cortex, guided by auditory feedback (Clancy, 2014). This approach provides long-term access to up to thousands of neurons, referenced to an easily quantified output layer of a few neuron's activity.
We find that the local cortical neural population in which these BMI output neurons are embedded rapidly converges to form reproducible activity patterns in order to achieve arbitrary, experimenter-defined neuroprosthetic actions. We find that a sparse population of indirect neurons form sequential patterns that precede and follow the activity of output neurons, and possibly contribute to the coordination of timing of cells that directly drive the effector. In this poster we examine the formation, structure and stability of local and distant neural populations that appear to coordinate individual output neurons in greater detail.

Disclosures: W.A. Liberti: None. **L. Xue Gong:** None. **A. You:** None. **N. Vendrell Llopis:** None. **T. Roseberry:** None. **R.M. Costa:** None. **J.M. Carmena:** None.

Poster

591. Behavioral Neuroendocrinology: Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 591.01/VV4

Topic: F.02. Behavioral Neuroendocrinology

Support: CSUPERB

Title: The effects of a hypocretin receptor 1 antagonist on a pup retrieval task relevant for maternal motivation

Authors: A. SELKE, 92096[1], C. J. WHITTEN, 92096[1], *K. L. D'ANNA[2]
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Abstract: Hypocretin (HCRT) is a neuropeptide that is released from the lateral posterior hypothalamic region and has two receptors types, HCRTR-1 and HCRTR-2, with HCRT-1 having a strong affinity for both receptors. The HCRT-1 neuropeptide system has been associated with reward and motivation in drug-seeking behaviors, but unclear if this extends to other reward-linked behaviors such as pup retrieval in lactating dams. Therefore this study was conducted in order to investigate if the HCRT-1 system might be related to reward and

motivation in maternal care. We hypothesized that dams given the HCRT-1 receptor antagonist (HCRTR1A) would retrieve pups less quickly and show less overall more maternal behaviors than those given the control. Lactating dams were given either a HCRTR1 A (n=7) or vehicle control (n=7) and total time in or out of the goal box and number pups retrieved were recorded in a Tmaze. No significant differences in retrieving pups were found between the vehicle and the HCRTR1A groups ($F(22, 22.11) = .046$, $p = .831$). However, dams that were given the HCRTR1A stayed in the goal box longer (M = 490.43) than dams given a vehicle (M = 249.57; $F(12, 10.18) = .892$, $p < .01$). This work suggests that in a novel environment, HCRT-1 neurotransmission may play a role in exploratory behavior in the postpartum period. Future work should include the HCRT-2 system is needed to delineate the separate roles of HCRT-1 and HCRT-2 in our understanding of the neural networks involved with maternal behavior.

Disclosures: A. Selke: None. **C.J. Whitten:** None. **K.L. D'Anna:** None.

Poster

591. Behavioral Neuroendocrinology: Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 591.02/VV5

Topic: F.02. Behavioral Neuroendocrinology

Title: The role of hypocretin in postnatal anxiety and reward responses to pup sensory stimuli

Authors: *G. H. LEE[1], J. KUSKE[2], K. L. D'ANNA-HERNANDEZ[2]
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Abstract: Anxiety is associated with heightened arousal impairing daily-life functioning and maternal behavior. Although we are unclear of its underlying neuromechanisms, the hypothalamic neuropeptide hypocretin (HCRT) has been implicated in both anxiety and maternal behavior. During the perinatal period, several changes occur in the mother's body including increased arousal and wakefulness linked with lactation. Past literature has shown that HCRT modulates arousal and influences maternal behavior (e.g., nursing and nesting behavior in lactating dams). In the present study, fifteen lactating dams were injected with HCRTR1-antagonist (SB-334867; n=8) or saline (n=7), tested on the elevated zero maze (EZM) for five minutes, and scored on time and latency to head poke and enter open/closed arms. Time spent in open and closed arms in the EZM were not significant, whereas latency to head poke into the open was significant $t(13) = 2.28$, p = .04. Dams injected with the HCRTR1 -antagonist had a shorter latency to head poke into the open arms than those injected with saline. This suggests that blocking HCRT decreases arousal and anxiety-like behavior as the dams appeared less fearful to begin exploring the exposed areas. As dams had spent limited time on the open arms, a follow-up study was conducted using pups (on postnatal days 3-4) as a potential motivator on the EZM and

another measure of anxiety, the light/dark box (LBD). Transparent boxes were attached on the apparatuses to secure pups and placed in the exposed areas. About 27% and 80% of dams retrieved pups on the EZM and LDB, respectively. Altering anxiety-like tests to include pup retrieval may be more ecologically relevant measures of maternal anxiety. Given that HCRT has been linked to reward and motivation and pups are highly rewarding, HCRT may alter anxiety-like behavior and/or pup retrieval on these modified measures. To determine what aspects of pup stimuli activate HCRT neurotransmission in the brain, dams were exposed to either both their own pups and soiled bedding (n=6), soiled bedding only (n=7), pup-shaped items without bedding (n=9) or a control with no stimuli (n=7). Brains were double-labeled for cFos and prepro-HCRT. Cell counting is ongoing. This work suggests HCRT may play a role in the regulation of postnatal anxiety and reward.

Disclosures: G.H. Lee: None. **J. Kuske:** None. **K.L. D'Anna-Hernandez:** None.

Poster

591. Behavioral Neuroendocrinology: Parental Behavior

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Program #/Poster #: 591.03/VV6

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF IOS 1455960

Title: Corticosterone alters genomic activity of the reproductive axis

Authors: *A. M. BOOTH[1], S. AUSTIN[2], A. S. LANG[3], V. FARRAR[2], O. CALISI[4], T. CHEN[2], B. NAVA[2], M. MACMANES[3], R. M. CALISI[2]
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Abstract: Stress is a well-known cause of reproductive dysfunction in many species. The stress response generally involves an increase in adrenal glucocorticoid secretion, which can reduce activity of the reproductive, or hypothalamic-pituitary-gonadal (HPG), axis. Previously, we leveraged a highly replicated and sex-balanced experimental approach using the model of the rock dove (*Columba livia*) to understand how males and females respond to restraint stress at the level of their HPG transcriptome. In this follow-up experiment, we determined the role of the glucocorticoid corticosterone in HPG genomic differential expression. We injected male and female rock doves with corticosterone to mimic natural levels following 30 minutes of restraint stress. We found sex-biased changes in genomic activity specific to our corticosterone manipulation as compared to controls. Our data provide a vital genomic foundation on which sex-specific reproductive dysfunction attributable to corticosterone can be further studied, as well as novel gene targets for potential genetic intervention and therapy investigations.

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Poster

591. Behavioral Neuroendocrinology: Parental Behavior

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Program #/Poster #: 591.04/VV7

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant 5R25NS080686 – 07
NIH Grant HD088411

Title: Assessing behavioral preference for mouse pup calls as a function of maternal experience and oxytocin signaling

Authors: *K. L. FURMAN[1,2], I. CARCEA[2], A. C. MAR[2], R. C. FROEMKE[1,2,3]
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Abstract: When a mouse pup is removed from its nest, it emits ultrasonic vocalizations (USVs) to signal distress. Mother mice (dams) hear USVs, and respond by retrieving pups into the nest almost immediately. While dams will retrieve pups with 100% accuracy, virgin female mice without previous maternal experience fail to exhibit retrieval behavior. But after several days of cohousing with a dam and pups, virgins can begin retrieving pups reliably (Marlin et al., 2015). However, it is unclear if the USVs themselves have some degree of behavioral salience, are attractive or aversive to adult mice, and how this sensation might be affected by maternal experience.

Here we investigated innate and learned behavioral responses to auditory pup USVs, when these acoustic stimuli are presented through speakers in isolation, i.e., without pups or other pup-related sensory information (visual, olfactory). We behaviorally assessed the salience of these auditory cues in dams, pup-naïve virgins, and experienced (cohoused) virgins.

We performed two behavioral tests of animal spatial preference. First we used a spatial orientation behavioral paradigm, where USVs recorded from isolated pups were presented via ultrasonic speakers on one side of a modified T-maze with acoustically-isolated chambers at either end. We quantified the time animals spent in the chamber with the USV sounds vs the time spent in the other chamber or elsewhere in the T-maze. We found that pup-naïve virgins orient towards pup USVs, while dams and pup-experienced virgins show no orientation trend in either direction. This suggests that the USV stimulus, when played in isolation, is more salient for female mice with less maternal experience, possibly due to its novelty especially outside of behavioral context.

Second, we played different sounds in each chamber at the end of the T-maze arms. In one

chamber, USVs were presented, in the other chamber, pure tones were presented. Animals were sequentially placed in each chamber for 10 minutes during each stimulus presentation. After these two exposures, animals were given access to both chambers with no auditory cue. We found that animals tended to avoid the chamber in which they heard USVs, irrespective of maternal experience. This suggests that the USV stimulus is aversive to all groups, despite being salient to inexperienced virgins in the first behavioral test. Given these results, we are interested in investigating the role of oxytocin signaling in the preference or aversion towards pup USVs. In the future we will use optogenetic and pharmacogenetic approaches to investigate how formation of preference or aversion is affected by alterations to endogenous oxytocin systems.

Disclosures: K.L. Furman: None. **I. Carcea:** None. **A.C. Mar:** None. **R.C. Froemke:** None.

Poster

591. Behavioral Neuroendocrinology: Parental Behavior

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Program #/Poster #: 591.05/VV8

Topic: F.02. Behavioral Neuroendocrinology

Support: DGAPA UNAM, project IN-200317
CONACYT fellowship number 660096 to MOV

Title: Late emerging effects of perinatal undernutrition on the dendritic spines of BLA neurons underlying the maternal response in the rat

Authors: *M. ORTIZ, M. REGALADO, C. TORRERO, M. SALAS
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Abstract: Perinatal undernutrition (PU) interferes with the morphofunctional organization of the brain. The long-term effects of PU include alterations in the maternal response of the rat that correlates with perikaryal and dendritic arbor in the multipolar neurons of the basolateral amygdala (BLA) on days 4 and 12 of lactation. The mothers (F0) of the DPP group of (F1) rats, during pregnancy, received food restriction on different percentages (50, 70 and 100) throughout this period. After birth, undernutrition continued by ligating the milk ducts of one mother of a pair and changing dams between the litters every 12h, until weaning at 25 days of age. At 90 days of age, the F1 females were pregnant, and at the delivery evaluated for maternal behavior. Results showed significant prolonged latencies with abnormal retrieval pups; while on the frequency was only significant on day 12, for the DPP group. Reductions in the area and perimeter of perikaryal BLA multipolar neurons were identified on day 4, as well as alterations in the number of crossing and dendritic orders, although less consistent to the reductions in the number of spines in the PU group. The findings suggest that alterations in postsynaptic organization may affect the course of neuronal excitability, for the integration and coding of

signals that trigger the maternal response components altered in the early underfeed.
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Disclosures: M. Ortiz: None. **M. Regalado:** None. **C. Torrero:** None. **M. Salas:** None.

Poster

591. Behavioral Neuroendocrinology: Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 591.06/VV9

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF IOS 1455960

Title: What makes a parent? Genome to phenome changes in parental care of rock doves (columba livia)

Authors: *S. AUSTIN[1], A. LANG[2], M. MACMANES[2], R. M. CALISI[3]
[1]UC Davis, Davis, CA; [2]Univ. of New Hampshire, Durham, NH; [3]Neurobiology, Physiol. and Behavior, Univ. of California - Davis, Davis, CA

Abstract: The transition to parenting requires major changes to the physiology and behavior of an organism in order to promote offspring survival. Research continues to elucidate crucial endocrine players and pathways associated with this fundamental transition to parenthood, yet we know less about the underlying genomic activity that drives these behaviors. Using a socially monogamous species with biparental care, the rock dove (*Columba livia*), we ask, are the genetic mechanisms that facilitate similar behaviors in males and females the same across sex, or do they differ? Conversely, is the genomic origin of sex-specific behaviors the same or different? To address these questions, we used high-throughput sequencing to determine sex-biased differences in gene activity over the course of parental care. At nine different time points that range from non-breeding through to neonate care, we assessed levels of gene transcription in tissues critical for reproduction in vertebrates: the hypothalamus and lateral septum in the brain, the pituitary gland, and the testes and ovaries. We found a diversity of similar and sex-biased changes in gene expression across parental care stages. For instance, both sexes experience the same amount of differential gene expression in their brains and pituitaries when they transition from incubation to nestling care. However, males and females differ in the genes they express at the nestling care stage sampled by approximately 150 genes in the hypothalamus and 600 genes in the pituitary. We report changes in the activity of genes identified a priori for their known role in facilitating parental care, and we identify novel targets for further investigations. The results of this large-scale study offer significant insight into the genomic mechanisms driving maternal versus paternal care behaviors, from genome to phenome.

Disclosures: S. Austin: None. **A. Lang:** None. **M. MacManes:** None. **R.M. Calisi:** None.

Poster

591. Behavioral Neuroendocrinology: Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 591.07/VV10

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF IOS 1455960

Title: Lactating birds: Gene expression of prolactin and its receptor in male and female rock doves

Authors: *V. S. FARRAR[1], B. M. NAVA ULTRERAS[1], S. H. AUSTIN[1], M. MACMANES[2], R. M. CALISI[3]
[1]Univ. of California Davis, Davis, CA; [2]Univ. of New Hampshire, Durham, NH; [3]Neurobiology, Physiol. and Behavior, Univ. of California - Davis, Davis, CA

Abstract: The hormone prolactin (PRL) plays a role in many physiological functions, but it is best known for its role in facilitating lactation and parental care behaviors. In birds, PRL helps initiate and maintain incubation and offspring provisioning. However, in rock doves (*Columba livia*), PRL also drives crop milk production in both sexes, a process akin to lactation in mammals. We asked how the gene activity of PRL and its receptor, PRL-R, change across the parental care stage in the brain and reproductive tissues, and if these changes are driven by an internal clock mechanism or the external environment (offspring presence). We quantified gene expression of PRL and PRL-R in male and female rock dove hypothalamus, pituitary, gonad, and crop organ at multiple time points across the parental care stage. To understand if changes in gene expression are internally or externally driven, we experimentally manipulated egg and hatchling presence. We found sex-, tissue-, and time-specific changes in PRL and PRL-R over the course of parental care. These data offer the highest resolution to date in any species of the behavior of PRL and PRL-R genes during the parental care stage. Specifically, our data offer significant insight into the regulatory mechanisms of avian crop-milk production and parental care.

Disclosures: V.S. Farrar: None. **B.M. Nava Ultreras:** None. **S.H. Austin:** None. **M. MacManes:** None. **R.M. Calisi:** None.

Poster

591. Behavioral Neuroendocrinology: Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 591.08/VV11

Topic: F.02. Behavioral Neuroendocrinology

Title: Effects of pregnancy stress on postpartum socioemotional behaviors and central serotonin 2A and 2C receptor expression

Authors: *E. M. VITALE[1], J. S. LONSTEIN[2]
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Abstract: Mammalian mothers show a unique suite of behavioral responses beginning around the time of parturition, including increased offspring caregiving and low anxiety. The neurotransmitter serotonin (5-HT), which is synthesized by cells in the midbrain dorsal raphe nucleus (DR) and projects to many forebrain sites, regulates many of these postpartum socioemotional behaviors. Recent findings from our lab have revealed normal reproductive state-dependent changes in the expression of central 5-HT receptors, including a decrease in serotonin 2C receptor (5-HT2C) mRNA in the DR and an increase in serotonin 2A receptor (5-HT2A) mRNA in the medial preoptic area (mPOA) at parturition and early lactation. Interestingly, others have found that systemic activation of 5-HT2C or blockade of 5-HT2A during early lactation disrupts maternal behaviors. Stress during pregnancy also reduces maternal caregiving behaviors and leads to long-term changes in serotonergic signaling, suggesting that stress-induced alterations in this system may contribute to the resulting behavioral disruptions. The aim of the current study is to determine whether disrupted maternal caregiving and emotional behaviors due to pregnancy stress are associated with derailments in the normative expression of central 5-HT receptor expression. Repeated variable stress was employed during pregnancy and caregiving, anxiety-like, and depression-like behaviors were observed after parturition. RT-qPCR and western blotting was used to analyze 5-HT2C in the DR and 5-HT2A in the mPOA of stressed and unstressed dams. We predict that pregnancy stress will prevent or even reverse the normal peripartum changes in DR 5-HT2C and mPOA 5-HT2A receptor mRNA, which will be associated with reduced caregiving and increased anxiety. This work could reveal that disruptions in the normative expression of serotonin receptors in the DR and mPOA across reproduction may contribute to the stress-induced maladaptions in maternal caregiving and socioemotional responses often displayed during postpartum depression and anxiety.

Disclosures: E.M. Vitale: None. **J.S. Lonstein:** None.

Poster

591. Behavioral Neuroendocrinology: Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 591.09/VV12

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant K99 HD085188

Title: Molecular and functional profiling of neural populations involved in parental behavior

Authors: V. M. SEDWICK, I. CARTA, *A. E. AUTRY
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Abstract: Parenting behavior is obligatory in many species and is particularly critical in mammals that rely on nursing for nutrition during early development. In laboratory mice, both fathers and mothers show behaviors associated with parental care such as nest building, pup retrieval, pup grooming, and crouching over pups. Virgin males and females, however, do not show robust parental behaviors and typically show neglect or even attack behavior toward pups. This behavioral change suggests that there are alterations in neural mechanisms that underlie social behaviors toward infants. In our lab, we aim to uncover the molecular and functional changes that lead to these opposite behavioral responses toward infants relative to the physiological status of the adult. In the present study, we profile candidate neural populations involved in both positive and negative regulation of parental behavior. In addition, we manipulate these neurons to determine how they impact parental behavior in various physiological conditions.

Disclosures: V.M. Sedwick: None. **I. Carta:** None. **A.E. Autry:** None.

Poster

591. Behavioral Neuroendocrinology: Parental Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 591.10/VV13

Topic: F.02. Behavioral Neuroendocrinology

Support: NARSAD Young Investigator Grant from the Brain & Behavior Foundation award to Dr. Mariana Pereira

Title: RNAseq analysis of the mPOA in early postpartum Wistar-Kyoto rats reveals candidate genes associated with parenting deficits characteristic of postpartum depression

Authors: *S. B. WINOKUR, M. PEREIRA
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Abstract: Postpartum depression (PPD) is a serious psychiatric disorder affecting 10-15% of mothers and their children worldwide. PPD causes deleterious effects on the mother's health and parenting abilities, posing a risk for the mother-infant relationship and infant developmental outcomes. While previous research suggests underlying cognitive, motivational, and affective dysfunctions contribute to the underlying pathology of PPD, little is understood about the underlying neurobiological mechanisms of PPD symptomatology that specifically impact parenting abilities. The present study used the Wistar-Kyoto (WKY) genetic rat model of depression in comparison to control Sprague-Dawley (SD) and Wistar (WIS) rats to identify candidate genes in the medial preoptic area (mPOA) that underlie the cognitive and parenting deficits that are representative of PPD symptomatology. These regions were selected because the mPOA plays a major role in orchestrating cognitive and motivational aspects of maternal behavior. Gestational stress (a known risk factor for PPD) was additionally used across an experimental group containing all strains, with the aim of exacerbating a PPD-like phenotype in SD and WIS mothers and creating a robust high severity PPD phenotype in WKYs. Comparisons across strains and stress vs. non-stress groups allows us to pinpoint possible candidate genes from multiple symptomatic angles. RNAseq transcriptomic analysis was used to identify differentially expressed genes (DEGs) in the mPOA and mPFC of all subjects. RNAseq revealed over 500 genes in the postpartum mPOA that had at least a 2-fold-change in expression between WKY and SD mothers, including oxytocin, mitogen-activated protein kinase, the monoamine signaling genes vesicular monoamine transporter 2 (Vmat2) and tyrosine hydroxylase (Th), and the immediate early genes Fos, FosB, and Egr1. Gene Ontology (GO) and enrichment analyses on DEGs identified signaling pathways associated with cellular metabolic and biological processes, including chromatin organization, synaptic plasticity, and response to stress and hormones. Together, these results provide insight into pathology of key symptoms of postpartum depression.

Disclosures: S.B. Winokur: None. **M. Pereira:** None.

Poster

591. Behavioral Neuroendocrinology: Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 591.11/VV14

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R01DC008343

Title: Auditory cortex dependent reprogramming of an innate maternal behavior

Authors: A. G. DUNLAP[1], *R. C. LIU[2]
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Abstract: Recent research into the neurobiology of rodent maternal care has revealed how its active motor components, like pup approach and retrieval, can be unlocked by the activation of subcortical circuits involving the medial preoptic area. When animals are in the appropriate internal state (e.g. mediated endogenously by reproductive hormones), natural pup cues trigger the activation of these circuits, releasing these maternal motor programs. However, the degree of flexibility in how these innate behaviors can be triggered by sensory stimuli is not well understood. Presumably, the ability to release these behaviors in response to novel cues that are predictive of infants would be highly adaptive for mothers, but how such new pup-associated stimuli would come to elicit maternal motor programs is unclear. Here, we use a pup reinforcer-based training paradigm to investigate whether female mice can incorporate a new sensory cue to guide their maternal motor program for pup retrieval. Trials begin with mice at its nest in the base of the T-maze. Two speakers are placed at either ends of the T's two arms, and a synthetic, amplitude-modulated noise stimulus is played from one of the two speakers to indicate which arm the mouse should enter to be given a pup. Pups, which are initially held outside the T-maze, are placed at the end of the arm associated with playback only after the mouse enters this "Correct" arm. The mouse then retrieves the pup back to the nest. If the mouse initially chooses the incorrect arm, they are allowed to subsequently investigate the other arm and receive the pup then without any punishments. We found that in this paradigm, mice have an innate strategy on the first day where on ~80% of the trials they return to the last arm where they previously received a pup on the last trial. In contrast, they correctly enter the playback arm on only ~40% of the trials on the first day, suggesting they do not use the novel sound to guide retrieval. Over the course of 8 days of training, the initial strategy shifts, wherein performance based on choosing the playback arm increases to ~60% of trials, while a strategy based on the last rewarded location drops to ~60%. After reversible, bilateral inactivation of auditory cortex with muscimol, we find that mice return to their initial strategy, and performance based on sound playback drops significantly ($n=3$, $p<0.05$). These results indicate that auditory cortex is required for using a learned, pup-associated sound to overcome an innate strategy for guiding pup approach. Our paradigm allows investigating how subcortically mediated maternal behaviors can be "reprogrammed" to utilize cortical representations of pup-associated cues.

Disclosures: A.G. Dunlap: None. **R.C. Liu:** None.

Poster

591. Behavioral Neuroendocrinology: Parental Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: The Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science and the Ministry of Education Culture, Sports, Science and Technology (24119005) to MM
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Title: Effects of breastfeeding and oxytocin on the perception and recognition of facial expressions in mothers

Authors: *M. MATSUNAGA[1], T. KIKUSUI[2], R. OOYAMA[2], M. MYOWA[1]
[1]Kyoto Univ., Kyoto, Japan; [2]Azabu Univ., Kanagawa, Japan

Abstract: Breastfeeding, which is a highly conserved element of maternal care in mammals, is the first turn-taking interaction between a mother and infant. One mechanism for lactation, *oxytocin,* is necessary for milk let down. Past research has shown that exogenous oxytocin can enhance several social behaviors, including emotion perception and recognition. In this study, we investigated two topics: (i) the relationship between tonic/phasic breastfeeding behaviors and changes in endogenous oxytocin levels and (ii) whether tonic/phasic breastfeeding experience and oxytocin levels could enhance emotional processing (i.e., attention regulation in detecting emotional signals, recognition of emotion category, and/or arousal rating for facial expressions). Thirty-eight primiparous mothers (mean age = 33.29, range 28–43 years; SD = 4.85 years) participated in this study. The mean age of their infant was 6.6 months (19 boys and 19 girls; range 4–8 months; SD = 1.4 months). All participants were Japanese. Two kinds of emotional tasks were conducted, specifically “the emotion perception task” and “the emotion recognition task.” Each mother completed the tasks twice, before and after the manipulation phase. Half of the mothers breastfed (i.e., breastfeed manipulation) and the other half only held their infant (i.e., hold manipulation). In order to measure oxytocin level change, saliva was collected at two time-points: before manipulation (i.e., baseline oxytocin) and after manipulation. Additionally, accumulated breastfeeding experience was assessed by a questionnaire. Data showed the following three findings: (1) no relationship between tonic breastfeeding and baseline oxytocin level; (2) phasic oxytocin levels did not differ between the two manipulation types (i.e.,

breastfeed or hold). Half of mothers increased their oxytocin level just after manipulation, but the other half decreased their oxytocin level after manipulation. (3) Both tonic and phasic breastfeeding affected the detection of emotion signals (i.e., attention regulation). As a tonic effect, levels of accumulated breastfeeding and higher baseline oxytocin levels moderated the sensitivity to detect negative emotions. As a phasic effect, breastfeeding enhanced the sensitivity to detect both positive and negative emotional signals. These findings suggest that nurturing experience may change mother's emotional perception, with the possible mechanism of this effect being endogenous oxytocin function.

Disclosures: M. Matsunaga: None. **T. Kikusui:** None. **R. Ooyama:** None. **M. Myowa:** None.

Poster

591. Behavioral Neuroendocrinology: Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 591.13/VV16

Topic: F.02. Behavioral Neuroendocrinology

Title: Altered maternal investment in vasopressin 1b receptor knockout mice

Authors: *E. A. AULINO[1], S. K. WITCHEY[1], A. R. FREEMAN[2], H. K. CALDWELL[1]
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Abstract: Many mammal species, including mice, alter their reproductive investment based on social and environmental cues. One well-studied example of reduced investment is the Bruce effect, whereby there is the spontaneous termination of pregnancy if a female is exposed to unfamiliar male early in pregnancy. Similarly, there is evidence that exposure to a novel male late in pregnancy can result in diminished maternal care, representing a shift in maternal investment. Interestingly, female vasopressin 1b receptor knockout (Avpr1b –/–) mice do not exhibit the Bruce effect; i.e. no pregnancy block. Thus, we hypothesized that Avpr1b –/– females would not have impaired maternal investment if exposed to a novel male in late pregnancy. To test this hypothesis Avpr1b wild type (+/+) and Avpr1b –/– mice were mated and exposed to familiar or unfamiliar males beginning at approximately gestational day 14. Once born, litter weights were taken every three days, as a proxy of maternal investment, until pups were weaned at postnatal day 21. Weaned mice continued to be weighed until two months of age. Contrary to our hypothesis, preliminary data suggest that Avpr1b –/– dams exposed to novel males have reduced maternal investment, as their pups have low weights compared to controls. This genotypic difference in pup weights is only observed during nursing and is reconciled by weaning age, which is consistent with the literature on maternal investment.

Disclosures: E.A. Aulino: None. **S.K. Witchey:** None. **A.R. Freeman:** None. **H.K. Caldwell:** None.

Poster

591. Behavioral Neuroendocrinology: Parental Behavior

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Program #/Poster #: 591.14/VV17

Topic: F.02. Behavioral Neuroendocrinology

Support: JSPS KAKENHI 16K08531

Title: The effect of maternal experiences on spatiallearning and hippocampal neural plasticity

Authors: *M. FURUTA, A. FUKUSHIMA, T. AKEMA, T. FUNABASHI
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Abstract: It is well documented that, during postpartum period, many behaviors are affected. For example, an increased spatial learning ability is reported, but mechanisms for this change is obscure. Maternal experiences consist of a series of events including pregnancy, delivery, lactation and rearing. It remained to be determined which events can change the neural system of mother rats to govern their behavior. To determine the role of each maternal event in working memory tests, we studied in four experimental groups: nulliparity control, nulliparity that reared foster pups, primiparity without rearing experience and primiparity with all maternal events of pregnancy, delivery, lactation and rearing. In the present study, postpartum rats that resumed estrus cycle after weaning and nulliparity controls at the same age were subjected. Behavior was analyzed by Y-maze test for spatial learning assessments. In electrophysiological experiments, we induced long-term potentiation (LTP) by stimulating presynaptic fiber at 10 Hz (1.5 min duration) paired with postsynaptic depolarization in CA3 hippocampus using whole-cell patch-clamp. Further, the expression level of GluR1 and 2 was analyzed in the hippocampus by Western blot. As the results, LTP was induced, rectification index (RI: response at -60 mV/that at 40 mV) was high, AMPA/NMDA ratio was high, GluR2 expression was increased and spatial learning score was high in the primiparity group compared to those in the nulliparous group. The primiparity without rearing experience group also showed a significantly higher score of spatial learning than the nulliparity control group. The results suggest that maternal experience of delivery coupled with rearing most drastically change hippocampal function leading to improved spatial learning.

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Poster

591. Behavioral Neuroendocrinology: Parental Behavior

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 591.15/VV18

Topic: G.03. Emotion

Support: UCI School of Medicine

Title: Impaired aspects of maternal behavior in virgin mice lacking melanin concentrating hormone receptors

Authors: *L. ALHASSEN, A. ALACHKAR, K. ONOUYE, H. SHAHARUDDIN, A. LO, O. CIVELLI
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Abstract: Melanin concentrating hormone (MCH) has been implicated in the onset of maternal care in the postpartum period. However, it is not known whether MCH regulates components of maternal behavior that are independent of the hormonal and neurochemical changes associated with pregnancy and parturition. Here, we examined the effects of the deletion of MCH receptors (MCHR1) on maternal-related behaviors in virgin female mice. Our results reveal that deletion of MCHR1 impairs maternal behavior that is induced spontaneously upon pups' exposure. The MCHR1 KO mice spent a longer time in retrieving the pups compared with the WT mice. In support of this finding, we found that, in the three-chamber social test, MCHR1KO female mice spent similar time interacting with the pups-containing cup and the empty cup, indicating a lack of interest in interacting with pups. MCHR1 KO females were unable to detect pups' chemosensory signals and displayed impaired general olfactory discrimination. The number of Fos-positive neurons in brain regions known to be critical for maternal behavior was lower in MCHR1KO mice than WT mice. Our findings indicate that the lack of MCHR1 causes defects in maternal behavior in non-sensitized virgin mice, and that modulation of the olfactory signaling and reward system might be the mechanisms, through which MCH regulate maternal behavior in virgin mice.

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Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 592.01/VV19

Topic: F.02. Behavioral Neuroendocrinology

Support: Lundbeck Foundation
Hvidovre Hospital
Rigshospitalet

Title: Neuroticism predicts the impact of serotonin challenges on fear processing in subgenual anterior cingulate cortex

Authors: *O. B. PAULSON[1], B. HORNBOLL[2,3], J. MACOVEANU[2], A. NEJAD[2,4], J. B. ROWE[5], R. ELLIOTT[6], G. M. KNUDSEN[7,3], H. R. SIEBNER[2,3]
[1]Rigshospitalet & Univ. of Copenhagen, Copenhagen, Denmark; [2]Danish Res. Ctr. for Magnetic Resonance, Hvidovre Hosp., Hvidovre, Denmark; [3]Clin. Med., Univ. of Copenhagen, Copenhagen, Denmark; [4]Child and Adolescent Mental Hlth. Ctr., Capital Region Psychiatry, Copenhagen, Denmark; [5]Cambridge Univ. Dept. Clin. Neurosciences, Cambridge, United Kingdom; [6]Div. of Neurosci. and Exptl. Psychology, Univ. of Manchester, Manchester, United Kingdom; [7]Neurobio. Res. Unit, Righospitalet, Copenhagen, Denmark

Abstract: Background: The personality trait neuroticism is associated with increased vulnerability to anxiety and mood disorders, conditions linked with abnormal serotonin neurotransmission and emotional processing. The interaction between neuroticism and serotonin during emotional processing is however not understood. Here we investigate how individual neuroticism scores influence the neural response to negative emotional faces and their sensitivity to serotonergic tone. Methods: Twenty healthy participants performed an emotional face task under functional MRI on three occasions: increased serotonin tone following infusion of a selective serotonin reuptake inhibitor (SSRI), decreased serotonin tone following acute tryptophan depletion (ATD) protocol, and no serotonin challenge (control). During the task, participants performed a gender-discrimination task of neutral, fearful or angry facial expressions. Results: Individual variations in neuroticism scores were associated with the neural response of subgenual anterior cingulate cortex to fearful facial expressions. The association was however opposite under the two serotoninergic challenges. The fear-related response in this region and individual neuroticism scores correlated negatively during the citalopram challenge and positively during the ATD. Conclusions: Neuroticism scales with the relative impact of serotonin challenges on fear processing in subgenual anterior cingulate cortex. This finding may represent a neural mechanism for the variable therapeutic effect of SSRI treatment observed in clinical populations.

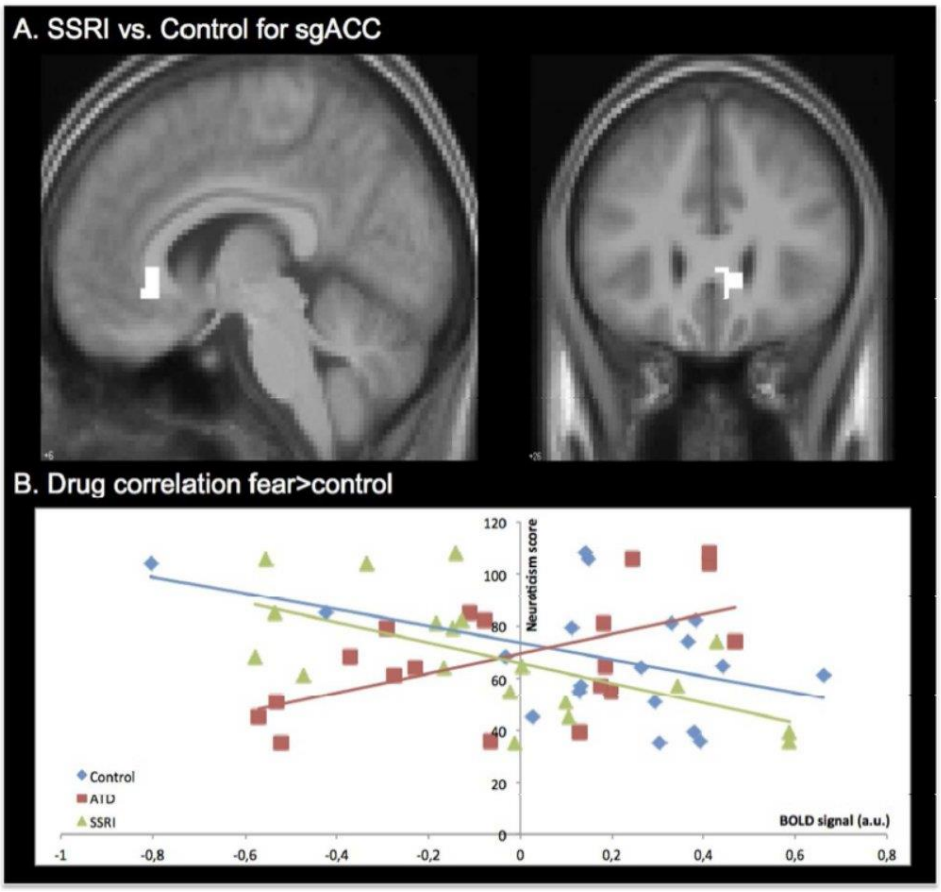

Figure 2: (A) Statistical parametric map (SPM) showing changes in activation for fearful face expressions relative to neutral faces during the SSRI challenge compared to baseline (control) in the subgenual cortex (sgACC). The SPM indicate changes in BOLD signal and are thresholded at p<0.001 (uncorrected). (B) Shows the correlation between the individual neuroticism scores and the BOLD response in the STG for SSRI, control and ATD challenges.

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Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

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Topic: F.02. Behavioral Neuroendocrinology

Support: The National Natural Science Foundation of China 31471022
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Title: Identify the whole brain inputs to different cell types in the LDT

Authors: *W. XIAOMENG, H. YANG, S. HAO, H. WANG
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Abstract: The laterodorsal tegmentum (LDT) has been recently recognized as a key brain structure involved in distinct behaviors including arousal, reward and innate fear when researchers using optogenetics to strictly manipulate different cell types in LDT. Identification of the cell type's specific inputs is the basis to clearly understand the distinct circuit's functions. Although previous studies have shown the whole brain inputs to the LDT, specific afferents of different cell types in the LDT are still unknown. In this study, using a modification of the rabies virus, we were able to apply a monosynaptic retrograde tracing technique to the whole brain to examine the cell type specific upstream nuclei of the LDT. Overall, the LDT receives very strong midbrain afferents and moderate hindbrain and hypothalamus innervations but exhibited weak connections to both of the cortical areas and the thalamus. Although different cell populations received qualitatively similar inputs, dominated by the afferents from the periaqueductal gray area (PAG), interstitial nucleus (In) and the LDT itself, significant differences were observed in that pavalbemin-positive (PV^+) GABAergic cells were preferentially projected by the local LDT neurons. Additionally, for different subtypes of GABAergic cells, a considerable number of nuclei, including those of the later habenular (LHb), central amygdaloid nucelus (Ce), lateral hypothalamus (LH) and zona incerta (Zi), made greater inputs to somatostatin-positive (SOM^+) cells than to PV^+ cells. Our study reveals a diverse input to the LDT on a system wide level.

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Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

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Title: Nucleus accumbens cell-type specific control of operant aggression reward and relapse in hybrid transgenic mice

Authors: *S. A. GOLDEN, M. JIN, C. HEINS, M. MICHAELIDES, Y. SHAHAM
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Abstract: Background: We recently reported that adult male outbred CD-1 mice will lever-press for the opportunity to attack younger male inbred C57 mice. These mice also relapsed to aggression seeking during abstinence. Here we studied the role of nucleus accumbens (NAc) D1- and D2-expressing medium spiny neurons (MSNs) in aggression self-administration and relapse. **Methods**: We first validated a transgenic hybrid breeding strategy, crossing male C57-based inbred D1-Cre and D2-Cre transgenic mice with female outbred CD-1 mice, and tested the hybrid male mice for aggression self-administration or relapse to aggression seeking in an extinction test on abstinence day 1. Next, we validated the use of sub-threshold clozapine as a ligand for DREADD activation by determining the effect of different clozapine doses on aggression self-administration. Finally, we tested the effect of sub-threshold clozapine (0.1 mg/kg) on aggression self-administration and relapse in D1- and D2-Cre hybrids, injected with either DIO-h4MDi or DIO-mCherry in NAc. **Results**: D1-Cre and D2-Cre transgenic mice showed robust aggression self-administration and relapse to aggression seeking. Additionally, systemic clozapine injections decreased aggression self-administration and relapse in D1- but not D2-Cre mice injected with DIO-h4MDi in NAc; clozapine had no effect in D1- or D2-Cre transgenic mice injected with DiO-mCherry. **Conclusions**: Our results indicate that NAc D1- but not D2- MSNs are critical for both aggression self-administration and relapse to aggression seeking. Our study also suggests that hybrid F1 crosses between outbred CD-1 mice and inbred C57-based Cre lines can be used to study mechanisms of operant aggression reward and relapse. This work was supported by NIDA/NIH.

Disclosures: S.A. Golden: None. **M. Jin:** None. **C. Heins:** None. **M. Michaelides:** None. **Y. Shaham:** None.

Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 592.04/VV22

Topic: F.02. Behavioral Neuroendocrinology

Support: KAKENHI 17H04766
KAKENHI 15K12773
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NIH 2R01MH090264-06
NHI 5R01MH104559-02

Title: Individual difference of aggression and interleukin 1 beta in the dorsal raphe nucleus

Authors: *A. TAKAHASHI[1,2,3], H. ALEYASIN[2], M. A. STAVARACHE[4], M. E. FLANIGAN[2], A. BRANCATO[2], C. MENARD[2], M. L. PFAU[2], G. E. HODES[2], S. OGAWA[1], B. S. MCEWEN[3], S. J. RUSSO[2]

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Abstract: It has been shown that the level of interleukin 1 beta (IL-1β) in the periphery or cerebrospinal fluid correlates with aggressive traits in humans. Here, we aimed to study the functional role of IL-1β in mediating individual differences in aggression using a resident intruder mouse model. Like humans, outbred CD-1 mice show individual differences in aggressive behavior with two third of mice exhibiting a spectrum of aggressive behavior (termed Aggressors: AGG) and one third of mice showing no aggressive behavior (termed non-aggressors; NON). We first measured peripheral cytokines, and observed a phasic increase in IL-1β in the blood following an intruder encounter in both AGG and NON; however, there was no difference between groups. By contrast, we found significantly higher levels of central IL-1β in the dorsal raphe nucleus (DRN) in NON compared to AGG. To examine the role of IL-1β in the DRN, we injected an IL-1 receptor antagonist into the DRN. Our result showed that intra-DRN microinjection of IL-1 receptor antagonist increased aggressive behavior of male mice. Furthermore, knockdown of the IL-1 receptor (IL-1R1) in the DRN by injecting IL-1R1 shRNA expressing AAV caused an increase in aggressive behavior. Currently, we are examining the possible involvement of 5-HT neuron activity modulated by IL-1β on aggressive behavior. Together, our results indicate that IL-1R mediated pathways in the DRN have an inhibitory role on aggressive behavior.

Disclosures: A. Takahashi: None. **H. Aleyasin:** None. **M.A. Stavarache:** None. **M.E. Flanigan:** None. **A. Brancato:** None. **C. Menard:** None. **M.L. Pfau:** None. **G.E. Hodes:** None. **S. Ogawa:** None. **B.S. McEwen:** None. **S.J. Russo:** None.

Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 592.05/WW1

Topic: D.05. Olfaction and Taste

Title: Aggressive behavior in forebrain-specific Ctgf knockout mice

Authors: *H.-C. CHANG[1], L.-J. LEE[1,2,3]
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Abstract: In the nervous system, connective tissue growth factor (CTGF) is expressed in some distinct areas, such as the olfactory bulb, endopiriform nucleus and cortical subplate, but its

function is still largely unknown. We have generated forebrain-specific *Ctgf* knockout (Fb*Ctgf* KO) mice to investigate the role of CTGF in the brain. Fb*Ctgf* KO mice, being apparently normal, exhibited typical activity in the open field test; while displayed a sign of anxiety in the elevated-plus maze test. In the present study, we further explored the social behaviors of these mice. In the resident-intruder test, Fb*Ctgf* KO male mice showed greater aggressive behaviors than control mice, such as shorter latency of the first attack, greater attack counts and longer attack period. We then selected two aggression-related brain regions, medial amygdala and orbitofrontal cortex, for further exploration. In the medial amygdala, greater number of c-fos-positive cells was observed in Fb*Ctgf* KO mice compared with controls after the intruder test, while the basal levels of c-fos expression and numbers of NeuN-positive cells were similar between two genotypes. In the orbitofrontal cortex, the basal levels of c-fos- and NeuN-positive cells were comparable between control and mutant mice. After intruder stimulus, the numbers of c-fos-positive cells in both genotypes were increased to similar levels. The greater aggressive behaviors in Fb*Ctgf* KO mice might be resulted from by greater neural activity in the medial amygdala which is connected to CTGF-expressing olfactory structures. Our findings suggest a role of neuron-derived CTGF in modulating aggression-related behavior.

Disclosures: H. Chang: None. **L. Lee:** None.

Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 592.06/WW2

Topic: F.02. Behavioral Neuroendocrinology

Support: SNSF NCCR-Synapsy P28 LSYM (to R.S.)

Title: Role of medial amygdala - hypothalamic GABA projections in aggression control

Authors: A. BALEISYTE, R. SCHNEGGENBURGER, *O. KOCHUBEY
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Abstract: Aggression is a behaviour that is misregulated in many psychiatric conditions such as autism, schizophrenia and bipolar disorder, but our knowledge about the neuronal circuits and synaptic pathways that control and modulate aggression is still incomplete. The medial amygdala (MeA) integrates socially relevant information from olfactory and pheromonal sensory inputs, and regulates social behavior. The majority of neurons in the posterodorsal MeA are GABAergic inhibitory neurons (~70%). However, the role of genetically defined sub-populations of these MeA-GABA neurons in social behavior has not been addressed; a recent study showed that stimulating MeA-GABA neurons led to increased aggression (Hong et al., 2014). Using a Cre-driver mouse line for Somatostatin (SOM-Cre), we found that ~20% of MeA-GABA neurons

express SOM. Surprisingly in light of the previous study, optogenetic activation of SOM+ MeA-GABA neurons *in-vivo* interrupted ongoing inter-male aggression and prevented the onset of new attacks in the resident-intruder test. To test whether the SOM+ sub-population might act antagonistically to all MeA-GABA neurons, we next stimulated all MeA-GABA neurons under the VGAT promoter, which similarly led to a suppression of ongoing aggression. To investigate the mechanism of how MeA-GABA neurons suppress aggression, we investigated the long-range output projections of these neurons. Using virus-mediated anterograde tracing, we found putative output synapses of VGAT+ and SOM+ neurons to similar degrees in the bed nucleus of stria terminalis and in some hypothalamic areas, whereas in the ventro-medial hypothalamus (VMH), a region previously identified in aggression control (Lin et al., 2011), SOM+ fibers from the MeA were sparser than VGAT+ fibers. In *ex-vivo* optogenetic mapping of synaptic connections, GABAergic inhibitory output connectivity in the VMH was sparser for SOM+ than for VGAT+ GABAergic fibers originating from the MeA. Using a second viral vector that drives the expression of eGFP under the VGluT2 promoter in the VMH, we found that MeA-GABA neurons directly inhibit glutamatergic neurons in the VMH. Thus, long-range inhibition from MeA-GABA neurons onto excitatory neurons in the VMH is likely one mechanism of how MeA-GABA neurons suppress inter-male territorial aggression.

Disclosures: A. Baleisyte: None. **R. Schneggenburger:** None. **O. Kochubey:** None.

Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 592.07/WW3

Topic: F.02. Behavioral Neuroendocrinology

Support: ERC Grant 261286
Swedish Research Council 2014-3906

Title: Maternal aggression depends on a prolactin- and oxytocin-sensitive switch in ventral premammillary nucleus (PMv) network behaviour

Authors: *A. S. STAGKOURAKIS[1], P. WILLIAMS[1], G. SPIGOLON[1], S. KHANAL[2], K. ZIEGLER[3], L. HEIKKINEN[1], G. FISONE[1], C. BROBERGER[1]
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Abstract: Aggression is common among male laboratory rodents, but rarely observed in virgin females. Curiously, the female behavioural repertoire changes dramatically at the end of pregnancy and the birth of pups, when the dam can express intense aggression against both male and female intruders. The central adaptation underlying this phenotypic switch is poorly

understood. Dopamine-transporter-expressing neurons in the hypothalamic ventral premammillary nucleus ("PMv^{DAT} cells") have recently been shown to drive intermale aggression, and PMv^{DAT} excitability correlates to aggression phenotype in males. We therefore hypothesized that functional plasticity in PMv^{DAT} neurons may underlie the emergence of aggression in dams. Aggressive encounters in the resident-intruder test induced activation of PMv^{DAT} neurons in dams, in the form of c-fos immunoreactivity. Optogenetic activation of PMv^{DAT} neurons in lactating dams triggered attack in the resident-intruder test; optogenetic inhibition of these cells reduced attack duration. Maternal care was assessed in the pup retrieval test, where optogenetic PMv^{DAT} activation resulted in impaired performance, by a reduction of successful retrieval episodes and an increased latency to the first pup retrieval. Genetic (caspase3-medited) ablation of PMv^{DAT} neurons in dams resulted in reduced maternal aggression, but had no impact on maternal care. We hypothesized that the increased circulating levels of the hormone, prolactin, typical of the lactating dam, might be involved in these behavioural changes. Notably, PMv^{DAT} cells in lactating dams exhibited elevated pSTAT5 immunoreactivity compared to virgins, indicative of increased prolactin receptor-mediated activation. In whole-cell patch clamp slice recordings, application of prolactin (500 nM) to PMv^{DAT} neurons resulted in depolarization and action potential discharge. Similar effects were observed with the hormone oxytocin, which also peaks during nursing. This augmented excitability was associated with a post-synaptic potentiation of T-type Ca^{2+} currents and an increase in synaptic input. Lastly, we tested if maternal behaviours can be induced in virgin females by local application of maternal hormones into the PMv (via bilateral osmotic minipumps over 30 days). Both prolactin and oxytocin inhibited pup retrieval, but did not induce aggression, similar to the effects of optogenetic PMv^{DAT} stimulation in virgins. These results reveal that a *post-partum* switch in PMv^{DAT} neuron activity, which can be stimulated by prolactin and oxytocin, is instrumental in driving maternal aggression, and has a concurrent inhibitory effect on maternal care.

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Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 592.08/WW4

Topic: F.02. Behavioral Neuroendocrinology

Support: NSF 1355163

Title: Intranasal vasopressin increases aggression during courtship in a dose-dependent manner in California mice (peromyscus californicus)

Authors: *E. KASTAR, C. D. GUOYNES, A. P. AUGER, C. MARLER
Univ. of Wisconsin-Madison, Madison, WI

Abstract: Intranasal vasopressin increases aggression during courtship in a dose-dependent manner in California mice (*Peromyscus californicus*)
Erin Kastar, Caleigh Guoynes, Anthony Auger, Catherine Marler
Vasopressin (AVP) is a neuropeptide that modulates complex social behaviors such as pair bonding, aggression, and stress. Previous studies have shown different behavioral effects of AVP, possibly because some studies use different doses of AVP or because of cross talk with oxytocin (OT) receptors. There is a gap in our understanding of how AVP in males and females influences aggression in courtship. Here we use a dose response study to examine how different doses of AVP influence social interactions between males and females. In addition, we use intranasal AVP to mimic administration and doses used in human research with potential applications for therapy of social disorders. We assess AVP's effects on aggressive, affiliative, and stress-related behaviors during an encounter with a novel member of the opposite sex in the strictly monogamous and territorial California mouse (*Peromyscus californicus*). Previous studies in our lab demonstrated that OT
decreased aggression during courtship in males, suggesting that neuropeptides may play an important role in social receptivity toward a potential mate. Using a similar paradigm as in the OT study, males and females were administered one of four treatments using intranasal infusions: saline control, 0.05 IU of AVP (low), 0.5 IU of AVP (medium), or 5.0 IU of AVP (high) (N=8 per group). We found that low and medium doses of AVP significantly increased aggression and significantly decreased stress-related behaviors during courtship in both males and females. The high dose in both males and females did not differ from saline. There was no effect on affiliation in either males or females at any dose. We will also examine which brain areas are activated by the different doses of AVP by CREB and p-CREB in social and decision-making brain areas such as the central and medial amygdala, ventral hippocampus, and medial prefrontal cortex using Western blots. We hypothesize that while the saline and high dose of AVP are behaviorally similar, they will be activating different brain regions. Overall, these studies will illuminate our understanding regarding the complex interactions of neuropeptide systems on both affiliative and aggressive behavior.

Disclosures: C.D. Guoynes: None. **A.P. Auger:** None. **C. Marler:** None.

Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 592.09/WW5

Topic: F.02. Behavioral Neuroendocrinology

Support: R21MH110678

Title: Prefrontal cortex exerts top down control over the ventromedial hypothalamus to regulate aggressive behaviors

Authors: *N. MACK[1], B. XING[2], W.-J. GAO[3]
[1]Dept. of Neurobio. and Anat., [2]Neurobio. & Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [3]Dept Neurobiol & Anat., Drexel Univ. Col. Med., Philadelphia, PA

Abstract: Many neuropsychiatric disorders such as schizophrenia, depression, and drug addiction involve abnormal social behavior, increased aggression, and hypofunction of the medial prefrontal cortex (mPFC). As an executive center for top-down control of social behavior in humans, how the mPFC regulates aggressive and social behaviors via subcortical structures remains elusive. The ventromedial hypothalamus (VMH) is well characterized with the ventrolateral sub region (VHMvl) being of a critical aggression locus in rodents. In this study, we explore whether and how the mPFC controls aggression in both male and female mice. Using Cre-dependent retrograde tracer and excitatory DREADD viral vector, we identified several projection cells to the VMHvl from lamina 5/6 of the mPFC. We found that c-fos expression in the mPFC overlaps with the mPFC-VHMvl projection cells following a male-male attack (n=4). With a chemogenetic approach to functionally activate the pathway, our preliminary data suggest that activating the mPFC to VHMvl pathway increases the number of attacks in males (n=6) but not female (n=4) mice subjected to the resident-intruder task without affecting locomotion, indicating a sex difference. Future directions include increasing sample size, confirming the results with optogenetic stimulation, characterizing electrophysiological properties of the projections cells in the mPFC, and identifying the mechanism of sex difference.

Disclosures: N. Mack: None. **B. Xing:** None. **W. Gao:** None.

Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 592.10/WW6

Topic: F.02. Behavioral Neuroendocrinology

Support: MH1R01MH093362
T32 NS 007453

Title: Novel mechanistic insights on panic disorder

Authors: *A. WINTER[1], R. AHLBRAND[1], R. SAH[2]
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Abstract: Panic disorder (PD) is a prevalent anxiety disorder. Existing treatments are limited and many patients are unresponsive, highlighting the need for improved mechanistic understanding and identification of therapeutic targets. PD's hallmark is recurrent panic attacks; episodes of extreme fear and physical discomfort. Often, panic attacks occur spontaneously without external threats, suggesting internal homeostatic disturbances as triggers. Accordingly, PD patients are sensitive to pH imbalances, as panic attacks are triggered by acidosis-evoking agents such as carbon dioxide (CO_2). *We hypothesized that specialized homeostatic sensory regions in the brain would play a relevant role*. The subfornical organ is a homeostatic regulatory area devoid of a blood brain barrier. Previous work from our lab reported unique CO_2 detecting mechanisms within the SFO. Utilizing, site-directed brain infusions and transgenic mice, coupled with FOS mapping as methods, the current project a) investigated whether acidosis within the SFO was sufficient to evoke fear b) identified angiotensin receptors as a novel target in CO_2-evoked fear c) mapped contributory circuits. We report that infusion of acidified aCSF into the SFO evoked significant freezing in mice. Importantly, mice lacking CO_2-chemosensing receptors within the SFO did not elicit this response. Infusion of angiotensin receptor antagonist, losartan, near the SFO resulted in significant attenuation of CO_2-evoked fear. FOS mapping identified forebrain (IL, BNST) and midbrain (PAG) fear regulatory areas as significant contributors to the behavioral effects. Ongoing studies are utilizing chemogenetic and optogenetic manipulations to modulate identified circuits. Our studies identify novel mechanisms and circuits (Fig 1) by which homeostatic pH imbalance may trigger extreme fear associated with panic attacks, leading to therapeutic interventions for PD.

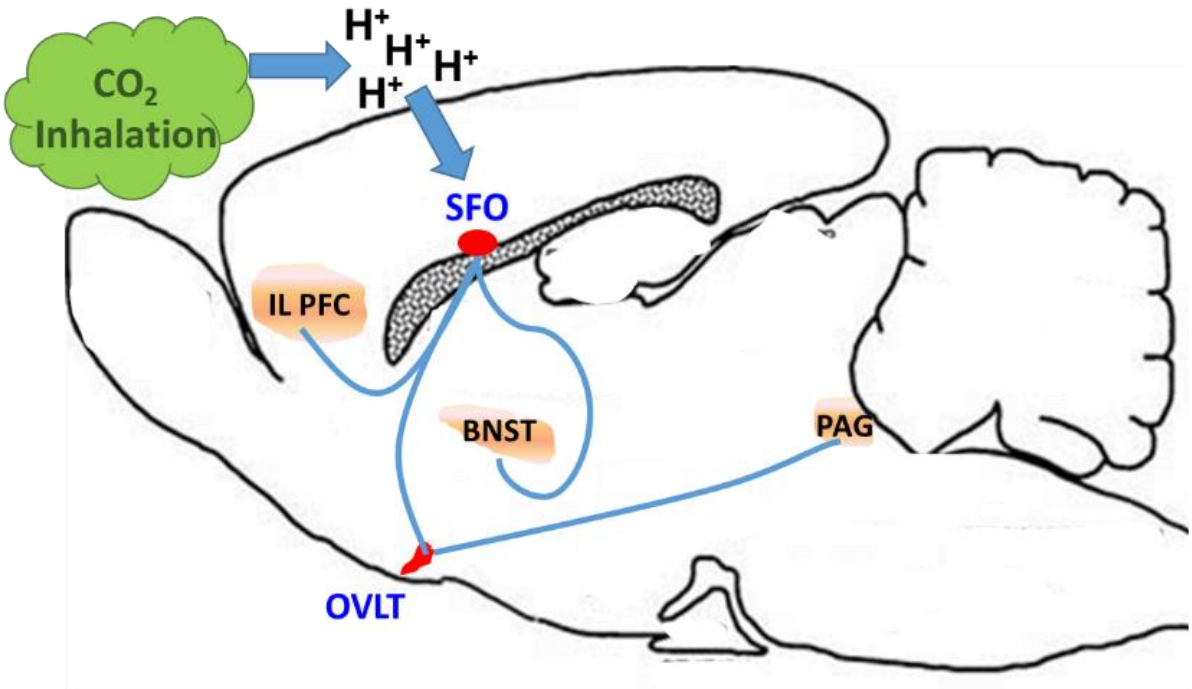

Figure 1: Circuits recruited in homeostatic pH threat-evoked fear: SFO detects acidosis and engages downstream fear regulatory regions. (IL, infralimbic cortex; BNST, bed nucleus of the stria terminalis; PAG, periaqueductal gray)

Disclosures: A. Winter: None. **R. Ahlbrand:** None. **R. Sah:** None.

Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 592.11/WW7

Topic: F.02. Behavioral Neuroendocrinology

Title: Effects of prenatal exposure to endocrine disruptors and chronic exposure to estradiol in adulthood on stress-related behavior in female rats

Authors: *A. KAIMAL[1], J. M. HOOVERSMITH[2], A. D. CHERRY[1], N. M. MARTIN[1], H. E. BUECHTER[1], P. V. HOLMES[2], S. M. MOHANKUMAR[1], P. S. MOHANKUMAR[1]
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Abstract: Developmental exposure to low doses of endocrine disrupting chemicals (EDCs), including the widely prevalent bisphenol A (BPA) and di(2-ethylhexyl) phthalate (DEHP), has been shown to produce a predisposition to behavioral disorders in later life. Additionally, changes in estrogen are associated with the development of mood and anxiety disorders in women. We examined the combined effects of prenatal exposure to these chemicals followed by chronic estradiol exposure in adulthood on behavior in female rats. Dams were orally administered saline (control; 10 µL/kg), BPA (5 µg/kg), DEHP (7.5 mg/kg) or a combination of BPA+DEHP during days 6 through 21 of pregnancy. Adult female offspring were sham-implanted or implanted with pellets that release 17β-estradiol (E2) for 90 days (20 ng/day; Innovative Research America). Rats were tested in the shock probe defensive burying (SPDB) paradigm. E2 treatment reduced rearing and probe exploration and increased immobility time in control animals, compared to control shams. This suggests that E2 exposure may induce a phenotypic shift to a passive threat response. Prenatal exposure to BPA had no effect on any of the parameters in the SPDB compared to control shams. E2 treatment did not change the response in BPA-exposed animals. This suggests that prenatal BPA exposure may reduce the sensitivity to E2 in adulthood. Prenatal exposure to DEHP reduced rearing and probe exploration compared to control shams. E2 treatment reduced these behaviors even further. However, the DEHP-treated groups resembled the control groups in the amount of time spent burying and immobile. Prenatal exposure to BPA+DEHP did not affect any of the measures except for reducing rearing frequency after E2 treatment. In conclusion, 1) E2 exposure induces differential threat responses in control offspring 2) offspring prenatally exposed to BPA appear to be desensitized to these estrogenic effects, 3) prenatal exposure to DEHP produces a partially aberrant response, and 4) BPA+DEHP offspring do not show robust differences in any behavioral measure, but exhibit similarities to BPA-exposed offspring in the SPDB.

Disclosures: A. Kaimal: None. **J.M. Hooversmith:** None. **A.D. Cherry:** None. **N.M. Martin:** None. **H.E. Buechter:** None. **P.V. Holmes:** None. **S.M. Mohankumar:** None. **P.S. Mohankumar:** None.

Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 592.12/WW8

Topic: F.02. Behavioral Neuroendocrinology

Support: EY019049
EY022478

Title: Tactile stimulation facilitates flight responses via ventral zona incerta

Authors: *X. WANG[1], X. CHOU[2], L. I. ZHANG[2], H. TAO[3]
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Abstract: Zona incerta (ZI), a functionally mysterious subthalamic nucleus, receives extensive inputs from cortical regions and projects to both the motor thalamus and midbrain nuclei. Previous studies have suggested involvements of ZI in several physiological functions including defense regulation. The present study provides evidence that the parvalbumin positive neurons in the ventral division of ZI (ZIv) directly modulate sound-induced flight response via its projection to the motor part of the posterior complex of the thalamus (POm). Moreover, we found that tactile stimulation could enhance sound-induced flight response, likely through the activation of the projection from the somatosensory cortex to ZIv. Together these findings suggest that somatosensory information may modulate sound-induced flight through recruitments of ZIv.

Disclosures: X. Wang: None. **X. Chou:** None. **L.I. Zhang:** None. **H. Tao:** None.

Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

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Topic: F.02. Behavioral Neuroendocrinology

Support: ERC Advance Grant 341139
FNS Advance Mobility P300PA_177897

Title: Subcortical circuits balancing attack and defense during predatory hunting

Authors: *D. ROSSIER, V. LA FRANCA, C. GROSS
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Abstract: Adaptation of innate behaviors to their potential risk and benefit is essential for the survival of most animal species. In predatory hunting, prey defensive responses can represent a significant danger to the predator. To engage in hunting, the predator's motivation to hunt needs to overcome prey fearing. The circuits involved in balancing fear and attack during predatory hunting remain largely unexplored. When mice are given access for the first time to cockroaches, a natural murine prey, they show intense defensive behaviors that are gradually replaced, with experience, by predatory behaviors. Using mice as a model for predatory hunting, we found that optogenetic stimulation of a subset of GABAergic neurons in the *lateral hypothalamus* (LHA) specifically drives hunting behaviors. Stimulation of these neurons' axonal projections to the *periaqueductal grey* (PAG) decreased defensive behaviors to prey and drastically reduced the latency to hunt in naïve animals. We compared neuronal activity in the LHA of naïve and

hunting-trained animals using *in-vivo* calcium imaging. Consistent with our optogenetic experiments, we observed that the activity of a defined subset of LHA GABAergic neurons correlates with hunting episodes. Together, our results suggest that this neuronal subpopulation encoding the motivation to hunt inhibits defensive behaviors through projections to the PAG to counter-balance fear associated with attacking prey.

Disclosures: D. Rossier: None. **V. La Franca:** None. **C. Gross:** None.

Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

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Program #/Poster #: 592.14/WW10

Topic: F.02. Behavioral Neuroendocrinology

Support: ERC Advanced Grant 341139
Croucher Scholarship for Doctoral Studies

Title: Functional mapping of periaqueductal gray cell types identifies innate defense circuits

Authors: *E. F. TSANG, I. PRANKERD, C. T. GROSS
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Abstract: The midbrain periaqueductal gray (PAG) is commonly recognised as the exit relay for the coordination and execution of a wide range of instinctive behaviours, such as defense, reproduction and predation. In line with its functional diversity, are the range of inputs it receives from higher cortical and subcortical areas as well as ascending spinal pathways, and the various neurotransmitter and neuromodulatory mechanisms active in its different subregions. However, the lack of a comprehensive cell-type classification of the PAG hinders systematic investigations into the intricacies of its many behavioural roles. Here, we applied high-throughput single neuronal nucleus RNA sequencing to profile transcriptomes of adult mouse PAG neurons. In addition, using a combination of optogenetically manipulations and a carefully designed defense test battery, we identified key excitatory and inhibitiory PAG neuronal populations important for triggering and modulating defensive behaviour. Our work aims to provide a comprehensive transcriptional perspective of the PAG, and demonstrates a framework towards a systematic dissection of cell-type specific functions of complex brain regions.

Disclosures: E.F. Tsang: None. **I. Prankerd:** None. **C.T. Gross:** None.

Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 592.15/WW11

Topic: F.02. Behavioral Neuroendocrinology

Support: ERC advanced grant

Title: Social challenge: Understanding dynamics of VMH in defence and aggression

Authors: *P. KRZYWKOWSKI[1], C. T. GROSS[2]
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Abstract: Exposure to a threatening conspecific can elicit either avoidance or aggression depending on the context and history of the encounter. Neural activity in the ventrolateral division of the ventromedial hypothalamus (VMHvl) has been shown to be both necessary and sufficient for defensive and aggressive behavioural responses to conspecific threats. In male mice, inhibition of neural activity in VMHvl reduces avoidance behavior following exposure to an aggressive male as well as attack behavior following exposure to a subordinate male. However, it remains unknown whether the same or different neurons in VMHvl are responsible for defense and aggression toward social threat and how experience affects these responses. We performed serial cFos labelling experiments and found that defence and aggression recruited partially overlapping populations in VMHvl, consistent with a recent study. Using in vivo calcium endoscopy of VMHvl neuron activity during social defense and aggression we found that strong calcium responses were elicited upon exposure to the social stimulus and these were further modulated as the animal exhibited defensive or aggressive behaviours. Notably, specific calcium responses were identified that were entrained to defensive behaviours and these could be tracked across multiple exposures to the social stimulus. In parallel, we performed optogenetic stimulation of cell-types in VMHvl and identified two populations that elicited defensive responses to a social threat. These results demonstrate that the VMHvl encodes and controls both specific and overlapping features of defensive and aggressive behavioural responses to social threat.

Disclosures: P. Krzywkowski: None. **C.T. Gross:** None.

Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 592.16/WW12

Topic: F.02. Behavioral Neuroendocrinology

Support: PEACE project from Japan International Cooperation Agency

Title: Localization of aggression-induced c-Fos immunoreactivity in the brain of male layer chicks

Authors: *S.-I. KAWAKAMI
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Abstract: Although aggression is a normal display of an instinct in almost all animals, excessive aggression leads to serious economic problems in poultry industries. We have recently reported that a kind of behavioral test, named resident-intruder test, is an effective tool for monitoring aggressive behavior of male layer chicks (J. Poult. Sci., 54: 296-302, 2017), the aim of the present study was, therefore, to examine the localization of aggression-induced c-Fos immunoreactivity in the brain of male layer chicks under the test. From 3 days of age, the chicks were divided into 2 groups, isolated-housing (residents: 1 chick per cage, as aggressors) and grouped-housing (intruders: 3 or 4 chicks per cage, as opponents), and a group-raised chick (opponent) was transferred to the home cage where a chick of isolated-raising (aggressor) was reared. The frequencies of aggressive behavior (pecking, biting, kicking, threatening, and leaping) and latency of both the aggressor and the opponent were recorded for 10 minutes. After 2 h of the treatments, the aggressor chicks were anesthetized with sodium pentobarbital and then perfused transcardially with phosphate buffered saline followed by 4% paraformaldehyde. The brains were coronally cut using a cryostat to make 20 μm-frozen sections. The sections were incubated with mouse primary antibody against c-Fos, then with biotinylated horse anti-mouse IgG followed by incubation in avidin-biotin-peroxidase complex. Digital images were captured using fluorescence microscope and analyzed with image processing software (ImageJ). The sum of the frequencies of aggressive behavior significantly increased and latency significantly decreased in the aggressors compared to those in the opponents. Aggression-induced c-Fos immunoreactivities were mainly observed in the hypothalamus and limbic system of the chick brain. In the hypothalamus, c-Fos immunoreactivities were localized in the median preoptic nucleus, the nucleus of the hippocampal commissure, the paraventric nucleus rostral and parvicellular part, the arcuate hypothalamic nucleus (ARC), and the ventromedial nucleus of hypothalamus (VMH). c-Fos immunoreactivities were localized sparsely in the VMH and densely in the ARC as compared to the previous reports of rodents. In the limbic system, c-Fos immunoreactivities were localized in the amygdalohippocampal area and mammillary body.

These results suggest that the localization of aggression-induced c-Fos immunoreactivities in chick brain, except in the VMH and ARC, corresponded approximately to the brain area in which the immunoreactivities had been previously reported on rodents.

Disclosures: S. Kawakami: None.

Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 592.17/WW13

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH/NIGMS R35 GM119844-01
Japan Society for the Promotion of Science
The Naito Foundation

Title: The *Drosophila* nervy gene functions in octopaminergic neurons to suppress aggressive behaviors

Authors: *K. ISHII, K. ASAHINA
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Abstract: Aggressive behaviors are generally important for animals to gain competitive advantages over other individuals. On the other hand, excessive aggression is energetically costly and often interferes with other behaviors. To appropriately balance aggressive and non-aggressive behaviors, animals must have evolved sophisticated systems for modulating action choices. Recent studies have focused primarily on the activation of aggression by neuromodulatory systems, but how aggression is suppressed remains poorly understood.
We performed an RNAi-based behavioral screen in *Drosophila* to identify genes that suppress aggressive behaviors. Among >1,400 RNAi strains, we found that pan-neuronal RNAi against the gene *nervy* significantly increased aggression. Temporal RNAi of *nervy* during larval and pupal stages was sufficient to induce an aggressive phenotype in adults, suggesting it plays a role in neuronal wiring during development.
We generated CRISPR/Cas9-mediated knockout mutants of *nervy* (*Δnervy*), and confirmed that *nervy* was indeed necessary to dampen aggressiveness. Interestingly, not only males but also females were more aggressive in *Δnervy* than in the wild-type. The hyper-aggressive phenotype of *Δnervy* flies were rescued by pan-neuronal expression of the full-length *nervy* gene. Genetic rescue was not achieved by expressing a truncated version of Nervy that lacked its conserved NHR2 domain, which was required to form homo-multimers of Nervy proteins. Furthermore, expression of *MTG8* and *MTG16*, human homologs of *nervy*, reduced levels of aggression in *Δnervy* mutants.

To determine the neurons in which *nervy* acts, we performed a screen using selected GAL4 lines combined with a *nervy* RNAi line. Expressing the *nervy* RNAi construct via Tdc2-GAL4, which specifically labels 30-40 octopaminergic neurons in the adult brain, increased aggressiveness. In addition, expressing the full-length *nervy* gene via Tdc2-GAL4 resulted in cell type-specific rescue of the *Δnervy* hyper-aggressive phenotype.
To our knowledge, *nervy* is the first *Drosophila* gene implicated in the quantitative regulation of aggressive behavior in both sexes. The *nervy* gene therefore provides an entry point for uncovering the conserved, sex-invariant genetic mechanisms that control aggression.

Disclosures: K. Ishii: None. **K. Asahina:** None.

Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 592.18/WW14

Topic: F.02. Behavioral Neuroendocrinology

Support: IN224417
IA207316

Title: Characterization of the olfactory response in the establishment of hierarchical order in crayfish

Authors: I. HERNÁNDEZ-PRIOR, Z. PEÑA-LEAL, F. U. ROSAS-VALDÉZ, Y. PITALUGA-JAVIER, K. MENDOZA-ÁNGELES, *J. HERNANDEZ-FALCON
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Abstract: Sensory systems provide an organism with information about both the inner and the external environments. This information is then processed in higher centers of the brain in order to produce an adequate response. Agonistic behavior in crayfish seems to depend mainly on olfactory information carried out by a putative compound released in urine streams that each contender release during social interactions. This putative compound has not been identified but the lack of olfactory information, by the blockade of olfactory receptors, induces long-lasting fights between contenders even after the hierarchical order was previously established. The main goal of this work was to identify brain and chemoreceptor, electrical responses during the establishment of dominant-submissive hierarchical order in adult crayfish.We used adult male crayfish implanted, under cold anesthesia, with a brain electrode, on the olfactory lobe, and simultaneously a peripheral electrode that recorded chemoreceptor activity from the ipsilateral antennule. In triads of crayfish we videotaped agonistic interactions until a hierarchical order was established. Then we applied urine from the dominant animal on the recorded antennule of a submissive one and recorded the central and peripheral response as well as the behavior of the

animal. We also recorded the response of the dominant animal when urine of a submissive one was applied on the antennule. Urine from the dominant animal induced a bimodal electrical response, an immediate reduction in the electrical activity of both the brain and the antennule, followed by bursts of discharges with variable duration. Behaviorally, the stimulated animal increased locomotor activity and showed even an escape response. Urine from a submissive crayfish applied to a dominant one, was accompanied by less intense electrophysiological response without behavioral changes. These results point out to a putative compound released in urine during agonistic encounters that allow the recognition of conspecifics and, probably, the hierarchical status.

Disclosures: I. Hernández-Prior: None. **Z. Peña-Leal:** None. **F.U. Rosas-Valdéz:** None. **Y. Pitaluga-Javier:** None. **K. Mendoza-Ángeles:** None. **J. Hernandez-Falcon:** None.

Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 592.19/XX1

Topic: F.02. Behavioral Neuroendocrinology

Title: Analytical and behavioral characterization of procambarus clarkii after chronic serotonin exposure

Authors: *N. K. MCLAUGHLIN, I. J. HARRIS, K. STUMPO
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Abstract: Crayfish often serve as a common model of agonistic behavior due to their innate aggression towards each other. As recorded, both sexes show similar characteristics of behavior when fighting each other. Crayfish aggression can be classified based on its interaction allowing for simplistic observation and recording of data. Additionally, crayfish offer an elementary and well understood nervous system that is prime for chemical manipulation. With these factors in mind, crayfish are a suitable choice for experimentation in this research.In this experiment, crayfish(n=16) were immersed to 424 ng/L of serotonin(5-HT) that was directly injected into their water. Additional crayfish (n=16) were also subjected to standard conditions (tap water) with no drug injection. These conditions were maintained everyday for two weeks, with daily water changes. Ideally, this concentration represents the amount of serotonin that selective serotonin reuptake inhibitors (SSRI) would provide crayfish in natural locations owing to the rise of antidepressant usage and the subsequent release of these compounds from wastewater treatment plants After the prolonged period of drugging, the crayfish were then fought and their aggression score was recorded. As hypothesized, the preliminary behavioral data shows that the serotonin does have an effect on the crayfish aggression score. Additionally, this experiment will serve as a baseline comparison to that of SSRIs.Chemical analysis of the crayfish brain will

provide additional understanding into the neuromodulatory system of crayfish. Due to the chronic effect of 5-HT and Sertraline it is unknown what kinds of effects are present. Further work will be done to quantify and localize serotonin in the brain of crayfish. Analytical chemistry techniques such as High Performance Liquid Chromatography with electrochemical detector (HPLC-ECD) and Mass Spectrometry Time-of-Flight (TOF-MS) will be used to achieve such goals

Disclosures: N.K. McLaughlin: None. **I.J. Harris:** None. **K. Stumpo:** A. Employment/Salary (full or part-time):; University of Scranton.

Poster

592. Behavioral Neuroendocrinology: Modulation of Defensive and Aggressive Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 592.20/XX2

Topic: F.02. Behavioral Neuroendocrinology

Title: Effects of testosterone manipulation and personality traits on arginine vasopressin immunoreactivity in brook trout (salvelinus fontinalis)

Authors: *E. G. PLOPPERT[1], C. GOWAN[2], M. BARDI[1]
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Abstract: In social animals, decision-making regulatory systems encode the salience of social stimuli that ultimately determine the adaptive response of the animal (O'Connell & Hofmann, 2011). The preoptic area (POA) is a central node in these neural systems, which regulates both sexual male behavior and aggressive behavior in most vertebrate species (Hull & Dominguez, 2007). The activity of sex steroids such as testosterone (T), peptides hormones such as oxytocin (OT) and arginine vasopressin (AVP), and various monoamines (such as dopamine) may play a crucial role in the decision-making during agonistic encounters. Little is known, however, of how individual personality influences these neural processes. Personality is defined as consistent behavior of an individual within a given context. Previous studies in our laboratory have identified a significant correlation between personality and aggressive behavior in social context (White & Gowan, 2012). In the first phase of the current study, 20 brook trout were exposed to the open field test (OFT) for three consecutive days. Subsequently, pairs of subjects were size matched to eliminate the effect of fish size on behavior and introduced to a novel arena where social interactions were recorded over a 2.5 hour period. Blood was drawn from each fish at the end of the period and blood was analyzed for levels of testosterone and cortisol. A significant negative relationship was found between cortisol and testosterone. Additionally, although no relationship was found between hormone levels and OFT scores or the amount of aggression displayed during agonistic interactions, using multidimensional scaling we were able to correctly predict the decision-making of individuals on the basis of their personality and T levels.

Following this phase, exogenous T was administered to the fish. A significant spike in peripheral T levels was observed after the injection, which was related to a significant increase in aggressive behavior during the OFT. In the final phase of the study, brains will be exposed to immunocytochemistry to determine the effects of exogenous T and personality on c-Fos-, glucocorticoid receptor-, and AVP-immunoreactivity in the POA, as a way to assess the role of personality in encoding information from the social environment and in shaping adaptive behavior.

Disclosures: E.G. Ploppert: None. **C. Gowan:** None. **M. Bardi:** None.

Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.01/XX3

Topic: F.02. Behavioral Neuroendocrinology

Support: Thomas Hartman Center for Parkinson's Research
IMSD MERGE Fellowship to MC

Title: Expanding the use of recognition memory paradigms with the what-when-where episodic-like memory task to assess effects of gonadal hormones on cognitive function in adult rats

Authors: *M. CONNER[1], B. J. ANDERSON[2], M. F. KRITZER[1]
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Abstract: Gonadal hormones influence complex cognitive and mnemonic function of the prefrontal cortex and hippocampus. However, obtaining clear behavioral evidence for this influence in rodents can be difficult due to hormone effects on non-cognitive endpoints such as sensitivity to stress and reward. These actions can impact behavioral performance in positively or negatively motivated tasks in ways that are independent of any effects on cognition. However, a recent review highlights the benefits of object recognition memory tasks for isolating neuroendocrine influence on cognitive behaviors; as described, novel object recognition and location paradigms are low stress and rely on rodents' natural preference for novelty, making them especially well suited for parsing estrogen and androgen effects on higher order processes (Luine, Behav. Brain Res., 285, 2015). Here, we used a different sort of object recognition paradigm-- the What-Where-When (WWWhen) episodic-like memory task, to further explore hormone effects on cognitive and mnemonic operations. The WWWhen task involves sequentially exposing rats to two distinct arrays of four identical objects and a third test exposure where two objects from the second array are presented in original positions and two objects from the first array are presented with one in a new position. Using adult male, female and gonadectomized (GDX) male rats with and without estradiol (GDX-E) or testosterone propionate

(GDX-TP) we analyzed rats' differential object explorations during the test trial. As expected, gonadally intact rats preferentially explored objects from the first over the second array (What, When) and among first array objects further preferred those that were displaced (Where). GDX rats, however, showed no object preferences based on spatial location or recency. Finally, data from hormone-replaced GDX rats showed that both E and TP rescued recognition of novelty based on 'What' and 'When' while TP alone preserved recognition of novelty based on 'Where'. Together these data support the utility of recognition memory tasks for studying hormone effects on cognition and link estrogens and androgens to processes of object recognition memory and spatial cognition, respectively, for the first time in the context of an episodic-like memory task. We further suggest that the co-occurrence of discrete estrogen- and androgen-sensitive behaviors in the WWWhen task may confer unique advantages to this paradigm over other recognition memory tasks that should be exploited in future studies using rodents to examine hormone effects on cognitive function and its dysfunction in preclinical models of neuropsychiatric disease.

Disclosures: M. Conner: None. **B.J. Anderson:** None. **M.F. Kritzer:** None.

Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.02/XX4

Topic: F.02. Behavioral Neuroendocrinology

Title: Structural changes of songs in adult zebra finch by chronical application of thyroid hormone

Authors: *M. IWANAGA[1], K. HOTTA[2], K. OKA[3]
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Abstract: It has been well known that song learning in zebra finches (*Taeniopygia guttata*) has similar mechanism to speech acquisition in humans (Doupe and Kuhl, 1999). Male juveniles first learn songs from tutors, adult birds nearby, and around 30 days post-hatching, they start imitating songs (Immelmann, 1969; Tchernichovski *et al*., 2001). They correct their vocal outputs by feedback mechanism; they compare their own songs with tutor songs (Immelmann, 1969; Konishi, 1965; Tchernichovski *et al*., 2001). Once they acquire their own songs, they will never sing any other songs (crystallisation). In zebra finch, sensitive period for song learning lasts 90 to 100 days after hatching (Lipkind *et al*., 2017). On the other hand, chicken (*Gallus gallus domesticus*) is a typical example to show sensitive period, and new-born chicks recognise objects moving in front of them as their parents and start following them (imprinting). We tested whether application of thyroid hormone (T_3) can change the structure of bird song because it is

known that application of thyroid hormone triggers reopening of learning period after its closure around 2 to 3 days post-hatching (Yamaguchi *et al*., 2016). In this study, we measured T_3 effects on song structures in adult zebra finches. We continuously injected T_3 into adult male zebra finches, and followed song changes by recording their songs with software, Audacity. Songs were then analysed by Sound Analysis Pro (SAP) 2011. Data were processed with Principal Component Analysis (PCA) using R. We found that daily injection of 50 ng hormone has affected mean syllable structures. However, birds showed significant decrease in the number of motifs. Now we are investigating an optimal injection condition to acquire continuous recording from the same bird with its song structure changing.

Disclosures: M. Iwanaga: None. **K. Hotta:** None. **K. Oka:** None.

Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.03/XX5

Topic: F.02. Behavioral Neuroendocrinology

Support: This research was supported by NASA Grants NNX13AB73G and NNX16AE06G

Title: Role of estrogen in mediating the effects of exposure to space radiation on cognitive performance in female rats

Authors: *B. M. RABIN[1], M. G. MILLER[2], E. M. HAWKINS[1], A. N. LARSEN[1], C. SPADAFORA[1], N. N. ZOLNEROWICH[1], L. DELL'ACQUA[1], W. PAGDEN[1], V. ROTTMAN[1], B. SHUKITT-HALE[2]
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Abstract: During exploratory class missions to other planets, astronauts will be exposed to types and doses of radiation not experienced in low earth orbit, where the space shuttle and International Space Station operate. While it is likely that both male and female astronauts will comprise the crew on these missions, the majority of the research using animal models has utilized only male subjects.
The subjects were ovariectomized (OVX) and intact female rats, approximately two months of age at the time of irradiation. The OVX subjects were given implants of estradiol or vehicle. Following head-only exposure to ^{12}C or ^{4}He particles at Brookhaven National Lab, the rats were shipped to UMBC and tested on novel object recognition and operant responding on an ascending fixed-ratio schedule. All behavioral tests were conducted at least 2 months after irradiation, such that the hormonal status of all OVX rats was the same.
Results indicate that the effects of exposure to these particles on the cognitive performance of

female rats varied as a function of the specific particle, hormonal status at the time of irradiation, and the specific task. Exposure to either ^{12}C or ^{4}He did not cause a reliable disruption of performance on the novel object task. Estradiol provided partial protection against the deleterious effects of exposure on novel object performance. The presence of estradiol at the time of exposure did not prevent the disruption of operant performance by exposure to either particle. In the intact rats, exposure to both particles increased the responsiveness of the subject to the changes in reinforcement contingencies.
These results suggest that the effects of exposure to space radiation on cognitive performance among female subjects are more variable than they are for males. While hormonal status at the time of irradiation may be a factor influencing the effects of exposure on cognitive performance, the mediating effect of female hormones are not uniform, and vary as a function of the specific particle and task.

Disclosures: B.M. Rabin: None. **M.G. Miller:** None. **E.M. Hawkins:** None. **A.N. Larsen:** None. **C. Spadafora:** None. **N.N. Zolnerowich:** None. **L. Dell'Acqua:** None. **W. Pagden:** None. **V. Rottman:** None. **B. Shukitt-Hale:** None.

Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

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Program #/Poster #: 593.04/XX6

Topic: F.02. Behavioral Neuroendocrinology

Support: Grants: CONACYT 1134291 (OGF)
PROMEP/103,5/09/1294.
Beca CONACYT 277841 (CEAP) Doctorado en Ciencias Biológicas, UATx.

Title: Rapid ejaculator rats have more copulatory analgesia that intermediate and sluggish ejaculator rats

Authors: *C. E. AGUILAR PÉREZ, SR[1], R. A. LUCIO[2], J. C. MORALES-MEDINA[3], P. GÓMORA ARRATI[4], O. GONZÁLEZ FLORES[4]
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Abstract: Analgesia is the absence of the pain perception without loss of consciousness, and can be produced by different factors, such as copulatory behavior. In male rats, analgesia is produced during the execution of mounts, intromissions and immediately after ejaculation. However, as humans, male rats have different copulatory phenotypes depending on the duration of the

ejaculation latency, i.e., rapid (200-400 sec), intermediate (700-900 sec) and sluggish (1200-1400 sec). Additionally, the rapid males have a shorter inter-intromission interval (iii) compared to intermediate or sluggish males. These data allow us to hypothesize that rapid-ejaculators will present more analgesia than the intermediate/sluggish ejaculators during copulation. To demonstrate this hypothesis, we used adult Wistar male rats with sexual experience after a copulatory training of six tests. Afterwards male rats were identified as rapid (n=22), intermediate (n=7) or sluggish (n=5). In the 7th copulatory test, males were behavioral registered in another laboratory equipped with an analgesia system. Only in the 8th copulatory session, we recorded mount, intromissions and ejaculation latencies, and the number of mounts, intromissions, and it was calculate the iii. We measured the vocalization threshold to tail shock test (VTTS) before, and during the copulatory behavior of rapid, intermediate and sluggish males during two consecutive ejaculatory series including their respective post-ejaculatory intervals. Rapid ejaculators increased their VTTS (44.16% compared to their basal value), also the sluggish ones (4.86% compared to their basal value) during the first ejaculatory series. The same happened during the second series (44.86%, 15.31%, rapid and sluggish, respectively). At the first postejaculatory interval the rapid and intermediate ejaculators increased their VTTS (21.08%, 3.59%, respectively). However, the sluggish males decreases it (-3.62%). In addition, a simple linear regression analysis indicated a stronger association between copulatory induced analgesia and rapid ejaculation. These results suggest that the nervous pathway of analgesia and that of the sensory genital information are close related.

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Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.05/XX7

Topic: F.02. Behavioral Neuroendocrinology

Support: CONACyT KCS 554743

Title: Physiological markers and personality differences in first and lastborn students

Authors: *K. C. SÁNCHEZ[1], V. REYES[2], R. HUDSON[3], A. BAUTISTA[4]
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Abstract: From an evolutionary psychological perspective, family structure has been suggested as a source of individual differences among siblings, particularly effects of birth order on

personality traits. Traditionally, firstborns are considered more conscientious, introverted and reserved than lastborns. However, other studies suggest this to be an artefact due to methodological limitations of classical studies. We reinvestigated the relation between birth order and individual differences in personality, as well as potentially associated differences in three physiological markers: heart rate variability, skin conductance, and skin temperature. We predicted that birth order would be associated with personality traits as traditionally suggested, and with differences in physiological responses in three stressful contexts: a) talking about oneself before a camera, b) solving mathematical problems, c) speaking about a controversial issue before judges. We predicted that firstborns will score higher in consciousness and will be more introverted in front of the camera than lastborn. Additionally, firstborn will increase their hearth rate variability, skin conductance, and skin temperature more than lastborns. We used the Ten Item Personality Inventory, the psychophysiological measuring device NEXUS, and a thermographic camera to register individual differences in personality traits and physiological responses, respectively. Preliminary results showed no significant differences in personality traits; physiological data are being analyzed.

Disclosures: K.C. Sánchez: None. **V. Reyes:** None. **R. Hudson:** None. **A. Bautista:** None.

Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

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Program #/Poster #: 593.06/XX8

Topic: F.02. Behavioral Neuroendocrinology

Support: Japan Science and Technology Agency
Japan Grant-in-Aid for Scientific Research

Title: Hippocampus-synthesized estrogen and androgen modulate dendritic spines and LTP in non-genomic manner

Authors: *S. KAWATO[1], Y. KOMATSUZAKI[2], M. SOMA[3]
[1]Univ. of Tokyo, Tokyo, Japan; [2]Dept. of Physics, Col. of Sci. and Technology, Nihon Universit, Tokyo, Japan; [3]Dept. of Cognitive Neurosci., Teikyo Univ., Tokyo, Japan

Abstract: We have demonstrated (1) hippocampal synthesis of estrogen and androgen, and (2) non-genomic synaptic modulation by these sex-steroids. [Synthesis] We showed expression as well as neuronal/synaptic localization of essential enzymes (mRNA and protein) in the adult male rat hippocampus. Mass-spectrometric analysis demonstrated that exact levels of estradiol (E2), testosterone (T), dihydrotestosterone (DHT) were 8 nM, 18 nM and 7 nM, respectively, which are much higher than their levels in plasma. Castration significantly decreased T and DHT in the hippocampus, indicating that plasma-derived T is efficiently converted to DHT within the

hippocampus. Even after castration to deplete circulating T, the male hippocampal E2 level was not decreased, indicating that E2 is mainly synthesized from hippocampal T. Female hippocampal levels of E2 (0.5-4 nM), and T (1 nM) were less than those of male, but much higher than those in female plasma. [Synaptic Modulation] E2-induced rapid non-genomic modulation (1- 2 h) was demonstrated by analysis of spinogenesis and LTP of adult male rat hippocampal 'acute' slices (steroid-depleted slices). Spine analysis was performed for pyramidal neurons in hippocampal slices. The density of spines and their head diameters were obtained by mathematical and automated software Spiso-3D which identifies spines by calculating geometrical parameters. E2 at 1 nM rapidly increased the density of small-head spines, in CA1 pyramidal neurons. T and DHT at 10 nM increased the density of small-head spines and large-head spines, respectively. Signaling pathways are: synaptic ERalpha or AR→LIMK, MAPK, Src, PKA, PKC →cofilin or cortactin → actin polymerization→ new spines. LTP analysis showed that 1 nM E2 induced full-LTP (E2-LTP) upon weak sub-threshold stimulation, although without E2 the weak sub-threshold stimulation did not induce full-LTP. Kinase inhibitors against MAPK, PKA, PKC blocked E2-LTP. Only 20 min application of letrozole (aromatase inhibitor) suppressed full-LTP upon full teta-burst stimulation, indicating that rapid E2 synthesis is necessary for LTP in hippocampal slices. References: Kawato et al., 2002 Methods in Enzymol, Hojo et al., 2004 PNAS, Mukai et al., 2007 J. Neurochem, Hojo et al., 2009 Endocrinology, Mukai et al. 2011 Cerebral Cortex, Ooishi et al. 2011 Cerebral Cortex, Komatsuzaki et al., 2012 PLoS-ONE, Okamoto et al., 2012, PNAS, Kato et al., 2013, Frontier Neurosci. Hasegawa et al., 2015 Brain Res., Hatanaka et al., 2015 Brain Res., Murakami et al., 2015 Brain Res., Soma et al., 2018 Frontier. Neurosci.

Disclosures: S. Kawato: None. **Y. Komatsuzaki:** None. **M. Soma:** None.

Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.07/XX9

Topic: F.02. Behavioral Neuroendocrinology

Support: GRFT 2018, Science and Technology Ministry of Córdoba.
University Siglo 21

Title: Chronic stress and mental health from a psychobiological approach

Authors: *L. P. MORERA, SR[1], M. TRÓGOLO[2], L. LAPUENTE[3], L. A. MEDRANO[3]
[1]Univ. Siglo 21, Argentina; [2]Univ. Siglo 21, Córdob, Argentina; [3]Univ. Siglo 21, Córdoba, Argentina

Abstract: Historically, stress has been an object of attention and interest for the scientific community due to the important impact this variable has on people's health and functioning (Moretti & Medrano, 2014). Chronic stress is associated with both physical and psychological problems. Specifically, the influence of stress on the immune system (Lorentz, 2006), somatic problems and low antibody response (Phillips, Burns, Carroll, Ring and Drayson, 2005) have been corroborated, among other factors. Scientific evidence also corroborates the role of stress as a predictor of different psychological disorders, such as generalized anxiety (Hoehn-Saric, McLeod, FunderBurk and Kowalski, 2004), panic attacks (Wood, Cano-Vindel, and Salguero, in press), depression (Hammen, 2005; Liu and Allow, 2010) and interpersonal problems. In the present work we applied a comprehensive survey that included clinically validated scales for anxiety (GAD07) and depression (PHQ09) in sample of 100 postgraduate students from the National University of Córdoba, Argentina. We also assessed stress biomarkers, such as Cortisol (C) from saliva. Methods: A survey was delivered in person to each postgraduate student from different Institutions within, National University of Córdoba (UNC). Participants were instructed to collect all samples during a regular day, avoiding stressful situations and intense physical activity, and refraining from eating, drinking, smoking, or brushing teeth in the 15 min prior to saliva collection. Saliva samples were obtained at awakening, 30 and 50 min after awakening by passive drooling into a plastic tube. This collection method was also recommended by previous studies because other collection methods, such as the use of Salivette, which uses cotton, interfere with the salivary immunoassay results for IL-6. HPA axis function was assessed using CAR (Cortisol awakening response) test. It represents a discrete and distinct component of the cortisol circadian cycle, with characteristics unrelated to those of cortisol secretion throughout the rest of the day. In the present study, the CAR was calculated using the area under the curve with respect to the increase, as suggested by Pruessner et al. (Pruessner et al., 2003), and included three sampling points (awakening, 30′ post-awakening, and 60′ post-awakening). IL-6 levels were assessed from the same samples by salivary immunoassay. In this study we found a high prevalence of anxiety and depression in the population of graduate students from UNC, Argentina. Also, a high correlation between anxiety or depression and C levels or IL-6 its observed.

Disclosures: L.P. Morera: None. **M. Trógolo:** None. **L. Lapuente:** None. **L.A. Medrano:** None.

Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.08/XX10

Topic: F.02. Behavioral Neuroendocrinology

Title: Oxytocin decreases impulsive choice in rats

Authors: *M. D. SINGSTOCK, D. TAPP, M. S. MCMURRAY
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Abstract: Gambling disorders are characterized by an increase in risky behavior due to the progressive loss of impulse control, even in the presence of negative financial and social consequences. Despite their prevalence in society and the severity of their consequences, few targeted pharmacological therapies exist to combat them, and current treatments are not widely effective. Alternative approaches to treatment need to be considered, which rely on new pharmacological targets. One such target may be the oxytocin system. Oxytocin (OT) is a hormone implicated in social behavior, reward preference, motivation, and modulation of dopaminergic neurons in the mesocorticolimbic system. These relationships suggests that OT may play a role in decision-making and impulsivity, although this relationship has yet to be investigated. The purpose of this study was to determine if OT administration influences impulsive decision-making in rats. We predicted that increased OT receptor activation would significantly decrease impulsive decisions. To assess its effect on impulsivity, animals were tested daily on probability and delay discounting tasks. In the probability discounting task, animals chose between a small-certain reward or a large-risky reward, which paid off at declining probabilities across days of testing. In the delayed discounting task, animals chose between a small-immediate or a large reward delivered with increasing delay across days. Reward sensitivity was also analyzed using the intracranial self-stimulation rate frequency (RF) curve-shift method. Animals received OT or vehicle immediately prior to testing (0.1ug OT, 10ug OT, or vehicle intracerebroventricular (ICV); or 6mg/kg or vehicle intraperitoneal). We found that the oxytocin (regardless of administration route) dose-dependently decreased impulsivity in both tasks. ICV infusions also dose-dependently decreased reward sensitivity in RF curve-shift. These results suggest that oxytocin could be an effective treatment for gambling disorders and other impulse control disorders.

Disclosures: M.D. Singstock: None. **D. Tapp:** None. **M.S. McMurray:** None.

Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.09/XX11

Topic: F.02. Behavioral Neuroendocrinology

Support: NSERC 400212

Title: Effects of dorsal hippocampal inhibition of actin polymerization or protein synthesis on rapid estrogen-facilitated social recognition, dendritic spines, and Arc protein expression in ovariectomized female mice

Authors: *P. A. SHEPPARD[1], H. A. ASLING[2], S. E. ARMSTRONG[1], V. M. ELAD[3], A. WALCZYK-MOORADALLY[2], J. LALONDE[2], E. CHOLERIS[1]
[1]Psychology, [2]Dept. of Mol. and Cell. Biol., [3]Dept. of Biomed. Sci., Univ. of Guelph, Guelph, ON, Canada

Abstract: While the long-term, genomic mechanisms of estrogens are established, the mechanisms by which these hormones rapidly affect different forms of cognition are not yet fully understood. Estrogens can rapidly facilitate social recognition - the ability of an animal to recognize another. In ovariectomized female mice, social recognition was facilitated within 40 min of systemic (Phan et al., 2012) or dorsal hippocampal (Phan et al., 2015) administration of 17β-estradiol (E2). Within the same timeframe, E2 increases dendritic spine density in CA1 dorsal hippocampal neurons (Phan et al., 2012; 2015). Mechanisms underlying these effects remain unclear. Estradiol rapidly stimulates changes in actin cytoskeletal dynamics through rapid enhancement of actin polymerization (Briz & Baudry, 2014), increases dendritic spine scaffolding protein PSD-95 expression in an Akt pathway-dependent manner in cultured NG108-15 neurons without a concurrent increase in PSD-95 mRNA (Akama & McEwen, 2003), and increases translation of dendrite-localized mRNA in an ERK-dependent manner in primary cultured hippocampal neurons (Sarkar et al., 2010). Although we previously found dorsal hippocampal activation of both the ERK and Akt pathways is necessary for the rapid facilitation of social recognition by E2 in ovariectomized female mice (Sheppard et al., 2016; 2017), the necessity of protein synthesis or actin polymerization has not yet been examined. Here, we first determined the highest doses of either actin polymerization inhibitor latrunculin A (LAT) or protein synthesis inhibitor anisomycin (ANI) that do not block social recognition when infused into the dorsal hippocampus of ovariectomized female mice 15 min prior to testing. We then determined whether these treatments could prevent the enhancing effects of E2 (as in Phan et al., 2015) in a task where control mice do not typically perform social recognition. The paradigms are completed within 40 minutes of E2 administration, thus enabling investigation of rapid effects of estrogens. Both actin polymerization and protein synthesis were found to be necessary for E2 to rapidly facilitate social recognition. Brains from these animals were collected and either stained with Golgi-Cox solution to evaluate dendritic spine density and length in the dorsal CA1 or were used to determine the effects of treatment on Activity-regulated cytoskeleton-associated protein (Arc) expression, as a potential target of estrogen action. These studies provide a mechanism through which estrogens rapidly facilitate social recognition.

Disclosures: P.A. Sheppard: None. **H.A. Asling:** None. **S.E. Armstrong:** None. **V.M. Elad:** None. **A. Walczyk-Mooradally:** None. **J. Lalonde:** None. **E. Choleris:** None.

Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.10/XX12

Topic: F.02. Behavioral Neuroendocrinology

Support: NSERC
FRQNT
Canada Foundation for Innovation

Title: Genomic and non-genomic effects of progesterone on memory bias in female rats

Authors: *J. M. LACASSE[1], W. G. BRAKE[2], S. PATEL[2], V. PERONACE[2], A. LESTAGE[2], C. GAGNE[2]
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Abstract: When ovariectomized female rats are given low levels of 17B-estradiol (E2) they are biased towards using response memory while navigating a T-maze. Contrarily, if they are given high levels of E2, they are biased towards using spatial memory to navigate. Here we examine the effects of E2 and progesterone (P) on memory bias. Ninety-six female Long-Evans rats were split into 4 groups: low E2 (n =24), high E2 (n=24), high E2+P (4h; n=24), and high E2+P (15min; n=24). All rats were tested in an ambiguous T-maze to differentiate whether they were predominantly using place or response memory while navigating. No statistically significant differences were found between the four hormone conditions. However, when odds ratios were calculated for each hormone condition, some clear differences between conditions emerged. Rats in the low E2, as well as when P was administered 4h and 15min prior were 2.78x more likely to use response memory. Whereas rats in the high E2 alone condition were 2.78x more likely to use place memory.

Disclosures: J.M. Lacasse: None. **W.G. Brake:** None. **S. Patel:** None. **V. Peronace:** None. **A. Lestage:** None. **C. Gagne:** None.

Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.11/XX13

Topic: F.02. Behavioral Neuroendocrinology

Title: Modulation of postmenopause and premenopause on interhemispheric electroencephalographic activity on resting-state in women

Authors: *E. G. GONZÁLEZ-PÉREZ[1], M. S. SOLIS-ORTIZ[2]
[1]Med. Sci., Univ. de Guanajuato, León, Mexico; [2]Med. Sci., Univ. of Guanajuato, Leon, Mexico

Abstract: Postmenopause is characterized by a decrease in estrogen levels, while premenopause in its ovulatory phase, is characterized by elevated estrogen levels. The influence of these

different hormonal states on cortical electrical activity in women is not well known. Therefore, the aim of this study was to explore the modulation of postmenopause and premenopause on electroencephalographic activity (EEG) recorded during resting-state, as a measure of the functional state of the brain. EEG activity was recorded during resting-state in 10 postmenopausal healthy women between 48 and 60 years old and 10 premenopausal healthy women in ovulatory phase between 40 and 45 years old. All subjects were instructed to close their eyes during the recording of the EEG activity. Absolute power was obtained for delta, theta, alpha1, alpha2, beta1 and beta2 and were compared between both hemispheres in frontal, central, parietal and occipital regions. When compared to the premenopausal women in ovulatory phase, postmenopausal women displayed higher EEG power in the left hemisphere for alpha2 ($p = 0.001$) and beta1 ($p = 0.002$) bands in the frontal region. The present findings indicate a higher activation of left frontal region in postmenopausal women, which may suggest a compensatory brain mechanism for behavior and cognition functions.

Disclosures: E.G. González-Pérez: None. **M.S. Solis-Ortiz:** None.

Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.12/XX14

Topic: F.02. Behavioral Neuroendocrinology

Support: Sacred Heart University URI grant

Title: Exposure to bisphenol-a and estrogen during adolescence: Effects on behavior and spine density

Authors: *R. E. BOWMAN, E. MADDEN[1], J. HAGEDORN[1], M. FRANKFURT[2]
[1]Sacred Heart Univ., Fairfield, CT; [2]Sci. Educ., Donald and Barbara Zucker Sch. of Med. at Hofstra/Northwell, Hempstead, NY

Abstract: Bisphenol-A (BPA) is a mixed estrogen/androgen receptor agonist/antagonist known to be an endocrine disrupter that alters a variety of neural, physiological, and behavioral measures. We have previously shown that BPA exposure, in adolescent gonadally intact rats, increases anxiety, impairs spatial memory, and decreases dendritic spine density when measured in adulthood (Bowman et al, 2014, 2015). Additionally, in adult rats estrogen is neuroprotective, enhances memory, and increases dendritic spine density. However, BPA and estrogen effects in adolescence are limited. Thus, this experiment examined the effects of adolescent BPA exposure in juvenile rats under controlled hormone conditions on behavioral and neural alterations in adolescence. Female Sprague-Dawley rats were ovariectomized at postnatal day (PND) 21 and received subcutaneous injections of either BPA (40 μg/kg/bodyweight), 17β-Estradiol (EST, 50

µg/kg/bodyweight), or saline control during adolescence (PND 38-49). Immediately following injections, subjects were tested for anxiety and locomotor activity levels (elevated plus maze and open field), spatial memory (object placement), and non-spatial visual memory (object recognition) (PND 49-58). Animals were sacrificed at PND 59, trunk blood was collected for hormonal assay of estradiol and corticosterone and brains processed for Golgi impregnation. There were no significant group differences on any of the elevated plus maze or open field behavioral measures. Adolescent BPA exposure impaired spatial memory; however, all groups demonstrated intact object recognition performance. While there were no group differences in E2 or CORT levels, there were region specific effects of adolescent hormone treatment on dendritic architecture following behavioral testing. Basal and apical dendritic spine density in pyramidal cells in the CA1region of the hippocampus was increased by EST treatment and granule cells of the dentate gyrus was decreased by BPA. BPA also decreased basal and apical spine density in the mPFC. The current study provides novel data on the effects of adolescent BPA exposure and EST replacement in an adolescent OVX model.

Disclosures: R.E. Bowman: None. **E. Madden:** None. **J. Hagedorn:** None. **M. Frankfurt:** None.

Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.13/YY1

Topic: F.02. Behavioral Neuroendocrinology

Support: NARSAD Young Investigator Award
R01DK106188
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University of Michigan Rackham Merit Fellowship

Title: Cue-triggered food seeking is modulated by ovarian hormones in female rats

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Abstract: In females, naturally occurring elevations in estradiol reduce food intake and estradiol treatment in ovariectomized rats is sufficient to reduce food intake and body weight. However, stimuli paired with food (food cues) also influence feeding behavior, e.g., by increasing food-seeking, and the amount of food consumed. We previously found that male rats predisposed to obesity are more sensitive to the motivational properties of a food cue compared to obesity-resistant rats. However, whether a similar difference exists in females, and how cue-triggered motivation varies across the cycle are unknown. Here, we determined how conditioned approach,

a measure of cue-triggered food-seeking, and instrumental responding for food, differ between female selectively-bred obesity-prone (OP) and obesity-resistant (OR) rats as well as female Sprague Dawley rats. Additionally, we determined how these behaviors change across the estrous cycle. We found that female OP, but not OR rats, show greater conditioned approach during phases of the estrous cycle where estradiol is low (metestrus/diestrus) compared to phases where estradiol is high (proestrus/estrus). Interestingly, in both OP and OR females, motivation for food assessed by progressive ratio responding was lower during proestrus and estrus compared to metestrus and diestrus. Thus, in OP, cue-triggered food-seeking and consumption were similarly modulated by the cycle, whereas consumption but not food-seeking was affected in OR females. Furthermore, when OP and outbred rats were ovariectomized and given repeated cycles of hormone replacement (estradiol and progesterone) a reduction in conditioned approach was observed, demonstrating a role for ovarian hormones in modulating this behavior. This is the first demonstration that ovarian hormones modulate this behavior. In ongoing studies, we are dissecting whether estradiol, progesterone, or a combination of the two are needed to reduce conditioned approach. This study addresses interactions between individual susceptibility to obesity and incentive motivation within females and demonstrates a role of ovarian hormones in regulating food-seeking behaviors.

Disclosures: Y. Alonso Caraballo: None. **C. Ferrario:** None.

Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.14/YY2

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant 5R01GM102525-04

Title: 3beta-OH is a novel sedative/hypnotic with sex specific effects

Authors: *F. M. MANZELLA[1], D. WILKEY[2], D. F. COVEY[3], S. M. TODOROVIC[1]
[1]Univ. of Colorado Anschutz Med. Campus, Aurora, CO; [2]Univ. of Colorado, Boulder, CO; [3]Dept Developmental Biol., Washington Univ. Sch. Med., Saint Louis, MO

Abstract: A new anesthetic agent has not been approved for use in humans in over 20 years. Traditional sedative/hypnotic drugs (SHDs) that target $GABA_A$ and NMDA receptors, such as sevoflurane, propofol, and ketamine are associated with neurotoxicity and neurocognitive impairments in rodents and non-human primates. Moreover, traditional SHDs do little to treat post-surgical pain, a problem contributing to the opioid epidemic. Our group recently developed 3β-OH, a novel 5β-reduced neurosteroid that is a potent analgesic, which inhibits neuronal T-type calcium channels, as a safe alternative to traditional SHDs. However in basic science there

is a disparity in experiments using male and female models despite the importance of understanding the differential effects in men and women in clinic. Thus, it is important to recognize any sex differences of 3β-OH early in drug development in order to understand the best potential clinical use of this novel drug.
A dose response curve for 3β-OH-induced hypnosis was generated using both male and female 3-5 month old C57BL/6J mice. Mice were administered a range of doses between 20 and 120 mg/kg as a single intra-peritoneal (IP) bolus injection. After drug administration, animals were monitored for time to loss of righting reflex (LORR) and time to gain of righting reflex (GORR) as a measure of hypnosis. We found that females were significantly more likely to lose their righting reflex than males at lower doses. Effective dose 50 (ED_{50}) for females to achieve LORR was 48 mg/kg compared to males, which had an ED_{50} of 80 mg/kg. Time to LORR was also two-fold higher for males compared to females. Females also experienced longer duration of hypnosis (2- to 5-fold depending on dose). For example, ED_{50} to achieve maximal hypnotic effect was 72 mg/kg in females and 88 mg/kg in males. At these doses respectively, duration of hypnotic effect was 132 minutes in females, 2.6-fold longer than in males (51 minutes).
To test if this effect was hormonally dependent, we conducted a LORR experiment in male and female juvenile animals at postnatal day (P)21, before the time of sexual maturity. All animals were given a 100 mg/kg bolus of 3β-OH IP, which was the lowest dose in which all animals achieved LORR. Again, animals were monitored for time to LORR and time to GORR. In contrast to the adult animals, we found that at P21, males and females did not differ in achieving LORR, time to LORR, or duration of hypnosis.
Our results indicate that 3β-OH acts as a potent hypnotic in adult mice, with higher potency in females. These effects are dependent on sexual maturity. Further studies are needed to elucidate the mechanisms of sex differences and their potential impact on clinical practice.

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Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.15/YY3

Topic: F.02. Behavioral Neuroendocrinology

Title: Sex-specific effects of dietary isoflavones on peripheral estradiol and brain estrogen α and β receptor expression in the rat

Authors: *C. FINNEY[1], N. W. PROSCHOGO[2], N. M. HOLMES[1], R. F. WESTBROOK[1], K. J. CLEMENS[1]

[1]Psychology, Univ. of New South Wales, Randwick, Australia; [2]Sch. of Chem., Univ. of Sydney, Sydney, Australia

Abstract: Standard laboratory diets contain high levels of soy-based products, and are therefore high in phytoestrogens. Isoflavones, a class of phytoestrogen found in soy, are similar in conformation to estradiol and can act as selective estrogen receptor modulators at estrogen receptors (ER) α and β. However, little is known about the impact of dietary isoflavones on estrogen signaling in the brain and in the periphery. To investigate this, adult male and female Sprague-Dawley rats (n=10/group) were maintained on either a diet marketed as phytoestrogen free (AIN-93G), or one of two standard diets (Gordon's Premium Rat and Mouse Pellets or Specialty Feed's Irradiated Rat and Mouse Diet). We first examined the isoflavone context of these diets, and then assessed their effects on peripheral estradiol levels, as well as the impact of each diet on ERα and β mRNA expression in the hippocampus and prefrontal cortex, two brain regions known to be high in ERs. LC-APCI-MS analysis of isoflavone content in the diets revealed variations in the quantity of isoflavones present (Gordon's > Specialty Feeds > AIN-93G), and in the presence of individual isoflavones in each diet. Critically, the diet had doubly dissociable effects on peripheral estradiol (assessed through LC-APCI-MS of plasma) and brain ER expression (assessed through qRT-PCR) in male and female rats. In male rats, the standard Specialty Feeds diet increased peripheral estradiol levels, but had no effect on either ERα or β expression in the hippocampus or prefrontal cortex. In contrast, in female rats, diet had no effect on peripheral estradiol levels, but significantly affected ER expression in the hippocampus and prefrontal cortex: specifically, the Gordon's standard diet decreased both ERα and β in the prefrontal cortex, and ERβ (but not ERα) in the hippocampus. Combined, these data suggest that standard laboratory diets affect estradiol signaling in a sex specific manner. These results may have implications for rodent models of neurobiological disease that are influenced by estrogen signaling, such as anxiety and affective disorders.

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Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.16/YY4

Topic: F.02. Behavioral Neuroendocrinology

Support: Brain and Behavior Research Foundation
University of California, Santa Barbara

Title: Impact of oral hormonal contraceptives on the CNS: Developing a population neuroimaging study

Authors: *C. TAYLOR, E. G. JACOBS
Psychological & Brain Sci., Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: INTRODUCTION: Oral contraception (OC) is used by more than 100 million women worldwide. OC use suppresses the endogenous production of sex steroid hormones. Few human studies have investigated the impact of chronic ovarian suppression on brain regions modulated by these hormones. In animal models, estradiol and progesterone act on cortical and subcortical brain regions to alter synaptic morphology. A comprehensive study of the macro-structural brain changes that may result from long-term OC use is long overdue. To that end, we launched a large-scale neuroimaging database at UCSB dedicated to women's health research. By leveraging the activity of the UCSB neuroimaging community, we are pooling standard neuroimaging sequences collected on all Brain Imaging Center participants. We then pair participants' neuroimaging data with a detailed clinical-demographic battery. A core aim of the database is to understand OC's influence on regional brain morphology. We began by asking three questions: Does regional gray matter volume (GMV) differ between current OC users relative to never-users; does the duration of OC use impact regional GMV; and does OC use impact sex differences in regional GMV? METHODS: In a discovery dataset based on the first 150 database participants (aged 18-33), high-resolution T1 (MPRAGE) scans were analyzed in conjunction with clinical-demographic data. Participants were excluded for previous parity, psychiatric/mood disorder, substance use, or low-quality MPRAGE, yielding a sample of 48 women: 24 current OC users and 24 never-users, matched on age, age of menarche, education, and BMI. Age-matched men (n=27) were included for comparisons by sex. RESULTS: Whole brain analyses (VBM in SPM12, FDR-corrected) revealed greater cerebellar GMV in OC users compared to never-users. Further, duration of OC use (9-84 mos) was positively correlated with greater cerebellar GMV. Finally, sex differences in regional brain volume observed between men and never-users were obscured in OC users. Results are being tested for replication in additional cohorts.

Disclosures: C. Taylor: None. **E.G. Jacobs:** None.

Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

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Program #/Poster #: 593.17/YY5

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH 5R21HD076430-02

Title: Developmental exposure to the synthetic progestin 17α-hydroxyprogesterone caproate alters decision making in adulthood

Authors: *A. PHILLIPS[1], G. LI[2], C. K. WAGNER[3], R. I. WOOD[4]
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Abstract: 17α-hydroxyprogesterone caproate (17-OHPC) is a synthetic progestin commonly administered to women considered at risk for preterm birth. At this time, there is very little understanding of the potential effects of exposure to 17-OHPC on the developing fetus. Administration of 17-OHPC typically begins during the second trimester and can be found in both maternal and fetal plasma a month after the last injection. The period of administration coincides with critical stages of development of the mesocortical dopaminergic pathway, which originates in the ventral tegmental area (VTA) and projects to the medial prefrontal cortex (mPFC). This pathway is important for mediating complex cognitive functions and higher order executive tasks. In rodent models, progesterone receptors (PR) are expressed in dopaminergic cells of the VTA that project to the mPFC. Neonatal exposure to 17-OHPC in rats induced changes in both the innervation patterns and density of dopaminergic fibers at postnatal day 7 in the mPFC. In addition, 17-OHPC exposure during neonatal life impaired cognitive flexibility in adulthood, a complex cognitive behavior regulated by dopamine activity in the mPFC. Impulsive decision making is observed in children born prematurely and has been shown to be dependent on the mesocortical pathway. In this study, we examined the effects of administration of 17-OHPC during postnatal life (P1-14) on performance in a delay-discounting task, in which animals choose between a larger delayed reward or a small reward delivered immediately. Rats treated with 17-OHPC were significantly more likely to wait for the larger, delayed reward and were significantly less likely to choose the small, immediate reward with increasing delays. Interestingly, 17-OHPC treated rats were significantly more likely to not respond at all (omissions). Together, these results suggest that 17-OHPC may decrease impulsivity, but may also interfere with general decision making abilities.

Disclosures: A. Phillips: None. **G. Li:** None. **C.K. Wagner:** None. **R.I. Wood:** None.

Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.18/YY6

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH/NIMH
NIH/NIAAA

Title: Differential gene expression in response to estradiol withdrawal in perimenopausal depression

Authors: *S. A. RUDZINSKAS[1], J. HOFFMAN[2], D. R. RUBINOW[3], D. GOLDMAN[4], P. J. SCHMIDT[2]
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Abstract: The risk of depression increases 2-3 fold for women during the menopause transition compared to premenopausal women. Additionally, peri/postmenopausal women with even minor depression are at an increased risk of cardiovascular mortality (Wassertheil-Smoller et al, 2004). Clinical studies show both the therapeutic benefits of estradiol (E2) in perimenopausal depression (PMD) (Schmidt et al, 2000, Soares et al, 2001) and the symptom-provoking effects of E2-withdrawal (E2WD) in women with past PMD, which are not experienced by those without past PMD (Schmidt et al, 2015). It has been suggested that a heightened sensitivity to changes in ovarian steroids such as E2 may contribute to the onset of PMD. We hypothesized that the differential affective/behavioral responsivity to E2WD in PMD could be observed on a cellular level. To test this hypothesis, we used lymphoblastoid cell lines (LCLs) derived from women with a past PMD (n=8), or asymptomatic controls (AC) (n=9). These LCLs were examined in 3 different experimental conditions: 1) vehicle-treated media, 2) E2-treated media, or 3) E2-treated media which was changed to vehicle-treated media and collected 24 hours later, to mimic E2WD on a cellular level. Levels of E2 in cell culture media were confirmed using High Performance Liquid Chromatography/Tandem Mass Spectrometry. Cells were collected and examined for changes in gene expression levels using whole-transcriptome RNA sequencing. EDGE-R analysis of differential gene expression revealed significant transcript expression changes between women with PMD and AC in all three treatment conditions, as well as several molecular pathways that appear to be differentially altered in women with PMD. Of particular interest, the gene *CXCL10*, which has been previously linked to cardiovascular disease, is significantly upregulated in the cells of women with past PMD, and had the most extreme increase in transcription in the E2WD treatment condition. In contrast, a gene coding an enzyme *CYP7B1*, which is responsible for the metabolism of the steroids DHEA and pregnenolone, is also significantly upregulated in PMD, but E2-treatment or withdrawal had no further effect on transcript expression. We are currently working to replicate these findings in an independent cohort of AC and PMD cases. These data may suggest that both intrinsic genetic differences as well as differential sensitivity to E2WD could underlie the behavioral symptomology of PMD.

Disclosures: S.A. Rudzinskas: None. **J. Hoffman:** None. **D.R. Rubinow:** None. **D. Goldman:** None. **P.J. Schmidt:** None.

Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.19/YY7

Topic: F.02. Behavioral Neuroendocrinology

Support: CNPq
FAPERJ
INNT
NIH
ISN
HFSP
Alzheimer's Association Canada

Title: The role of brain FNDC5/irisin in synaptic plasticity and memory in mice

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Abstract: Irisin is an exercise-induced myokine released upon cleavage of a precursor protein termed FNDC5, recently reported to be expressed in the hippocampus. Irisin has been reported to regulate peripheral energy metabolism and to trigger neuroprotective mechanisms. However, physiological roles of FNDC5/irisin in the brain remain poorly understood. We used lentiviral vectors harboring two different shRNA constructs targeting FNDC5 to study the role of irisin in synapse plasticity and memory. Here we show that downregulation of brain FNDC5/irisin impairs hippocampal long-term potentiation and object recognition memory, but not contextual fear memory or radial arm water maze, in C57BL/6 mice. These data support the idea that FNDC5/irisin acts in the central nervous system and impacts selective forms of memory expression and hippocampal synaptic plasticity. Thus, boosting FNDC5/irisin pathway and/or using exercise-based therapies may offer new strategies to tackle memory loss in neurodegenerative diseases.

Disclosures: R.A. Lima-Filho: None. **M.V. Lourenco:** None. **O. Arancio:** None. **S.T. Ferreira:** None. **F.G. De Felice:** None.

Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.20/YY8

Topic: F.02. Behavioral Neuroendocrinology

Support: CIHR Operating Grant
CFI Grant

Title: Perinatal sucrose exposure in rats disrupts hormones, brain, and behavior in adulthood

Authors: *D. J. TOBIANSKY[1,2], G. KACHKOVSKI[1], R. T. ENOS[4], K. L. SCHMIDT[5], C. MA[1], J. E. HAMDEN[3], C. JALABERT[3], S. B. FLORESCO[1,2], E. A. MURPHY[4], K. K. SOMA[1,2,3]
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Abstract: The effects of maternal consumption of sucrose (table sugar) on offspring brain, hormones, and behavior are largely unknown. Here, we explored whether human-relevant levels of sucrose consumption by rat dams influenced the offspring phenotype. We examined offspring metabolic function, neurosteroid and dopamine signaling, and motivated behaviors. Dams were fed either a high-sucrose diet (25% of kCal) or an isocaloric, matched, control diet (0% sucrose) during gestation and lactation. After weaning, offspring were placed on standard lab chow until behavioral testing (~4 mo). When given a choice of diets [control diet (10% fat, 0% sucrose) vs. high-sucrose diet (10% fat, 25% sucrose) vs. high-fat diet (40% fat, 0% sucrose)] in a food preference test, sucrose-exposed male (but not female) offspring consumed more of the high-sucrose and high-fat diets. Motivation to obtain a sugar reward was assessed using a progressive ratio schedule of reinforcement. Sucrose-exposed male (but not female) offspring displayed increased motivation to obtain sugar. These behavioral differences might be related to changes in neurosteroid or dopamine signaling in the mesocorticolimbic system. We used ultra-sensitive liquid chromatography-tandem mass spectrometry to examine systemic and local levels of steroids (e.g., testosterone, estradiol, corticosterone), and we used qPCR to examine mRNA levels of steroidogenic enzymes, steroid receptors, and dopamine receptors. Perinatal sucrose exposure affected neurosteroids, steroidogenic enzymes, and dopamine receptors. In males, perinatal sucrose exposure decreased 17β-hydroxysteroid dehydrogenase I (*Hsd17b1*) mRNA in the nucleus accumbens and D1 dopamine receptor (*Drd1*) mRNA in the medial prefrontal cortex. Thus, early-life exposure to human-relevant levels of sucrose disrupts steroid and dopamine signaling in the mesocorticolimbic system. The results suggest that maternal sucrose

consumption has enduring sex-specific effects on offspring brain and behavior, particularly with regard to choosing and obtaining highly palatable foods. These findings might be critical for understanding and addressing the consequences of sugar overconsumption, including the current epidemics of obesity and Type 2 diabetes.

Disclosures: D.J. Tobiansky: None. **G. Kachkovski:** None. **R.T. Enos:** None. **K.L. Schmidt:** None. **C. Ma:** None. **J.E. Hamden:** None. **C. Jalabert:** None. **S.B. Floresco:** None. **E.A. Murphy:** None. **K.K. Soma:** None.

Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.21/YY9

Topic: F.02. Behavioral Neuroendocrinology

Support: Duke University Bass Connections

Title: Gradual loss of ovarian function exacerbates age-dependent cognitive dysfunction in an Alzheimer's disease mouse model

Authors: *S. V. MAURER[1], S. S. VASHISTH[1], C. GRANT[1], E. M. REYNOLDS[1], E. A. GRZESIAK[1], C. A. COLTON[2], E. A. FINCH[2], C. L. WILLIAMS[1]
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Abstract: Over two-thirds of individuals with Alzheimer's Disease (AD) are female, implicating biological sex as a major risk factor for the onset and progression of AD. Biological differences between males and females – most notably the gradual loss of ovarian hormones during the perimenopausal transition – are thought to be critical factors contributing to the greater female risk. To investigate the possibility that menopause exacerbates the development and progression of AD, we are inducing transitional menopause (TM) in female mice with the ovotoxin 4-vinylcyclohexene diepoxide (VCD). We are using the APPSwDI/mNos2$^{-/-}$ AD (CVN-AD) mouse model, which mimics familial AD with the expression of mutated APP and creates a human-like immune environment through lowered NOS2 expression. CVN-AD mice exhibit many of the neuropathological features of human AD, as well as exacerbated AD-like neuropathogenesis and resistance to therapeutic intervention in females. Both wild-type C57BL/6 and mNos2$^{-/-}$ mouse lines serve as controls. To evaluate cognitive function and the impact of TM in female CVN-AD mice, we are using a Barnes Maze task to evaluate spatial learning and memory. As expected based on their neuropathogenic progression, 4-month old CVN-AD mice do not differ from control mice on latency to locate the escape hole, while 14-month old CVN mice are significantly impaired. However, when we analyzed the strategies used to locate the escape hole we found that both young and old CVN-AD mice used a non-spatial,

serial-search strategy, whereas control mice were more likely to navigate to the hole directly using spatial cues. We also determined that while TM did not adversely alter spatial learning in control mice, loss of ovarian hormones in CVN-AD mice drastically impaired their ability to locate the escape hole. TM also increased the likelihood that CVN-AD mice used a serial search strategy. Our findings support previous reports that CVN-AD mice show progressive, age-related spatial learning deficits, and reveal that young CVN-AD mice are likely performing well on the Barnes Maze task by using a compensatory, non-spatial strategy to locate the escape hole. Moreover, our study demonstrates that a gradual, menopause-like loss of ovarian hormones exacerbates AD-like cognitive decline. Ongoing and future studies will investigate the effects of TM on other aspects of AD-like disease progression, and the response of females to therapeutic interventions at various stages of the menopausal transition.

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Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.22/YY10

Topic: F.02. Behavioral Neuroendocrinology

Support: OMHF
NSERC

Title: Prenatal testosterone affects social and anxiety-like behaviours in a sexually dimorphic and hormone-dependent manner

Authors: *E. R. MARTIN, C. S. WASSON, C. HOWES, A. J. GIUGA, M. CASTRO, H. A. WILSON, N. J. MACLUSKY, E. CHOLERIS
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Abstract: Gonadal hormones, such as testosterone (T), organize sexually dimorphic brain regions during development and consequently sex differences in behaviour later in life. Abnormal levels of prenatal T have been associated with Autism Spectrum Disorder (ASD), which is partially characterized by deficits in social communication and social interaction. We assessed the effects of elevated prenatal T on social learning (SL), object recognition, social recognition (SR), sociability, and anxiety-like behaviour, in mice. Pregnant CD1 female mice were treated subcutaneously with 10 µg of T propionate or sesame oil vehicle control on gestational days 12, 14 and 16. Experimental litters were assessed in the above listed behavioural

tests during adolescence (age 35-42 days; n=12-47). Mice then underwent sham surgery, gonadectomy (GDX), or GDX with silastic capsules, [T for males and estradiol (E) for females] and were re-tested for the same behaviours in adulthood (age 68-76 days; n=5-24). Prenatal T increased anxiety-like behaviour in adult male mice, but females were resilient to the effects of this treatment. Prenatal T enhanced SR in both males and females during adolescence. In adulthood, prenatal T impaired SR in gonadally intact and GDX males and this was reversed by T replacement. In females, GDX impaired SR but E replacement did not reverse this effect. For SL, castration improved learning in male controls but blocked SL in adult mice treated prenatally with T, an impairment that was not reversed by T replacement. Conversely, in ovariectomized mice, SL was impaired following prenatal T treatment, but recovered after estradiol replacement. These results are reminiscent of the effects of prenatal stress and suggest that prenatal T exposure may alter the development of normal social and anxiety-like behaviours, resulting in long-term effects that modify responses to gonadal hormone exposure in adulthood. The molecular mechanisms through which prenatal T acts remain to be elucidated, but may involve pathways similar to those activated by prenatal stress.

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Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.23/YY11

Topic: F.02. Behavioral Neuroendocrinology

Title: Assessment of peripheral BDNF variability over 30 days in healthy adults

Authors: S. HANG, J. RODRIGUEZ-ZAMORA, B. CHU, R. C. GARCIA, H. M. KILGORE, *E. B. GAHTAN
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Abstract: Brain-derived neurotrophic factor (BDNF) has been studied extensively for its potential role in brain and cognitive health. Lower levels of BDNF are associated with neuronal apoptosis, decreased hippocampal volume and conditions including schizophrenia, depression and Alzheimer's, whereas elevated levels are associated with neurogenesis and improved cognitive functioning, particularly memory. In most human studies, BDNF is measured in peripheral tissues, most commonly blood or saliva. Many studies have shown BDNF changes related to physical exercise, hormone levels, and gene polymorphisms, while others have not, signaling unknown sources of variability in peripheral BDNF levels. To better understand its normal degree of variability, the current study examined circulating BDNF in whole blood in healthy adults (N=9; 5 female) over 30 days. The main goal was documenting long-term BDNF

variability, but we also analyzed the relationship of BDNF levels to recent physical exercise, exercise intensity, sex, body mass, and (in female participants) hormone cycle. Samples were obtained through safety lancet finger-pricks three times per week over four weeks and participants' recent physical exercise information was recorded at each collection. Whole blood BDNF concentrations were quantified using enzyme-linked immunosorbent assay. Across 30 days, within-subjects BDNF levels fluctuated by an average of 5.92 pg/ml (SD) around the mean of 29.80 pg/ml, yielding a coefficient of variance (*CV*) of 19%. No association of BDNF to recent physical exercise, exercise intensity, sex, body mass, or hormone cycle was found. This estimate of variability in circulating BDNF levels is based on a longer duration of observation than previous reports and may be useful for assessing the statistical power of BDNF studies.

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Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.24/YY12

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH/NIMH
NIH/NIAAA

Title: Neuronal stem cell transcriptomic response to ovarian steroid hormones in women with Premenstrual Dysphoric Disorder: Beyond lymphoblastoid cell lines

Authors: *A. GOFF[1,2], H. LI[3], J. F. HOFFMAN[5], C. MARIETTA[4], P. E. MARTINEZ[6], D. R. RUBINOW[7], P. J. SCHMIDT[8], D. GOLDMAN[9]
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Abstract: Premenstrual dysphoric disorder (PMDD) is characterized by recurrent affective and behavioral symptoms during the luteal phase of the menstrual cycle. A study of lymphoblastoid cell lines (LCLs) of PMDD women revealed an intrinsic difference in ovarian steroid responsive genes at baseline. Clinical studies show that PMDD symptoms recur after re-exposure to estradiol (E2) or progesterone (P4) during GnRH-agonist-induced ovarian suppression.

Furthermore, women with PMDD show symptom reduction after blocking conversion of P4 to its neurosteroid metabolite, allopregnanolone (ALLO). These clinical studies suggest that *change* in steroid hormone levels leads to symptoms. To learn if there is an intrinsic difference in neural cells as well as LCLs, and to investigate effect of change in ovarian steroid levels, we differentiated neural stem cells (NSCs) from induced pluripotent stem cell (iPSC) lines from women with PMDD and asymptomatic controls (n=4, 4, respectively; 2 technical replicates per individual). Immunofluorescent staining of neuronal markers verified differentiated cells as NSCs. NSCs were exposed to vehicle (DMSO), E2, P4, or ALLO for 24hrs at 100nM, and examined for gene expression differences via AmpliSeqRNA Transcriptome Sequencing. EdgeR was used to detect differential expression, follow by DAVID and GSEA for pathways, and WGCNA for correlated gene clusters. Unsupervised hierarchical clustering revealed diagnosis- and hormone treatment-specific clusters. Genes were differentially expressed at baseline as well as in response to hormone. At baseline (i.e., vehicle), PMDD NSCs showed upregulation (FDR corrected p=0.04) of *STAR*, which enhances conversion of cholesterol to pregnenolone (a GABA receptor modulator and precursor of other neurosteroids), perhaps playing a role in differential sensitivity to changing steroid hormone levels. Furthermore, NSCs from women with PMDD showed upregulation of a similar subset of genes in response to treatment with either P4 or ALLO, suggesting the two steroids may affect gene expression through similar pathways. Presently, we are further investigating differentially expressed genes via qRT-PCR and protein analyses.

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Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.25/YY13

Topic: F.02. Behavioral Neuroendocrinology

Support: CIHR Operating Grant 133606

Title: Working to run: Assessing motivation for wheel running in female rats

Authors: *K. K. SOMA, W. A. KRIEGER, D. J. TOBIANSKY, M. A. TURCOTT, C. MA, S. B. FLORESCO
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Abstract: Running appears to be a rewarding activity for rodents. However, most rodent studies use reinforcers such as sugar pellets or drugs (e.g., cocaine) for instrumental learning. The use of running as a reinforcer in complex operant tasks and the neural mechanisms for motivation to

run have not been well examined. In this study, we explored a) whether running can be used as an effective reinforcer for operant tasks, and b) whether caloric restriction (CR) increases motivation to gain access to the running wheel. Adult female Long-Evans rats were group housed and given unlimited access to a running wheel for 2 wk (4 females/bin). Thereafter, females were pair-housed and randomly assigned to be CR (n = 10) or fed *ad libitum* (AL; n = 10). After 1 wk of exposure to the feeding paradigm, the subjects were introduced to an operant chamber with a running wheel attached. The subjects were trained to press a retractable level to open the guillotine door, which gave them access to a running wheel for 30 min on fixed ratio (FR) 1, FR2, and FR5 schedules of reinforcement. Motivation to gain access to a running wheel was assessed using a progressive ratio (PR) schedule of reinforcement. For each ratio, the subject was given 20 min to complete the number of lever presses required to gain access to the running wheel for 5 min. Subjects were exposed to the subsequent ratio on the following day until they failed to complete the task. All subjects readily learned to press the lever to gain access to the wheel. There was no difference in the number of ratios completed [mean = 13 ratios (62 lever presses)] between AL and CR subjects, but CR subjects ran significantly more than AL subjects during the 5-min running period. After all subjects completed the PR task, they were run on three (3) FR4 sessions with 5-min access to the running wheel and were euthanized at 90 min after the last running period. Local steroid concentrations and c-Fos expression will be examined in relevant brain regions to determine whether CR influences steroid signaling and neuronal activity. This study lays the groundwork for future studies examining the neuroendocrine regulation of the motivation for voluntary exercise.

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Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

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Program #/Poster #: 593.26/YY14

Topic: F.02. Behavioral Neuroendocrinology

Support: PAPIIT- DGAPA-UNAM-IN-216214
CONACYT-CB238744

Title: Gonadal status modifies the ratio of GABA expressing neurons in limbic areas of the rat brain

Authors: *V. S. HERNANDEZ, A. NAVA-KOPP, O. HERNÁNDEZ PEREZ, L. ZHANG
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Abstract: Gonadal status modifies the ratio of GABA expressing neurons in limbic areas of the rat brainAUTHOR BLOCK *V. S. HERNANDEZ1, A. NAVA-KOPP1, O. HERNÁNDEZ PEREZ1, L. E. EIDEN2, L. ZHANG1; 1Dept. of Physiology, Fac. of Med., Natl. Autonomous Univ. of Mexico, Mexico City, Mexico; 2Sec Molec Neurosci, NIH, NIMH-IRP, Bethesda, MDAbstract:Gonadal steroids act upon classical nuclear receptors to alter the function of many brain areas including limbic system areas involved in affective and cognitive processing. However, how the sexual steroids modulate the activity of the neurons that constitute the limbic system remains unclear. Previously we have reported the existence in the medial habenula of a population of neurons that receive inputs from hypothalamic homeostatic nuclei and may be induced to change to a GABAergic phenotype by local (synaptic) conversion of the gonadal steroid testosterone to estrogen vía aromatase contained in nerve terminals.In this study we used the RNAscope technique to evaluate the densities of neurons expressing the vesicular GABA transporter (VGAT) mRNA in several limbic regions, as well as the RNA of receptors for estrogen (ERa and ERb), testosterone (AR) and the enzyme aromatase. We compared male rats under four different gonadal status (castrated "GNX", sexually inactive "SI", sexually active "SA" and testosterone administration). Preliminary results show that GNX rats have diminished number of VGAT expressing neurons compared to SA rats in the following limbic structures: Anterior Cingulate Cortex (64% of that in SA rats); Anterior Olfactory Nucleus (44%); accumbens (56%); Lateral Septum (72%); Oval Nucleus of Stria Terminalis (72%). These data provide an evidence that gonadal status modify the emotion through GABAergic neuronal plasticity in limbic regions.

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Poster

593. Behavioral Neuroendocrinology: Hormones and Cognition II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 593.27/YY15

Topic: F.02. Behavioral Neuroendocrinology

Support: F32DA039715

Title: Estrogen, dopamine d_2-type receptors, and self-control

Authors: *N. ERTMAN[1], K. OKITA[4], M. E. FRY[1], Z. ZHANG[2], A. J. RAPKIN[2], M. A. MANDELKERN[5], B. BYCH[2], E. D. LONDON[3]
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Abstract: Estrogen has been directly and indirectly linked to dopaminergic signaling in preclinical literature - a finding with clinical relevance to addictive disorders, which differ in prevalence, course, and consequences between men and women. However, no human studies have tested whether circulating estrogen levels influence striatal dopamine D_2-type receptor availability, a marker of dopamine function that is significantly lower in people with addictive disorders compared to healthy controls. We tested whether dopamine D_2-type receptor availability, measured with [^{18}F]-fallypride and positron emission tomography, changed significantly over the course of the menstrual cycle to influence inhibitory control. The latter was measured with self-report inventories, a motor response inhibition task (Stop Signal), and an emotion regulation task using cognitive reappraisal. Contrary to our hypothesis that estradiol would decrease striatal dopamine and thereby reduce inhibitory control, there was no relationship between striatal D_2-type receptor availability and circulating 17β-estradiol levels, and no effect of menstrual phase on inhibitory control. An exploratory analysis, however, revealed a parabolic (inverted-U) relationship between the estrogen:progesterone ratio and thalamic D_2-type receptor availability. These data suggest that menstrual phase (and other neuroendocrine events) may not influence striatal D_2-type receptors, but may instead exert influences on behavior by affecting D_2-type receptors in the thalamus, which is structurally and functionally connected to both the striatum and cortical regions.

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Poster

594. Neuroimmunology: Regulating Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 594.01/YY16

Topic: F.05. Neuroimmunology

Support: UC MEXUS
CONACyT Grant CN-17-19
Escuela de Medicina, Universidad Anáhuac Mayab Grant PresInvEMR2017

Title: Artificial sweetener consumption induces changes in expression of c-Fos and NeuN in hypothalamus and hippocampus of rats

Authors: *L. E. MACIAS[1], M. DE LA CRUZ[2], D. MILLAN ALDACO[3], D. SORIANO NAVA[2], R. DRUCKER COLÍN[3], E. MURILLO RODRÍGUEZ[2]
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Abstract: Obesity is the result of the interaction of multiple variables, including the excessive increase of sugar-sweetened beverages consumption. Diets aimed to treat obesity suggest the uses of sugar substitutes, such as aspartame, sucralose, or saccharin. Despite current evidence suggests that consumption of these sugar substitutes may prevent obesity, the effects of intake of artificial sweeteners in biomarker expression of neurons remain unclear. This study was aimed to investigate the effects of consumption of artificial sweetener on c-Fos or NeuN expression in hypothalamus and hippocampus. Artificial sweetener was diluted in water (25,75 or 250 mg/100 mL) and orally given to rats during 2 weeks. Next, animals were sacrificed by decapitation and brains were collected for analysis of c-Fos or NeuN inmunoreactivity. Consumption of artificial sweetener provoked an inverted U-shaped dose-effect in c-Fos expression in ventromedial hypothalamic nucleus while similar findings were observed in dentate gyrus of hippocampus. In addition, NeuN inmunoreactivity was enhanced in ventromedial hypothalamic nucleus at 25 or 75 mg/100mL whereas an opposite effect was observed at 250mg/100mL. Lastly, NeuN positive neurons were increased in CA2/CA3 fields of hippocampus from rats that consumed artificial sweetener (25,75 or 250mg/100mL). Consuming artificial sweet tasting (no caloric/reduced-calorie beverage) in water induced effects in neuronal biomarkers expression. To our knowledge, this study is the first description of the impact of consumption of artificial sweetener on c-Fos and NeuN inmunoreactivity in hypothalamus and hippocampus.

Disclosures: L.E. Macias: None. **M. De La Cruz:** None. **D. Millan Aldaco:** None. **D. Soriano Nava:** None. **R. Drucker Colín:** None. **E. Murillo Rodríguez:** None.

Poster

594. Neuroimmunology: Regulating Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 594.02/DP11/YY17

Topic: F.05. Neuroimmunology

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HFSP Grant RG0063

Title: Discovery of a sensory pathway to detect pathogens invading the cerebrospinal fluid during meningitis

Authors: *C. WYART[1], A. E. PRENDERGAST[2], F. QUAN[2], K. JIM[3], L. DJENOUNE[4], L. DESBAN[5], H. MARNAS[5], Y. CANTAUT-BELARIF[1], C. VAN DEN BROUCKE-GRAULS[3]
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Abstract: Cerebrospinal fluid (CSF) is a complex fluid circulating around the nervous system whose composition dramatically changes as a function of the physiological state of an individual and in particular during bacterial meningitis. At the interface with the CSF in vertebrates, we discovered a singular class of spinal sensory neurons that surrounds the central canal and apically projects a ciliated extension into its lumen. We showed that these CSF-contacting neurons respond to changes in pH and CSF flow, and that their mechanosensitivity relies on the transient receptor potential channel PKD2L1. Here we investigated the physiological relevance of CSF-cN chemosensory properties. In particular, we tested whether CSF-cNs detect pathogen invasion in a zebrafish model of bacterial meningitis we previously developed by injecting red fluorescent mCherry labelled Streptococcus pneumoniae in the hindbrain ventricle. When injected in 2 days old larvae, the pathogen invades the entire CSF including the central canal within 24 hours. In response to the infection, we show that CSF-cNs undergo drastic changes in their activity. While basal activity goes down for most CSF-cNs, a fraction of CSF-cNs exhibits massive calcium transients lasting for tens of seconds. In order to identify novel receptors underlying CSF-cN activity, we isolated CSF-cNs by fluorescent-activated cell sorting and generated the transcriptome of CSF-cNs using RNAseq. Among genes enriched in CSF-cNs, we identified novel receptors modulating their activity by inactivating them with transient CRISPR/Cas9 injections and subsequently observing CSF-cN activity in vivo. This screen identified a novel receptor whose activation mimics the activation of CSF-cNs during the pathogen invasion. In addition, we found a multitude of peptides expressed by CSF-cNs known to carry antimicrobial functions and referred to as defensins. We are now investigating the molecular pathways enabling the massive activation of neurons contacting the CSF in vivo and the occurrence of peptidergic release to fight pathogen invasion in the CSF. Our study reveals a novel role for CSF-cNs in the detection of pathogens and deployment of the innate immune response during bacterial meningitis in vertebrates.

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Poster

594. Neuroimmunology: Regulating Systems

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Topic: F.05. Neuroimmunology

Support: Wellcome Trust Grant 099816/Z/12/Z

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Title: Complement and psychiatric disorder: The role of C3/C3aR in fear and anxiety

Authors: *L. J. WESTACOTT[1], N. HAAN[1], S. MITTON[2], E.-L. BUSH[2], T. HUGHES[3], J. HALL[1], P. MORGAN[3], W. GRAY[1], T. HUMBY[2], L. WILKINSON[1]
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Abstract: Recent genetic findings indicate that the complement system, a branch of the innate immune system, plays a causal role in risk for schizophrenia. However, the biological mechanisms that link complement and psychiatric disease remain unknown. The objective of this research is to investigate the impact of complement on behavioural phenotypes relevant to psychiatric disorders.

We studied the C3/C3aR pathway using two knockout mouse models; $C3^{-/-}$ and $C3aR^{-/-}$. C3aR is the canonical receptor for C3a, a breakdown product of C3 activation. Male mice ($C3^{-/-}$, $C3aR^{-/-}$, WT; 3-9 months of age; $n \geq 10$ per group) were tested in a battery of behavioural tasks to assay anxiety and learned fear. Additionally, subsequent to an acute stressor, blood samples were collected for analysis of corticosterone, and brain regions were dissected for gene expression analysis.

In the elevated plus maze (EPM), $C3aR^{-/-}$ mice were highly anxious, evidenced by markedly reduced open arm exploration compared to WT and $C3^{-/-}$ subjects. This pattern of results was replicated in the open field (OF) and other tests of anxiety, indicating an anxiogenic effect of C3aR deficiency that was not present in the $C3^{-/-}$ mice.

We next explored the sensitivity of this anxiety phenotype to the anxiolytic diazepam. The drug had no effect on the marked anxiogenic effects of C3aR deficiency at a dose that proved anxiolytic in WT subjects, and there were no effects in $C3^{-/-}$ subjects, suggesting an altered sensitivity to benzodiazepines and a potential alteration in the $GABA_A$ receptor system in the knockouts.

The fear potentiated startle paradigm was used as a test of learned fear. While both knockouts demonstrated elevated acoustic startle responses at baseline, indicating heightened fear, only $C3^{-/-}$ subjects demonstrated a significantly enhanced potentiation of the startle reflex in response to a conditioned stimulus, suggesting that C3, but not C3aR, is involved in learned fear.

These behavioural effects were accompanied by reductions in the reactivity of the HPA axis to an acute stressor in both knockouts, consistent with a chronic stress state. Pilot data also indicates that in both knockouts, there is altered expression of the glucocorticoid receptor gene NR3C1 in the brain following acute stress.

In conclusion, this research provides new insights linking manipulations of the complement C3/C3aR pathway and emotion. The behavioural differences between the mutants suggest dissociable mechanisms underlying the impact of discrete complement components on fear and anxiety, which are core symptoms of many psychiatric disorders, including schizophrenia.

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Poster

594. Neuroimmunology: Regulating Systems

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 594.04/YY19

Topic: F.05. Neuroimmunology

Support: SetPoint Medical, Inc.

Title: Two-year safety and efficacy of ultra-low duty cycle stimulation of the vagus nerve as a first-in-class bioelectronic therapy in rheumatoid arthritis

Authors: *Y. A. LEVINE, D. CHERNOFF
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Abstract: Introduction Despite the availability of multiple pharmaceutical and targeted biological drugs for treatment of rheumatoid arthritis (RA), significant unmet clinical need remains for those who are intolerant or fail to respond. Modulating innate anti-inflammatory neuro-immune pathways through electrical stimulation of the vagus nerve may represent a novel, nonpharmacological means of treating RA and other inflammatory diseases. We recently reported a 12-week proof of concept study with an implanted neuromodulation device, showing reductions in the DAS28-CRP disease activity score and in TNFα and IL-6 levels (PNAS, 2016. 113(29): 8284). To understand the long term safety and efficacy of this treatment approach, we followed the subjects for 24 months in a long term extension study. **Method** In the primary study, neuromodulation devices were implanted into 17 RA subjects, mostly with inadequate responses to multiple conventional and targeted biologic drugs. The cervical vagus nerve was stimulated at ultra low duty cycle (10 Hz, 1-4 min/day) with output current intensity titrated to subjects' upper comfort level. On completion, subjects were offered enrollment in a follow-up study, where study physicians were given flexibility to alter electrical dosing parameters and/or to add a biologic to the treatment regimen. DAS28-CRP and Health Assessment Questionnaire-Disability Index (HAQ-DI) were collected over 2 years. **Result** All subjects electively continued on neuromodulation treatment through 24 months follow-up study. Biologics were started in 9 of 17 subjects; of these, 4 were non-responders to therapy in the primary study, and 5 had improvement but had not yet achieved disease remission with neuromodulation alone. At the start of the follow-up study, the mean DAS28-CRP and HAQ-DI were significantly reduced compared to the pre-implant baseline, and the depth of effect was retained through 24 months. No association between DAS28-CRP and stimulation frequency (Range= 1-8X/day) was observed. At 24 months, there was no significant difference in DAS28-CRP between the subjects

treated with neuromodulation as monotherapy or those treated with a combination of neuromodulation and biologics. No difference in the adverse events profile between the two groups was detected. **Conclusion** These data demonstrate that this first in class bioelectronic therapy substantially reduced disease activity and disability in subjects with RA, and the therapeutic benefits were sustained for up to 24 months without untoward safety signals. These results support further development of implantable neuromodulation devices as an alternative therapeutic approach to treating RA.

Disclosures: Y.A. Levine: A. Employment/Salary (full or part-time):; SetPoint Medical, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SetPoint Medical, Inc. **D. Chernoff:** A. Employment/Salary (full or part-time):; SetPoint Medical, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SetPoint Medical, Inc., Adamas Pharmaceuticals, OLLY Nutrition, NAIA Pharma, Aquinox Pharma. F. Consulting Fees (e.g., advisory boards); Adamas Pharmaceuticals, OLLY Nutrition, NAIA Pharma, Aquinox Pharma, Crescendo Bioscience.

Poster

594. Neuroimmunology: Regulating Systems

Location: SDCC Halls B-H

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Program #/Poster #: 594.05/YY20

Topic: F.05. Neuroimmunology

Support: CONACyT PhD student grant to E.S.-T.
DGAPA IG-200417
CONACyT 220598

Title: Circadian gating of hepatic spinal inflammatory input shapes cytokine response by liver and spleen

Authors: *E. C. SOTO-TINOCO, E. SANTACRUZ, R. M. BUIJS
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Abstract: The autonomic nervous system (ANS) regulates the intensity of the inflammatory response to peripheral endotoxin exposure, but how is the brain informed about the immune challenge has remained largely elusive. We hypothesized that the sensory afferent part of the ANS is responsible for sensing lipopolysaccharide (LPS) and allow the efferent ANS to immediately mount an adequate inflammatory response. We show that sensory neurons in the spinal cord and not in the circumventricular organs become activated shortly after an LPS challenge by LPS-induced prostaglandin production. Denervation studies show that the inflammatory signal is transmitted by liver spinal afferents. The circadian system, which strongly

influences ANS output, imposes rhythmicity on the gating of hepatic sensory input, fine-tunning the sensitivity to LPS and allowing an efficient inflammatory response to happen during the active period of the animal, while preventing this process during the resting phase of the animal . This study unravels the circuit used to transmit the peripheral LPS signal to the brain. The hepatic spinal sensory nerves, strongly influenced by the circadian system, allow the spleen to mount an efficient inflammatory response only at the moment when it is most likely needed.

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Poster

594. Neuroimmunology: Regulating Systems

Location: SDCC Halls B-H

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Program #/Poster #: 594.06/YY21

Topic: F.05. Neuroimmunology

Support: the Landis-Berkman Family Fund

Title: A pilot randomized control trial investigating brain-body mechanisms of Qigong meditative movement practice for cancer-related fatigue

Authors: C. ZIMMERMAN[1], C. PENNER[2], *S. TEMEREANCA[2], D. DANIELS[2], B. CULLEN[3], S. JONES[2], C. E. KERR[1]
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Abstract: Meditative movement and exercise practices are both beneficial for health, and have been validated to improve fatigue levels in cancer survivors with cancer-related fatigue (CRF). Compared to exercise, less is understood about the specific brain-body mechanisms by which meditative movement practices may improve health. Qigong, a form of meditative movement, emphasizes gentle movements with a specific training of the mind to focus and engage those movements. In this pilot clinical trial, we employ a parallel, randomized non-inferiority design to test whether ten-weeks of Qigong training is not inferior to an exercise-nutrition control program in reducing fatigue (via the FACIT-Fatigue Questionnaire) in 48 female cancer survivors with CRF. While our primary hypothesis is that Qigong and exercise-nutrition will both reduce subjective reports of fatigue, we utilize multi-modal physiological measures of brain, cardiorespiratory, and muscle dynamics as well as inflammatory immune markers to assess whether Qigong improves fatigue via a distinct neuro-immune mechanism compared to exercise-nutrition. Specifically, this study utilized (1) Electroencephalography (EEG) during a tactile discrimination task to examine whether the meditative focus cultivated by Qigong enhances pre-stimulus control of alpha (7-14 Hz) rhythm over somatosensory cortex during a tactile acuity task (2) Simultaneous EEG and electromyography (EMG) during a precision grip task, to test

how Qigong differentially alters sensorimotor function indexed by enhanced beta (15-30 Hz) cortico-muscular coherence and decreased force variability (3) Electrocardiography (ECG) to assess whether high-frequency heart rate variability (HRV) as a measure of parasympathetic activity was correlated with peripheral inflammation and improved by the stress-reduction aspects of Qigong training (4) Laser Doppler Flowmetry (LDF) to evaluate effects on peripheral blood flow dynamics and (5) Neuroimmune interactions were tested to investigate if enhanced attentional modulation of alpha rhythms post-training were correlated with decreased inflammatory markers. Further, we examined if these post- vs pre-training effects on physiological measures were larger in the Qigong group compared to the exercise-nutrition control group. This study helps identify potential EEG and other physiological biomarkers of clinical effects of Qigong meditative movement therapy.

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Poster

594. Neuroimmunology: Regulating Systems

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Topic: F.05. Neuroimmunology

Support: 1R01AA024798

Title: Sexual dimorphism of the CCL2/CCR2 chemokine system in the lateral hypothalamus (LH) as it relates to the neuropeptide melanin-concentrating hormone (MCH), responds to ethanol exposure during pregnancy, and contributes to excess ethanol consumption in adolescent offspring

Authors: *S. F. LEIBOWITZ, G.-Q. CHANG, O. KARATAYEV, V. HALKINA, J. EDELSTIEN, E. RAMIREZ, V.-S. KEWALDAR
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Abstract: Ethanol consumption and inflammatory agents during pregnancy similarly increase alcohol drinking in adolescent offspring. To investigate how neuroimmune and neurochemical systems interact to mediate these behavioral disturbances, we examined a specific population of neurons in the LH which express the inflammatory chemokine CCL2 or its receptor CCR2 that are positively related to ethanol intake and also co-express the orexigenic neuropeptide MCH that similarly promotes ethanol consumption. Our published evidence in Sprague-Dawley rats (Chang et al., 2015) shows that maternal oral administration of ethanol at low-to-moderate doses (1-3 g/kg/day) from embryonic day 10 (E10) to E15 increases the expression and levels of CCR2 in the LH, its colocalization with MCH, and the drinking of ethanol in male offspring. We

additionally find that these neuronal and behavioral effects of maternal ethanol administration are blocked by a CCR2 antagonist administrated during pregnancy, suggesting that this CCL2/CCR2 chemokine system is involved in mediating ethanol's actions. Further investigations have yielded 3 main results: 1) Injection of CCL2 (4 and 8 ug/kg/day) during pregnancy (E10-E15) compared to its vehicle produces similar effects as maternal ethanol administration (2 g/kg/day, i.g.), significantly stimulating CCR2 and MCH neurons and their colocalization in the LH and ethanol intake in adolescent offspring; 2) These neuronal and behavioral effects of maternal CCL2 administration like ethanol are sexually dimorphic, consistently stronger in the female adolescent offspring, with both ethanol and CCL2 having a particularly strong stimulatory effect on CCR2 expression which is increased by ~250% in females compared to only 40% in males; and 3) Neurons expressing CCL2 are also detected in the LH, closely associated with MCH neurons, and increased by maternal administration of ethanol (2 g/kg/day). There are two distinct types of CCL2-expressing neurons stimulated by ethanol, namely, large CCL2 neurons that are concentrated in the LH and colocalize CCR2 along with MCH and small CCL2 neurons that while scattered throughout the hypothalamus are seen in the LH immediately adjacent to and surrounding the large MCH neurons. These results demonstrate that this neuronal CCL2/CCR2 system in the LH, which is closely linked to MCH neurons and involved in maternal ethanol's stimulatory effects on this neuropeptide and ethanol drinking in adolescent offspring, is sexually dimorphic with females showing greater responsiveness to ethanol and thus may contribute to the higher levels of adolescent risk factors for alcohol use disorders described in women.

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Poster

594. Neuroimmunology: Regulating Systems

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Program #/Poster #: 594.08/YY23

Topic: F.05. Neuroimmunology

Support: NIMH Grant MH66123
NIMH Grant MH108867

Title: The human brain microbiome; there are bacteria in our brains!

Authors: *R. C. ROBERTS, C. B. FARMER, C. K. WALKER
Psychiatry and Behavioral Neurobio., Univ. of Alabama, Birmingham, Birmingham, AL

Abstract: The gut-brain microbiome has received an abundance of attention recently. It is thought that gut microbiota can influence brain function and behavior, but how that happens is

still unknown. It has been proposed that bacteria can enter the brain through the blood brain barrier, and/or via nerves that innervate the gut. Here we show the presence of bacteria in the human and mouse brain under noninfectious or nontraumatic conditions. We first found the bacteria, identified by morphological criteria, in ultrastructural samples of human postmortem brain (n=34 cases). We did serial section analysis for identification and quantification. All cases contained bacteria in varying amounts. Bacteria were rod shaped, and contained a capsule, nucleoid, ribosomes and vacuoles. The average diameter of the short axis was 0.496um. Many were segmented, with the long axis averaging approximately 1.78um between segments. Others did not appear to be segmented and were approximately 0.866um in the long axis. The vast majority of the profiles had a thick capsule of approximately 100nm. The density of the bacteria varied according to the brain region, with abundant bacteria in the substantia nigra, hippocampus and prefrontal cortex but sparse numbers in the striatum. Bacteria were present in intracellular locations, predominantly in astrocytic end feet at the blood brain barrier, dendrites and the soma of glial cells. They were also abundant adjacent to and within myelinated axons. To address the possibility that the bacteria in human tissue was a result of postmortem artifact, we examined mouse brains that were fixed immediately at death (n=10); there were abundant bacteria in similar intracellular locations. To eliminate the possibility that the presence of bacteria was due to contamination, we examined germ free mouse brains (n=4) processed in an identical way; we did not detect any bacteria. The observation that the location of the bacteria was highly specific and deep within the specimens also argues against contamination. Interestingly, there were no structural signs of inflammation in any of the brains examined. It is presently unclear the route of entry bacteria take to the brain, but the evidence of them in axons and at the blood brain barrier supports previous speculation.

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Poster

594. Neuroimmunology: Regulating Systems

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Topic: F.05. Neuroimmunology

Support: JSPS KAKENHI Grant Numbers JP16K19752
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Title: The alternation of the molecules associated with chronic inflammation in the postmortem brains from patients with schizophrenia

Authors: A. WADA[1], *Y. KUNII[2,3], M. HINO[3], J. MATSUMOTO[3], A. NAGAOKA[3], S.-I. NIWA[2], A. TAKESHIMA[4], H. NAWA[5], A. KAKITA[4], K. KASAI[1], H. YABE[3]
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Abstract: The effectiveness of current drug therapy for schizophrenia based on the dopamine hypothesis combined with various psychosocial treatments is insufficient. Thus, there is a need to explore molecular targets for novel drugs for schizophrenia. Previous studies have indicated the possible involvement of chronic neuroinflammation in schizophrenia, with reports of elevated levels of proinflammatory cytokines. One of many similar reports, Zhang et al. found decreased levels of interleukin (IL)-2, IL-4, and IL-8 in patients with schizophrenia compared to healthy controls. Epidemiological studies have also shown a high risk of subsequent autoimmune disease in patients with schizophrenia. Meanwhile, genetic markers in the major histocompatibility complex locus appear to have a high genetic correlation with schizophrenia. However, a 2016 systematic review of 119 articles related to neuroinflammation in schizophrenia found no consistent results regarding levels of glial fibrillary acidic protein, microglial markers, cytokines, chemokines, products of the arachidonic acid cascade-related molecules, and substance P-related molecules in postmortem brains with schizophrenia. The present study aimed to elucidate part of the mechanism of schizophrenia onset using the postmortem brains from 24 patients with schizophrenia, 7 patients with bipolar disorder and 31 controls. Using the Luminex assay, the expressions of molecules involved in immunity and chronic inflammation were measured in postmortem brains with schizophrenia and the relationship between these levels and genetic polymorphisms was analyzed to investigate the association between inflammatory mediators and schizophrenia. The data obtained from the postmortem brains with schizophrenia were also analyzed regarding the relationship between each molecular level and clinical profile, including age, sex, duration of illness, antipsychotic dose, and postmortem interval. The following results were obtained. 1) The schizophrenia group had lower interferon-inducible protein (IP)-10 mass/mg total protein (pg/mg) compared to healthy controls ($p=0.012$). 2) The schizophrenia group had lower IL-17A protein mass/mg total protein (pg/mg) compared to healthy controls ($p=0.003$). 3) Data regarding antipsychotic dose were available for 22 patients in the schizophrenia group. Spearman's rank correlation analysis of the relationships of IP-10 and IL-17A protein mass/mg total protein with mean antipsychotic dose (chlorpromazine equivalent) over the 3 months antemortem found a positive correlation for IP-10 ($p=0.032$). No correlation was observed for IL-17A.

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Poster

594. Neuroimmunology: Regulating Systems

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Program #/Poster #: 594.10/ZZ1

Topic: F.05. Neuroimmunology

Support: NIH Grant P50AA017823
NIH Grant T32AA025606

Title: Sex differences in the expression of neuroimmune genes during withdrawal from acute or chronic ethanol exposure: A comparison of adolescent and adult ethanol exposures

Authors: *P. MARSLAND, A. S. VORE, A. GANO, T. DEAK
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Abstract: Prior work has shown that acute ethanol intoxication and subsequent withdrawal ("hangover") produces phase-specific fluctuations in neuroimmune genes. Although males and females did not differ in the acute rise in IL-6 during acute intoxication, subtle differences in neuroimmune gene expression during acute ethanol withdrawal were observed, warranting further examination of sex differences. In Experiment 1, male and female Sprague Dawley rats were injected with 3.5 g/kg of ethanol or saline (i.p.), with brains and blood samples collected at various time points post-ethanol (15, 18, 24, or 72 hr). Gene expression analyses in the paraventricular nucleus of the hypothalamus revealed increased expression of cytokines predominantly at the 15 hr time point (i.e., early in withdrawal), with TNFα being increased in both sexes. Sex-specific alterations were also observed at 15 hr (increased IκBα in males; increased expression of IL-1β in females). Consistent with prior studies, females displayed substantially higher ambient circulating corticosterone (CORT) than males, regardless of injection timepoint. However, progesterone showed a time-dependent increase during withdrawal that was only observed in females. Experiment 2 utilized a chronic ethanol exposure procedure in which early adolescent (starting at P30) or young adult (P70 start) male and female Sprague Dawley rats received 4.0 g/kg ethanol (i.g.) or water intubations for 3 days, followed by 2 days of withdrawal and abstinence. This cycle was repeated 4 times. Tissue was collected 24 hr after the final ethanol exposure to assess withdrawal from chronic ethanol exposure. Once again, female rats displayed higher circulating CORT than males, regardless of age. However, rats with a history of chronic vehicle exposure displayed significantly higher CORT than the ethanol group, an effect that was only observed in female rats, regardless of age. Sex differences were also observed in IL-6 expression, with adult males showing decreased IL-6 expression during ethanol withdrawal, and adult females showing increased IL-1β and TNFα expression. Taken together, these results demonstrate that withdrawal from acute ethanol exposure produces a relatively rapid increase in neuroimmune genes shortly after ethanol clearance, an effect that

appears to be sustained after repeated cycles of binge-like ethanol exposure. Although certain genes appeared to be mutually elevated in both males and females (TNFα), other neuroimmune genes appeared to be regulated in a sex-specific manner (IL-1β, IκBα). Finally, these data suggest that adolescents and adults do differ in the expression of withdrawal-related cytokines.

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Poster

594. Neuroimmunology: Regulating Systems

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Program #/Poster #: 594.11/ZZ2

Topic: F.05. Neuroimmunology

Support: NIH Grant AG043467

Title: Assessment of neuroinflammation in the aging brain using large-molecule microdialysis: Sex differences and involvement of purigenic receptors

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Abstract: Neuroinflammation has long been considered a hallmark characteristic of neurodegenerative disease, but recent work has suggested that inflammation may be a vital feature of natural aging as well. While much research has utilized animal models to investigate neuroinflammation, most studies have utilized static procedures such as immunohistochemistry (IHC) or *ex vivo* studies in which microglia or other resident immune cells of the CNS are extracted and stimulated in culture. Thus, very little is known about regulation of neuroinflammatory responses in the awake, behaving rat. Thus, the goal of the current study was to investigate time-dependent expression of extracellular cytokine and chemokine concentrations in young adult (3-month old) and aged (18-month old) Fischer 344 (F344) rats of both sexes using *in vivo* microdialysis. We further investigated the potential involvement of purinergic receptors in neuroinflammation by reverse dialysis of the purinergic P2X7 agonist Bz-ATP. Male and female F344 rats aged 3- or 18 months were acquired from the NIA colonies and underwent stereotaxic surgery to implant guide cannula into the dorsal hippocampus. On the day of testing, microdialysis probes were inserted and large molecule microdialysis was performed. Probe stabilization occurred for 3 hr followed by a 2-hr baseline period, after which BZ-ATP (100 mM) was delivered for 1 hr by reverse dialysis. Then, aCSF was administered for a 2-hr recovery period. Multiplex technology was used to simultaneously measure several analytes in dialysate samples. With the exception of IL-6, the release of cytokines, chemokines, and growth

factors was largely unaffected by age. The results demonstrated that males had greater overall release of IL-1β, IL-6, IL-10, MIP-1α and MIP-3α relative to females. Furthermore, IL-6, IL-10 and MIP-1α were greater in males relative to females following initial probe insertion, while this trend was seen in IL-10, IL-17, MIP-1α and TNF-α following drug administration. Lastly, modulation of cytokines by Bz-ATP (a P2X7 agonist) suggested selective regulation of individual factors by the P2X7 receptor (IL-1α, IL-1β, IL-6, IL-17, GRO-KC, MCP-1, MIP-1α and MIP-3α). Overall, this study revealed relatively few age differences in cytokine, chemokine, and growth factor release following probe insertion. However, males displayed enhanced reactivity in many of the target proteins relative to females. Overall, these findings provide critical information regarding characteristics of the individual (age, sex) that profoundly influence neuroinflammatory responses and their regulation by the P2X7 receptor.
Supported by NIH grant AG043467

Disclosures: T. Barney: None. **A.E. Perkins:** None. **M. Piazza:** None. **T. Deak:** None.

Poster

594. Neuroimmunology: Regulating Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 594.12/ZZ3

Topic: F.05. Neuroimmunology

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USDA intramural (DH)

Title: Negative association between T.gondii oocyst serointensity and cortical thickness in the Old Order Amish

Authors: *T. T. POSTOLACHE[1,6,7], D. HILL[8], J. CHIAPELLI[9], M. DAUE[2], A. DAGDAG[3], N. CONSTANTINE[4], L. A. BRENNER[10], J. W. STILLER[11,5], P. KOCHUNOV[9,5], L. E. HONG[9,5]
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Ctr. (MIRECC), Denver, CO; [7]VISN 5 Capitol Hlth. Care Network Mental Illness research Educ. and Clin. Ctr. (MIRECC), Baltimore, MD; [8]Agr. Res. Srvice, Animal and Natural Resources Institute, Animal Parasitic Dis. Lab., United States Dept. of Agr., Beltsville, MD; [9]Maryland Psychiatric Res. Ctr., Baltimore, MD; [10]Rocky Mountain MIRECC, Denver, CO; [11]Neurol. Consultation Service, St. Elizabeth Hosp., Washington, DC

Abstract: *Toxoplasma gondii (T.gondii)* is a neurotropic parasite with a life cycle involving oocyst-producing cats and any warm-blooded animals as intermediate hosts. Toxoplasmosis is commonly acquired via ingestion of oocysts (deficits in washing hands after gardening/farming, vegetables, contaminated water and food, cat litter) or tissue cysts (most common undercooked meat). Oocysts, more resilient to environment and disinfectants, have also been hypothesized to be more virulent and neurotropic than tissue cysts, although direct evidence is lacking. Although, in the immunocompetent host, *T.gondii* establishes latency in the CNS, brain-imaging data in chronic "latent" toxoplasmosis are scarce. Considering that cortical thinning has been previously reported in schizophrenia and suicidal behavior, conditions linked with *T.gondii*, we now relate the average whole brain cortical thickness (AWBCT) to *T. gondii* seropositivity and serointensity for IgG, IgM, and, oocyst-specific IgG, by ELISA. MRI scans were obtained from a convenience sample of 85 Old Order Amish with 48 (56.47%) women, with a mean age of 47.55 ± 17.95, with 10% diagnosed with any mental illness, and used to compute AWBCT (in mm). Linear regressions were used to analyze associations between T.gondii antibodies and AWBCT with adjustment for age, sex, diagnosis of any psychiatric disorder, and diagnosis of only bipolar or psychotic disorders. We found a negative association between AWBCT and oocyte IgG serointensity ($p= 0.0004$, $n=86$), robust to adjustment for age and gender ($p=0.0066$) and psychiatric diagnosis ($p=0.0151$). The IgM and IgG serointensity and seropositivity for all antibodies were not significantly related to AWBCT. Reverse causality potential and confounding by recency of infection notwithstanding, the link between *T.gondii* oocyst infection and cortical thinning uncovers a morphological connection, potentially through neuroimmune activation or neuroprotective defects, thus suggesting potentially modifiable preventative and treatment targets.

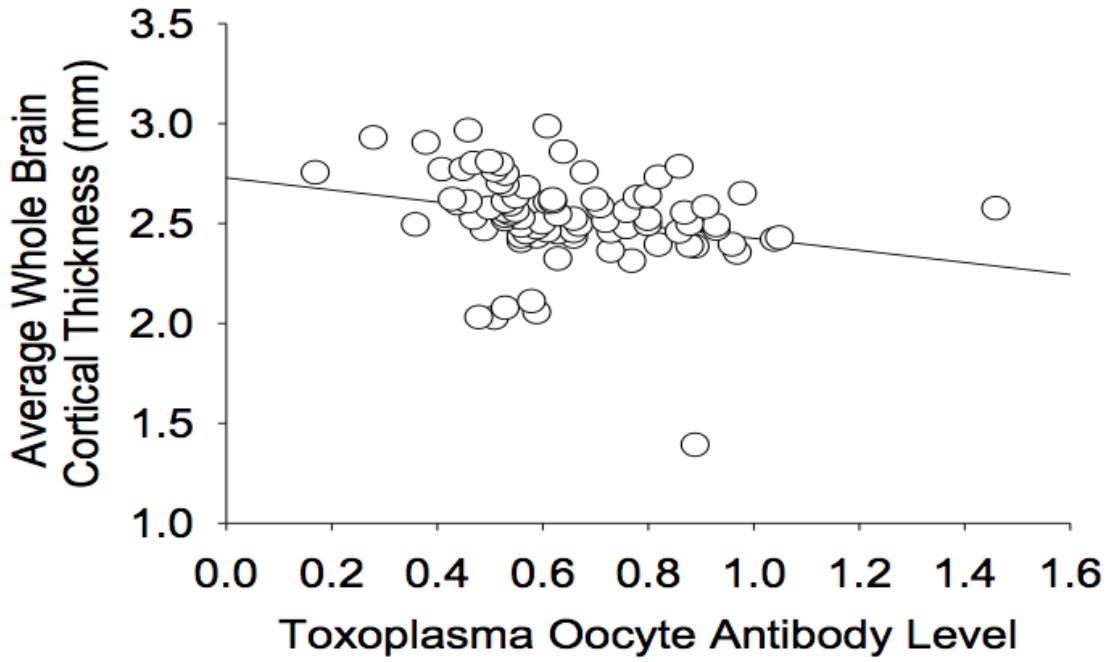

Figure 1: Association between T. gondii oocyte and Average Whole Brain Cortical Thickness: Spearman'rho = 0.37, p=0.0004, n=86

Corrected for age (partial correlation), partial r=0.31, p=0.003. Removing 'outlier' case with cortical thickness less than 1.5: Spearman's rho = 0.36, p=0.001, n=85. Corrected for age (partial correlation), partial r=0.30, p=0.006. Fully adjusted for age, sex and mental illness, p=0.015)

Disclosures: T.T. Postolache: None. **D. Hill:** None. **J. Chiapelli:** None. **M. Daue:** None. **A. Dagdag:** None. **N. Constantine:** None. **L.A. Brenner:** None. **J.W. Stiller:** None. **P. Kochunov:** None. **L.E. Hong:** None.

Poster

594. Neuroimmunology: Regulating Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 594.13/ZZ4

Topic: F.05. Neuroimmunology

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Title: Differential expression of TGFb and angiogenesis related genes between anterior and posterior areas of rats brain

Authors: *R. P. CARMEN[1], D. DAVILA[1], R. H. GILMAN[2], R. HOMERO[1], D. ANA[1], G. IZABO[1], M. VERASTEGUI[1]
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Abstract: Different brain areas present specific cellular and molecular response, here we examined the expression of modulatory cytokines and growth factors which can change their expression depending on cortex location. We used 3 months old 6 rats to study the gene expression of the anterior (sensorimotor cortex) and posterior (auditory and visual cortex) area of the cerebral cortex. Thirty-eight genes were evaluated involving cytokines, angiogenesis molecules, extracellular matrix components and oxidative nitric related genes by quantitative reverse transcription PCR (RT-qPCR). After Wilcoxon matched-pairs signed-ranks test for matched pairs of observations, we found that transforming growth factor beta (Tgfb-1), its receptor (Tgfbr1), VEGF receptor 1 and Angiopoietin-1 receptor changed their expression when compared anterior and posterior cortex area (P=0.046, 0.028, 0.028, 0.028, respectively). Those genes were upregulated in the anterior area of the brain and presented about 1.5 fold of increase. Collective this data report differential expression in normal brain areas which involve the regulatory cytokine Tgfb-1 and angiogenesis-related genes.

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Poster

594. Neuroimmunology: Regulating Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 594.14/ZZ5

Topic: F.05. Neuroimmunology

Title: Galantamine, a cholinergic drug for treating Alzheimer’s disease alleviates inflammatory responses and liver injury induced by APAP/Tylenol in mice

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Abstract: Galantamine is a centrally-acting acetylcholinesterase inhibitor and an FDA-approved drug for Alzheimer’s disease. We have previously demonstrated the anti-inflammatory and hepatoprotective effects of galantamine in mice with endotoxemia and metabolic syndrome. Here, we examined the effect of galantamine on liver injury and inflammation in a mouse model of APAP/Tylenol-induced hepatotoxicity. C57BL/6 male mice (10wks) fasted for 10hrs were

treated with either saline or galantamine (4mg/kg,ip) 1hr prior to APAP (350mg/kg, ip) or 1hr post-APAP (350mg/kg). 12h post APAP mice were euthanized; serum and livers were collected and assessed for liver injury (e.g. ALT, AST) and liver and systemic inflammation. Administration of galantamine before APAP significantly reduced liver injury. Moreover, galantamine given 1hr post-APAP significantly reduced APAP-liver injury. While no changes in serum IFNγ, IL-1β, IL-10, or TNF levels were observed 12h post APAP, serum IL-6 was significantly increased by APAP and this was reduced when galantamine was given 1hr post APAP ($P<0.05$). Similarly, APAP-induced liver IL-1β and TNF protein levels were significantly reduced by galantamine ($P<0.05$). qPCR analyses using liver RNA showed that liver *Il1b, Il6,* and *Tnfa* mRNA expression induced by APAP was significantly reduced by galantamine ($P<0.001$). Our results demonstrate that galantamine is hepatoprotective and anti-inflammatory when administered following APAP overdose. NAC (N-acetylcysteine) is the mainstay therapy for APAP overdose. However, not all patients benefit because it requires early administration, high doses, and long treatments for success. APAP overdose patients exhibit severe neurological pathologies and can die from brain edema and multi-organ failure caused, in part, by immune dysregulation and enhanced cytokine production. Our data support further investigating the role of galantamine and cholinergic modulation in reducing APAP-induced liver and brain injury.

Disclosures: V.A. Pavlov: None. **X. Xue:** None. **P.K. Chatterjee:** None. **M. Addorisio:** None. **K.J. Tracey:** None. **C.N. Metz:** None.

Poster

594. Neuroimmunology: Regulating Systems

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Topic: F.05. Neuroimmunology

Support: NIH Grant AG028271

Title: Characterization of white adipose tissue in young and aged rats and its possible role in neuroinflammation

Authors: *R. M. BARRIENTOS[1,2], L. S. TODD[1], M. KOVACS[1], E. R. LANGDON[1]
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Abstract: Aged rats are more susceptible than young adult rats to long-lasting memory impairments following acute inflammatory insults (e.g., bacterial infection, surgery, or high-fat diet). We have previously reported that this vulnerability is due to aging-induced sensitization of hippocampal and amygdalar microglia which produce exaggerated levels of proinflammatory

cytokines following these insults, and these in turn impede synaptic plasticity mechanisms such as LTP. However, what causes this microglial sensitization in aged rats in the first place remains elusive. An obvious, but over-looked characteristic of aged rats that may play a role in microglial sensitization is their increased body mass compared to young adult rats. White adipose tissue (WAT) is known to be proinflammatory, and depending on its anatomical location and the circulatory system into which they drain could differentially affect neural processes. Thus, we characterized distribution and inflammatory phenotype of several WAT compartments (retroperitoneal, epididymal, subcutaneous, and omental) of young and aged rats fed chow or high fat diet (HFD). We found that aged rats have significantly greater WAT than young rats in all compartments measured, with epididymal and subcutaneous compartments having the greatest percentage of WAT to body mass. Furthermore, HFD-fed aged rats showed an exaggerated increase in retroperitoneal fat over chow-fed controls after just 3 days on that diet. Cytokine expression in WAT from each compartment was also measured and will be reported and correlated to cytokine levels in the hippocampus and amygdala. We previously demonstrated that voluntary wheel running ameliorates contextual memory deficits induced by HFD in aged rats. Here we found that wheel running among chow- and HFD-fed aged rats significantly reduced WAT from the retroperitoneal compartment compared to sedentary HFD-fed aged rats. These findings suggest that WAT from the retroperitoneal compartment may be an important contributor to neuroinflammatory responses in aged rats.

Disclosures: R.M. Barrientos: None. **L.S. Todd:** None. **M. Kovacs:** None. **E.R. Langdon:** None.

Poster

594. Neuroimmunology: Regulating Systems

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Program #/Poster #: 594.16/ZZ7

Topic: F.05. Neuroimmunology

Support: NiH Grant P01HD085928

Title: Impact of neonatal hypoxia-ischemia and inflammation on cerebellum development

Authors: *S. E. ARAMBULA[1], E. L. REINL[1], J. WADDELL[2], M. M. MCCARTHY[1]
[1]Pharmacol., [2]Pediatrics, Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Neonatal Hypoxic-ischemic injury (H/I; concurrent oxygen/blood deprivation) occurs in approximately 2 out of 1,000 term births and is associated with an increased risk of death or major neurodevelopmental disability. Studies in rodent models suggest that preceding inflammatory events can increase the magnitude of brain injury caused by H/I and influence its response to therapeutic intervention. While clinical evidence demonstrates that gross cerebellar

development is profoundly but diffusely damaged by neonatal H/I, the cellular impact of H/I on the cerebellum is not well characterized. Here, we use a modified version of the Rice-Vannucci model of H/I that results in moderate brain injury to quantify the impact on cerebellar development in male and female rat pups with and without prior inflammation. To mimic an inflammatory reaction occurring prior to birth, Sprague Dawley rat pups were given a single intraperitoneal injection of lipopolysaccharide (200 μg/kg) or PBS (vehicle) on postnatal day (PN) 9. On PN10, animals underwent unilateral ligation of the carotid artery followed by exposure to 8% oxygen air for 60 min. Control animals for all experiments were sham-operated. On PN17, animals were euthanized and cerebellar vermis were dissected and processed for western blot analysis. Initial results show that H/I decreases both forms of glutamic acid decarboxylase (GAD65 and GAD67) in the anterior zone (lobules 1-5) of males only. This discovery, coupled with the knowledge that the second postnatal week is a sensitive period in cerebellar development, suggests that H/I may alter the maturation of GABAergic neurons. Further studies are underway to determine how H/I, with and without prior inflammation, affects astrocytes, microglia, Purkinje cells, granule cells and neurons of the cerebellar vermal zones. In addition clinical data indicates a sex-difference in H/I outcomes, with males exhibiting greater behavioral and cognitive deficits relative to females.

Disclosures: S.E. Arambula: None. **E.L. Reinl:** None. **J. Waddell:** None. **M.M. McCarthy:** None.

Poster

594. Neuroimmunology: Regulating Systems

Location: SDCC Halls B-H

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Program #/Poster #: 594.17/ZZ8

Topic: F.05. Neuroimmunology

Support: NIH 2RO1 MH52716-21

Title: Inflammatory mediators regulate DNA methylation during sexual differentiation of the brain

Authors: *E. L. REINL, C. L. WRIGHT, S. L. STOCKMAN, M. M. MCCARTHY
Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: The study of sex differences in healthy neural development can inform on the mechanisms leading to disorders with sex-differences in their susceptibility. The most well studied neural sex differences lie in the organization of brain regions necessary for sexual behavior, including the preoptic area (POA), established by the neuroendocrine milieu of the early postnatal period. The POA is masculinized by endogenous testosterone produced by the male testes in late gestation and shortly after birth, or by exogenous testosterone or estradiol in

females. Our group has shown that during this period estradiol masculinizes the POA by 1) recruiting degranulating mast cells to activate local microglia producing the pro-inflammatory prostaglandin E_2 (PGE_2), and 2) reducing DNA-methyltransferase (DNMT) activity leading to de-repression of a subset of masculinization genes. The work presented herein aimed to examine the connection between these two currently unrelated mechanisms. We hypothesize that estradiol inhibits DNMTs indirectly through mast cell degranulation and microglia PGE_2 production, resulting in DNA de-methylation in males. We measured DNMT activity in PN2 POA and hippocampus from males, females, females treated intracerebroventricular (ICV) on PN0 and PN1 with 1 µg 48/80 (a mast cell degranulation agent), and females treated ICV with 2.5 µg PGE_2 using the Epigentek EpiQuik DNMT Activity Assay Kit. Global DNA methylation (5mC) was measured in POA, hippocampus, and amygdala of males, males + PGE_2, females, and females + PGE_2 using the Epigentek MethylFlash Global DNA Methylation ELISA Kit. Our results show that treatment of females with PGE_2 decreased DNA methylation and DNMT activity in the POA to male levels. Females treated with 48/80 showed decreased DNMT activity even below male levels in the POA. In the hippocampus where DNA methylation and DNMT activity are also greater in PN2 females than males, PGE_2 had no effect in reducing DNMT activity, and 48/80 had only a subtle effect in doing so. Initial results show that PGE_2 also decreased 5mC in the female amygdala, and increased 5mC in both the male amygdala and POA. We conclude that inflammatory mediators may serve as a connecting point between estradiol and DNA demethylation. Future studies will extend this analysis to the prefontal cortex, will employ the use of HPLC to more precisely quantify 5mC, and will include treatment with the cox enzyme inhibitor indomethacin to probe the necessity of PGE_2 in masculinization.

Disclosures: E.L. Reinl: None. **C.L. Wright:** None. **S.L. Stockman:** None. **M.M. McCarthy:** None.

Poster

594. Neuroimmunology: Regulating Systems

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Program #/Poster #: 594.18/ZZ9

Topic: F.05. Neuroimmunology

Support: R01MH52716-020
R01DA0396062-01

Title: Microglial phagocytosis shapes the cellular composition of the neonatal rat amygdala in a sex dependent manner

Authors: *A. E. MARQUARDT[1], J. W. VANRYZIN[1], M. M. MCCARTHY[2]
[2]Dept. of Pharmacol., [1]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: The amygdala, a sexually dimorphic brain region, mediates a conserved male bias in juvenile social behavior in which males engage in more frequent and intense physical play. During the process of sexual differentiation of the amygdala, males exhibit fewer newborn cells than females across the first four days of life. We hypothesized that microglia, the brain's immune cells, may underlie this sex difference, as microglia are significantly more phagocytic in the male amygdala during this time period. Results from immunohistochemical analysis support this hypothesis, as the majority of Iba1-labeled microglial phagocytic cups in both sexes co-label with NucRed, a DNA binding dye, and PCNA, a marker of recently divided cells, indicating microglia actively phagocytose newborn cells in the developing amygdala. To explore how this sex difference in phagocytosis, mainly of newborn cells, affects the amygdala's architecture later in life, we used a fate mapping approach. Pups were treated with BrdU on postnatal day 0 (PN0) to PN4 to label newborn cells and sacrificed at PN26. Tissue sections were stained for BrdU and markers of various cell types. In the medial amygdala (MeA), the site of masculinization of play, BrdU+ cells predominantly (~80%) co-labeled with GFAP, an astrocyte marker, and this was true in both sexes. However, females had a higher density of BrdU+ cells including a higher density of GFAP+/BrdU+ cells specifically in the posterodorsal MeA (MePD). Because of this, we hypothesized that microglia generate the sex difference in postnatally born cells by phagocytosing cells fated to differentiate into astrocytes. To test this, we co-labeled histological sections from PN4 animals with Iba1 and the astrocyte-specific marker ALDH1L1, which is expressed much earlier in differentiation than GFAP. Microglia showed an enrichment for ALDH1L1 within the phagocytic cup and in their processes, and a greater percentage of phagocytic cups co-labeled with ALDH1L1 in males compared to females. This indicates that microglia phagocytose astrocytes in the developing amygdala and that they do so more frequently in males, likely producing the observed sex difference in postnatally-born astrocytes seen at the juvenile age. Future analysis will explore whether this developmental sex difference impacts the density of neurons, microglia, and astrocytes in the MePD and other MeA subregions.

Disclosures: A.E. Marquardt: None. **J.W. VanRyzin:** None. **M.M. McCarthy:** None.

Poster

594. Neuroimmunology: Regulating Systems

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Program #/Poster #: 594.19/ZZ10

Topic: F.05. Neuroimmunology

Support: R01MH52716
R01DA0396062

Title: Microglial phagocytosis of newborn cells is induced by endocannabinoids and sculpts sex differences in social play

Authors: *J. W. VANRYZIN, A. E. MARQUARDT, S. E. ARAMBULA, K. J. ARGUE, M. M. MCCARTHY
Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: The amygdala is a sexually dimorphic brain region important for juvenile social play behavior. During neonatal development, the male amygdala contains fewer newborn cells than females. This sex difference inversely correlates to the expression of juvenile social play, a process we demonstrated to be the result of a higher developing endocannabinoid (ECB) tone in the male amygdala (Krebs-Kraft et al. PNAS 107(47), 2010). We now report that microglia, the resident immune cells of the brain, are more phagocytic in the male amygdala during this postnatal window, suggesting a possible mechanism by which ECBs affect the number of newborn cells. Based on these data, we hypothesized that microglia control the number of newborn cells in the postnatal rat amygdala by phagoptosing (targeted phagocytosis of viable cells) newborn cells in an ECB-dependent manner. We find that males have more phagocytic microglia between postnatal day 0 and 4, during which they also have a higher ECB tone. Administering testosterone or cannabinoid receptor agonists to female pups masculinized the number of phagocytic microglia and correspondingly decreased the number of newborn cells as indicated by BrdU labeling. Further analysis found that phagocytic microglia engulf newborn cells, which are enriched for the complement protein C3b. To directly implicate microglia phagoptosis, we utilized a complement receptor 3 (CR3) function-blocking antibody to inhibit phagocytosis, which increased the number of BrdU+ cells only in males demonstrating that newborn cells can survive if phagocytic activity is prevented. Furthermore, administering the anti-CR3 antibody to neonatal males prevented the masculinization of their play behavior when grown to the juvenile age. Together, these data suggest that sex differences in the local environment of the developing amygdala instruct microglia to actively phagoptose newborn cells as a means to sculpt the later life architecture of the amygdala and produce sex differences in juvenile social play.

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Poster

594. Neuroimmunology: Regulating Systems

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Support: NIH/NIAID-5P01-AI073693-06 (PI Diamond)

NIH/NIAID-4P01-AI102852 (Core, PI Huerta)

Title: Disrupted place cell dynamics in the CA1 region of the hippocampus in long-term sepsis survivors

Authors: *J. J. STROHL, T. S. HUERTA, P. T. HUERTA
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Abstract: Sepsis is defined as a "life-threatening organ dysfunction caused by a dysregulated host response to infection" (Singer et al. JAMA 315:801 2016). Although the chance of surviving the initial shock has improved considerably, it has become apparent that long-term survivors suffer sepsis-related cognitive impairment. Preclinical studies using the cecal ligation and puncture (CLP) model of sepsis in mice reveal clear deficits in spatial memory tasks and contextual fear conditioning. Here, we use the CLP model to investigate the neural substrate of sepsis-induced memory impairment by studying place cell dynamics in the hippocampus. Male mice (Balb/C, n=8, C57BL/6, *n* = 8) underwent CLP or Sham surgery and allowed to fully recover. Following a 6-week survival period, mice were implanted with tetrodes lowered into dorsal CA1 and tested in open field environments. Recordings consisted of multiple *run* sessions interspersed with *rest* sessions in the homecage. Analysis included quantifications of mean and peak firing rates, in-field and out-of-field firing rates, place field size, stability, spatial coherence, spatial information, and navigational error rate calculated during path reconstruction (software: Cheetah, Spike2, NeuroExplorer, Matlab). Cells recorded during multiple sessions were categorized as active, emerging, or vanishing according to whether they remained active from one session to the next. CLP survivors have expanded place fields with lower spatial information and spatial coherence, and higher mean, in-field, and out-of-field firing rates compared to sham animals. Furthermore, when introduced to a new environment, CLP animals have a larger fraction of cells that remain active between the two environments. Finally, an algorithm (Bayesian path reconstruction, BPR) was implemented to test the ability of the ensemble of place cells to accurately determine the path traveled by the animal. The error between the BPR-estimated path and the actual path traversed by the animal was calculated. CLP animals demonstrated a significantly larger error rate compared to Sham. This indicates that the changes to CA1 place cell dynamics after CLP cause a distorted representation of the animals' location in its environment. These results suggest the dorsal CA1 network is disrupted in sepsis survivors and provide insight into potential therapeutics.

Disclosures: J.J. Strohl: None. **T.S. Huerta:** None. **P.T. Huerta:** None.

Poster

594. Neuroimmunology: Regulating Systems

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Topic: F.05. Neuroimmunology

Support: DARPA Grant HR0011-15-2-0016
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Title: Modulation of proinflammatory cytokine production by specific vagus nerve stimulation parameters

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Abstract: Vagus nerve stimulation (VNS) has been shown to be effective in treatment of inflammatory disease models. However, the impact of different electrical pulse parameters on the nerve activation and physiological responses is not understood. In the current study, we analyzed the effect of a range of stimulation parameters, which include amplitude, frequency, and pulse width on cytokine levels. The left cervical vagus nerve was stimulated for four minutes in naïve Balb/C mice using a range of asymmetric charge-balanced electrical pulses. The pulse parameters were: short (50µs) and long (250µs) pulse width, frequencies: 30Hz, 100Hz, 200Hz, and amplitudes: 50µA, 200µA, 750µA. Animals recovered for 2 hours, and circulating cytokine levels quantitated using multiplex ELISA assay. To assess the directionality of the signals, groups of animals were subjected to unilateral or bilateral vagotomy prior to vagus nerve stimulation. At 30Hz, amplitude-dependent increases in serum IL-6 and IL-10 were observed for both short and long pulses ($p<0.001$). Serum TNF levels were unchanged at 50uA and 200µA amplitudes with both pulses, however there was a significant increase with the long pulse at 750µA, compared to the short pulse. ($p<0.0001$). With unilateral vagotomy, levels of TNF and IL-6 were unchanged, whereas IL-10 levels increased significantly ($p=0.03$). With bilateral vagotomy, distal or cranial left vagus nerve stimulation did not affect TNF and IL-6 levels, but significantly increased serum IL-10 ($p=0.002$). Our data demonstrate that systemic cytokine levels can be modulated by selectively stimulating the vagus nerve using specific combinations of frequency, amplitude, and pulse width. Refinement of the specific parameters may enable controlled neuromodulation of immunity. This study was funded in part by DARPA (HR0011-15-2-0016) and NIH (1R35GM118182-01).

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Poster

594. Neuroimmunology: Regulating Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 594.22/ZZ13

Topic: F.05. Neuroimmunology

Support: NIH Grant 1R35GM118182-01

Title: Optogenetic stimulation of cholinergic neurons in the brainstem induces splenic nerve activity and attenuates systemic inflammation

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Abstract: The inflammatory reflex is a well-defined neural circuit composed of afferent and efferent fibers that travel via the vagus nerve to regulate peripheral tumor necrosis factor (TNF) production. Previous studies have demonstrated that electrical stimulation of the efferent fibers reduces splenic TNF output in an endotoxemia model. However, the exact origin of these vagus nerve fibers in the brainstem and the means by which they innervate the spleen to alter its activity remain incompletely understood. Using optogenetics, we selectively stimulated cholinergic neurons in the dorsal motor nucleus of the vagus nerve (DMV), the brainstem nucleus from which the vagus nerve fibers responsible for the inflammatory reflex may originate. A fiber-optic cannula was inserted using stereotactic guidance into the DMV of transgenic mice expressing channelrhodopsin under the choline acetyltransferase promoter (ChAT-ChR2-EYFP mice). Mice were subjected to either optogenetic stimulation or no light/sham surgery (n=15 per group) for five minutes (473nm laser, 20Hz, 25% duty cycle). During DMV stimulation, splenic nerve activity was recorded using a cuffed two-channel electrode and analyzed for changes in neural activity. After 24 hours, inflammation was induced with intraperitoneal lipopolysaccharide (0.25mg/kg) and blood was collected 90 minutes later for analysis. Systemic TNF was measured using a commercially available ELISA. Optogenetic stimulation of the cholinergic neurons in the DMV of ChAT-ChR2-EYFP mice significantly decreased endotoxin-induced serum TNF levels compared to sham controls ($p=0.0004$). Furthermore, splenic nerve activity during DMV optogenetic stimulation was significantly increased over baseline, demonstrating the physiological connection between the vagus and splenic nerves. Together, these studies demonstrate that cholinergic fibers originating in the DMV regulate splenic TNF production by means of splenic nerve activation. Understanding the anatomic pathway of the

efferent arc of the inflammatory reflex will further aid in therapeutic developments for patients with inflammatory conditions.

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Poster

594. Neuroimmunology: Regulating Systems

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Program #/Poster #: 594.23/ZZ14

Topic: F.05. Neuroimmunology

Support: 1R35GM118182-01

Title: Recording neural activity of intact nodose ganglia to examine TRPA1 in vagal afferent signaling

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Abstract: Electrical signals within central and peripheral nerves form the basis of communication throughout the brain and body. The vagus nerve is a major conduit for sensory information carrying electrical signals from the major internal organs, including the heart, stomach, lung, and abdominal viscera. Cell bodies of the vagus nerve fibers reside in the nodose ganglion at the base of the skull. In order to study how specific afferent information is organized within this vagus nerve, we developed an *ex vivo* preparation to image, record, and electrically stimulate intact NG and vagus nerve. To image calcium transients, which indirectly reflect action potentials, we created *Vglut2*-GCamp3 mice that express fluorescent calcium indicators in glutamatergic neurons. Then we examined the distribution of transient receptor potential ankyrin 1 (TRPA1) ion channels in the nodose ganglion. We observed that 8.3% of identified active neurons responded to polygodial (200 μM), a specific agonist of TRPA1, when applied directly to the perfusing solution. An additional 26.7% of neurons responded to capsaicin (100 μM) through activation of TRPV1 (vanilloid 1) channels. Amongst the TRPA1-responsive neurons, a subset responded with fast rise-time fluorescence intensity changes, while another subset exhibited a gradual intensity change. This may reflect differential activation of TRPA1 channels that reside at the cell bodies or on the vagus nerve fibers. TRPV1-selective responses were more uniform with a fast rise-time of fluorescence intensity change. Electrical stimulation of the attached vagus nerve via two-channel electrodes failed to replicate this selective activation.

Together, these results reveal a TRPA1-specific subset of sensory neurons in the nodose ganglion with varying neural response properties. We intend to utilize this *ex vivo* imaging preparation to dissect molecular details of afferent vagus circuitry by combining it with transgenic, knockout, or cre-loxP mice.

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Poster

594. Neuroimmunology: Regulating Systems

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Program #/Poster #: 594.24/ZZ15

Topic: F.05. Neuroimmunology

Support: Feinstein Institute Internal funds, General Electric (GE) and United Therapeutics (UT)

Title: Bench test validation of a novel flexible microelectrode for stimulating and recording from murine small diameter nerves for bioelectronic medicine

Authors: *T. LIU[1], J. D. FALCONE[1], J. WANG[1], M. OCHANI[1], D. D. POGUE[1], T. DATTA[1], R. SHARMA[2], H. S. SOHAL[1], L. RIETH[1]
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Abstract: Bioelectronic medicine requires the ability to monitor and/or stimulate neural signals longitudinally to treat disease states. Currently, there exists no effective electrode implants to record and stimulate autonomic nerves chronically in the mouse model. The implicit design challenges of chronically interfacing with the mouse cervical vagus nerve include developing an electrode that will be minimally invasive and conform to the 100 µm diameter nerve, as well as remain chronically viable for stimulation and recordings *in vivo*. Here we characterize a microfabricated flexible electrode through initial bench studies to validate the chronic insulation and stimulation integrity to determine device lifetime. Two- and three-contact flexible electrodes were fabricated using state-of-the-art iridium oxide for metallization and polyimide (PI-2611) for insulation. To evaluate the integrity of the polyimide, an accelerated soak test in phosphate-buffered solution (PBS) was conducted. Interdigitated electrodes (IDEs) were placed in PBS at 57°C (4x acceleration) for 20 days. The electrical impedance spectroscopy (EIS) was measured before, at regular intervals during, and after the test. No changes were observed in the EIS plots over time, suggesting insulation stability for > 180 days. To evaluate the stability of the iridium oxide, stimulation stability tests were performed. Flexible electrodes were placed in PBS and up to 100 million biphasic pulses (166 µs phase with a 66 µs interphase delay) with current

amplitudes ranging from 100 µA to 1000 µA were applied. EIS and cyclic voltammetry (CV) were measured before and after the pulse trains were applied. Initial results suggest that iridium oxide is a stable metal for long-term stimulation applications. These bench test results corroborate our *in-vivo* chronic performance, where viable SNR and impedances were obtained throughout a 21 day implantation period from the mouse cervical vagus nerve. Development of such technology will allow chronic electrophysiological experiments on mechanisms and treatments of disease and provide real-time closed loop treatment for bioelectronic medicine, with a goal of eventual clinical translation.

Disclosures: T. Liu: A. Employment/Salary (full or part-time):; Feinstein Institute for Medical Research. **J.D. Falcone:** None. **J. Wang:** None. **M. Ochani:** None. **D.D. Pogue:** None. **T. Datta:** None. **R. Sharma:** None. **H.S. Sohal:** None. **L. Rieth:** None.

Poster

594. Neuroimmunology: Regulating Systems

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Program #/Poster #: 594.25/ZZ16

Topic: F.05. Neuroimmunology

Support: NIH/NIAID-5P01-AI073693-06
NIH/NIAID-4P01-AI102852

Title: Disrupted place cell encoding and theta-gamma coupling in the hippocampus of a murine model of lupus

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Abstract: A poorly understood facet of lupus is its neurological component, known as neuropsychiatric systemic lupus erythematosus (NPSLE). Patients with NPSLE display severe cognitive impairment, particularly in the spatial domain. We have studied a mouse model of NPSLE in which animals carry a lupus antibody (termed DNRAb) that binds DNA and the GluN2A and GluN2B subunits of the NMDAR. Female mice (Balb/c, C57) are passively immunized over a 6-week period with either a lupus-inducing antigen (DNRAb+) or a control antigen (DNRAb–). A month later, the blood brain barrier is abrogated to allow antibody entry to the hippocampus. We measure spatial cognition with the object-place memory (OPM) task, consisting of 3 phases (T1, T2 and T3). For T1, mice navigate an empty chamber (40x40 cm) for 15 min. For T2 (5 min), mice are placed in the chamber with two identical objects. After 10-min rest, for T3 (5 min), they return to the chamber in which one of the objects has been moved. A discrimination ratio (based on the time exploring the objects in T3) reveals that DNRAb+ mice

examine the moved object significantly less than controls, indicating that they have a reduced ability to recognize the novel position of an object. To further investigate the neural substrate for this impairment, mice (9 DNRAb+, 11 DNRAb–) were implanted with tetrode arrays targeting the CA1 region of hippocampus, which is a crucial brain structure for spatial encoding. *In vivo* electrophysiology recordings were conducted during the OPM task and were analyzed via spike sorting (Spike2) to reveal place cell properties of CA1 neurons, as well as power spectral densities of network oscillations (Matlab, Chronux). We find abnormal place cell properties in DNRAb+ mice, such as expanded place field size, reduced stability, and lower spatial information when compared to DNRAb– mice. Vector analysis of object movement vs. place cell remapping (between T2 and T3) show that DNRAb– place cells shift in the direction of the moved object, whereas DNRAb+ place cells have no preferential shift directionality. Bayesian path reconstruction analysis reveal that DNRAb+ place cells have significantly higher error compared to the DNRAb– group. Moreover, we find significantly altered co-modulation of theta-gamma oscillations when the mice examine the moved object. Thus, our studies reveal that the CA1 ensemble encodes critical aspects of the OPM task through place cell dynamics and theta-gamma coupling. The disruptions of these processes caused by DNRAbs may explain the abnormal spatial encoding that occurs in NPSLE. Our data offer a neural substrate for bioelectronic therapies aimed to alleviate NPSLE-related cognitive impairment.

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Poster

594. Neuroimmunology: Regulating Systems

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Topic: F.05. Neuroimmunology

Support: Feinstein Institute Internal Funds from Center of Bioelectronic Medicine

Title: Validation of awake chronic functional recordings in the murine cervical vagus nerve with a low-cost, rapid prototype wrappable microwire electrode for high-throughput chronic interfacing

Authors: J. D. FALCONE[1], T. LIU[2], L. GOLDMAN[2], D. D. POGUE[2], M. STRAKA[2], L. RIETH[2], C. E. BOUTON[2], *H. SOHAL[2]
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Abstract: Bioelectronic medicine requires the ability to monitor and modulate nerve activity longitudinally for rectifying disease states, in a closed-loop manner. Currently, no effective rapidly-manufactured, low-cost chronic peripheral interfacing strategies exists in rodents,

particularly in mice. Additionally, for clinical relevance, preclinical recordings and stimulation should be conducted in awake animals to better mimic the clinical environment and to eliminate anesthesia as a confounding variable in preclinical studies. Here we present, to our knowledge, the first functional recordings for the cervical vagus nerve in an awake mouse model in a chronic period ranging up to 60 days.
BALB/c mice and Sprague Dawley Rats were implanted with custom-designed wrappable microwire electrodes on the cervical vagus nerve. Three or more teflon-insulated platinum wires (50 μ;m diameter) were de-insulated and wrapped around the vagus nerve in an overhand knot, after which kwik-sil (silicone elastomer) was applied to provide insulation. Electrodes were compared to commercial Cortec and Microprobe electrodes in an anesthetized acute model in terms of compound action potential (CAP) quality. Next we developed a novel awake model for recording from the mouse cervical vagus nerve and evaluated CAP quality.
The wrappable microwire electrode showed similar cervical vagus nerve recording performance (mean signal-to-noise (SNR) and mean peak-to-peak (P2P) amplitude) to commercial Cortec and Microprobes cuff in the acute anesthetized preparation for compound action potential (CAP) quality. For spontaneous chronic awake recordings, we recorded cervical vagus nerve activity across multiple days with all animals achieving recordings between 30 and 60 days (n = 8) with acceptable SNR (>1.3). There was no significant differences in SNR over time or between animals, showing the stability and reproducibility of this method. Further 5 out of 8 animal had stable impedances across the chronic implantation period hinting at a stable electrode-tissue interface. Initial Hematoxylin and Eosin staining of the electrode implanted nerve compared to the control naive nerve showed no significant differences in the number of visible axons. We translated this design into a chronic rat cervical vagus nerve interfacing model, where we recorded viable signals for a period of 105 days.
These initial results suggest an effective strategy for producing a rapidly-manufactured, low-cost chronic interface for the mouse cervical vagus nerve and other small nerves in acute and chronic experimental paradigms, which can be easily adopted by other research groups.

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Poster

594. Neuroimmunology: Regulating Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 594.27/ZZ18

Topic: F.05. Neuroimmunology

Support: Internal funds from Feinstein Institute for Medical Technology

Title: A novel flexible microelectrode for stimulation and recording in acute and chronic awake models in murine small diameter nerves for bioelectronic medicine

Authors: *J. FALCONE[1], T. LIU[1], J. WONG[1], M. OCHANI[1], D. POGUE[1], R. SHARMA[2], T. LEVY[1], T. ZANOS[1], T. DATTA[1], L. RIETH[1], H. SOHAL[1]
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Abstract: Bioelectronic medicine utilizes recording, stimulation, and modulation of neural tissue to monitor and relieve disease symptoms. Currently, there exists no effective electrode implant for autonomic nerves in the chronic mouse model, especially in models of inflammatory diseases (e.g. cervical vagus nerve). The design challenges of chronically interfacing with the mouse cervical vagus nerve include placement of electrodes to induce minimal damage while ensuring good electrical contact with the nerve (~100 μm in diameter). Here we characterize a microfabricated, flexible electrode designed to overcome these challenges with bench studies, and both acute and chronic preparations, in terms of stimulation and recording.
Two- and three-contact flexible electrodes were fabricated using state-of-the-art iridium oxide for metallization and polyimide (PI-2611) for insulation. Bench tests to evaluate the lifetime of the electrode included an accelerated saline soak and a stimulation stability test, and electrical impedance spectroscopy were measured. The recording and stimulation efficacy of the electrodes was tested in an acute *in vivo* mouse preparation. Briefly, the left cervical vagus nerve of the mouse was exposed, and the electrodes were placed around the nerve. The nerve was stimulated with a train of 10 biphasic pulses at current amplitudes from 200 to 1000 μA. The resulting compound action potentials (CAPs) were recorded. The electrodes were chronically implanted and characterized through weekly stimulation, and evoked CAP quality was accessed. Further, we tested a recently characterized novel awake model for spontaneous chronic recordings of the cervical vagus nerve in mice (this data is being presented at this conference).
Accelerated saline soak tests determined a lifetime over 180 days for the polyimide, which is compatible for chronic murine electrophysiology**.** Acute stimulation produced high-fidelity CAPs (SNR > 1.3) demonstrating initial validation of the device. In the chronic model, we were able to evoke and record CAP activity through stimulation. High fidelity CAPS were recorded from spontaneous activity for 21+ days along with acceptable *in vivo* impedances at 1 kHz (< 100 kΩ), showing viability of the implant over the indwelling period.
A flexible electrode for interfacing with the mouse vagus nerve has been developed. Bench testing and *in vivo* characterization demonstrated chronic stability at 21 days for both recording and stimulation. Development of such technology will allow for chronic electrophysiological studies on mechanisms and treatments of disease and provide real-time closed loop treatment for bioelectronic medicine.

Disclosures: **J. Falcone:** A. Employment/Salary (full or part-time):; Feinstein Institute for Medical Research. **T. Liu:** A. Employment/Salary (full or part-time):; Feinstein Institute for Medical Research. **J. Wong:** A. Employment/Salary (full or part-time):; Feinstein Institute for Medical Research. **M. Ochani:** A. Employment/Salary (full or part-time):; Feinstein Institute for Medical Research. **D. Pogue:** A. Employment/Salary (full or part-time):; Feinstein Institute for

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Poster

594. Neuroimmunology: Regulating Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 594.28/ZZ19

Topic: F.05. Neuroimmunology

Title: Electrical stimulation of the afferent cervical vagus nerve mediates skeletal muscle glucose uptake

Authors: *E. B. MASI[1,3], H. SILVERMAN[1], T. TSAAVA[1], J. NEWMAN[1], M. ADDORISIO[1], C. BOUTON[2], S. S. CHAVAN[1], K. J. TRACEY[1,2,3]
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Abstract: The central nervous system regulates glucose homeostasis. The vagus nerve is a major conduit of autonomic sensory and motor information that innervates all of the major organs involved in glucose homeostasis. Here, we describe a novel mechanism by which afferent vagus nerve signaling lowers circulating blood glucose by mediating increased glucose uptake in skeletal muscle. We observed that selective vagus nerve stimulation lowers blood glucose levels within 20 minutes. Interestingly, glucose modulating effects of vagus nerve stimulation is abrogated in animals subjected to proximal vagotomy, indicating that afferent vagus nerve signaling is required. No significant changes in circulating levels of insulin, glucagon, leptin or GLP-1 were observed following vagus nerve stimulation. Skeletal muscle glucose uptake through the GLUT4 transporter is a major contributor to maintaining glucose homeostasis. To determine whether glucose uptake in the muscle is necessary for this effect, we generated transgenic mice devoid of the GLUT4 transporter in MCK skeletal muscle using the cre-lox system. While littermate controls had a significant drop in circulating glucose after vagus nerve stimulation, the animals with the GLUT4 transporter deficiency in skeletal muscle failed to respond to vagus nerve stimulation. Next, to test whether vagus nerve stimulation can modulate glucose homeostasis during hyperglycemia, obese ob/ob mice with baseline glucose over 250 mg/dl were subjected to vagus nerve stimulation. A significant decrease, with an average of 70 mg/dl drop in blood glucose levels was observed in ob/ob mice as compared to the unstimulated

controls. Together, these findings describe an insulin-independent mechanism of increasing glucose uptake in skeletal muscle *via* afferent vagus nerve signaling, leading to an acute decrease in circulating glucose levels in hyperglycemic animals.

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Poster

594. Neuroimmunology: Regulating Systems

Location: SDCC Halls B-H

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Program #/Poster #: 594.29/ZZ20

Topic: F.05. Neuroimmunology

Support: NIH NS036960
P50 NS062684
NIH NS067469

Title: From the gut to the brain: Intestinal inflammation as a driver of parkinsonian neuropathology

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Abstract: The etiology of Parkinson's disease (PD) remains uncertain, and by the time the characteristic motor impairments manifest, extensive, irreversible neurodegeneration has already occurred. Gastrointestinal (GI) problems are also common features of PD, however, and they frequently manifest years before the development of motor symptoms. This has led to the theory that PD pathology could initiate in the intestine before advancing to the central nervous system (CNS). Given the abundant evidence supporting a role for inflammation in neurodegenerative disease, we investigated whether intestinal inflammation could mediate the progression from digestive dysfunction to CNS neuropathology in PD. In a large-scale human study, we confirmed that the majority of PD patients experienced GI problems, and we identified elevated levels of specific soluble inflammatory mediators in stool from PD patients compared to controls. We determined that this inflammatory profile did not emerge as a result of advanced age or disease duration, suggesting that GI inflammation is involved in earlier stages of PD. Evaluation of colonic biopsies from PD patients affirmed these findings, revealing evidence of substantially increased immune cell infiltration, proinflammatory activity, and oxidative stress in gut tissue

from PD patients compared to controls. We then utilized mouse models to evaluate the impact that colonic inflammation could exert on neuron health and function in the brain. Dextran sodium sulfate was used to induce colitis in wild type mice and mice lacking RGS10, a regulator of G-protein signaling which has been reported to suppress inflammatory activity in myeloid cells and to protect against inflammation-induced parkinsonian neuropathology. We discovered that the induction of damage and inflammation in the intestine was sufficient to perturb the functionality of dopaminergic neurons on its own, reducing levels of tyrosine hydroxylase and modulating dopamine transporter expression. We also found that colitis rendered mice more susceptible to the effects of the neurotoxic agent MPTP. The severity of certain neurological changes correlated with the severity of colitis in our model, further substantiating the relationship between GI inflammatory activity and central neuropathology. Our findings confirm that intestinal inflammation is present in PD and that such inflammation can induce dopaminergic neuropathology, lending support for the gut-to-brain theory of PD pathogenesis.

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Poster

595. Neuroimmunology: Behavioral Effects

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Topic: F.05. Neuroimmunology

Support: HHMI Grant 5007536
Pomona College Independent Research Grant

Title: Immune system response in zebrafish with disrupted sleep

Authors: A. R. PHILLIPS[1], C. N. NGO[2], D. A. LEE[3], G. OIKONOMOU[3], D. A. PROBER[3], *A. CHEN[4]
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Abstract: Immune interactions with the central nervous system (CNS) are critical nodes in neurological disease pathology; however immune-CNS interactions have been less studied in the role of sleep homeostasis. Recently, cytokines released from immune cells have been shown to act as neuromodulatory factors. In sleep regulation, acute sleep deprivation activates pro-inflammatory pathways; however, chronic sleep deprivation appears to suppress the immune system. Here, we utilize zebrafish to examine if cytokine expression differs between wild-type and zebrafish sleep mutants and whether newly identified sleep regulators modulate sleep-wake behavior through an immune-related mechanism. Zebrafish larvae serve as a simple and cost-

effective model to study the interaction between the immune system and neural circuits. Activation of the inflammatory pathway was examined by quantifying interleukin 1β (IL-1β), Interleukin 6 (IL-6) and nuclear factor κB (NF- κB) transcripts using qPCR. We report upregulation of IL-1β in serotonin-deficient (tph2-/-) mutants. We were unable to detect a difference in cytokine expression in our examination of zebrafish larvae with hypocretin overexpression (hsp:hcrt), neuropeptide VF overexpression (hsp:NPVF), deletion of neuropeptide VF (npvf -/-), deficiency of melatonin production (aanat2-/-), deficiency of histamine production (hdc -/-), or pharmacologically inhibited histamine receptor 1 (Hrh1) signaling when compared to wild-type relatives. Our data suggest that not all sleep regulators noticeably impact the immune system; however there may be an activated immune response in serotonin mutants. This data further validates the use of zebrafish in understanding vertebrate sleep mechanisms and opens new avenues in using zebrafish to explore neuroimmunological regulation of behavior.

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Poster

595. Neuroimmunology: Behavioral Effects

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Topic: F.05. Neuroimmunology

Support: NIH Grant R15AG052935

Title: Adult and aged TLR4 deficient mice show sex-dependent enhancements in spatial memory and alterations in interleukin-1 related genes

Authors: *R. A. KOHMAN, O. V. POTTER, M. E. GIEDRAITIS, C. D. JOHNSON, M. N. COX
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Abstract: Toll-like receptor-4 (TLR4) is a transmembrane receptor that initiates an immune response following a bacterial infection or host derived molecules associated with cellular distress. Beyond triggering inflammation, TLR4 has been implicated in modulating behavior and cognitive processes in a physiologically normal state, as young adult TLR4 deficient mice show learning enhancements in select tasks. Currently unknown is whether these benefits are present in both sexes and persist with aging. The present study evaluated spatial memory, anxiety-like behavior, and central levels of pro- and anti-inflammatory molecules in adult (4-5 months) and aged (18-19 months) TLR4 deficient (TLR4-/-) and wild type (WT) male and female mice. Results confirmed that TLR4-/- mice show enhanced spatial memory compared to WT mice.

These effects were age- and sex-specific, as memory retention was superior in the TLR4-/- adult males and aged females. While TLR4-/- mice showed aged-related changes in behavior, these changes were attenuated relative to aged WT mice. Further, aged TLR4-/- mice showed differential expression of molecules involved in interleukin (IL)-1 signaling in the hippocampus. For instance, aged TLR4-/- females showed heightened expression of IL-1 receptor antagonist (IL-1ra) and the IL-1 accessory proteins AcP and AcPb. Collectively, these data provide the initial evidence that TLR4 deficiency enhances cognitive function and modulates the inflammatory profile of the hippocampus in a sex- and age-dependent manner.

Disclosures: R.A. Kohman: None. **O.V. Potter:** None. **M.E. Giedraitis:** None. **C.D. Johnson:** None. **M.N. Cox:** None.

Poster

595. Neuroimmunology: Behavioral Effects

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 595.03/ZZ23

Topic: F.05. Neuroimmunology

Support: NICHD 083791-02

Title: Central immune alterations in a gestational stress model of postpartum depression

Authors: *C. POST[1], A. CASTANEDA[1], P. BANTA[2], L. NELSON[2], A. SAULSBERY[1], K. LENZ[3], B. LEUNER[3]
[1]Psychology, [2]Neurosci., [3]Psychology & Neurosci., The Ohio State Univ., Columbus, OH

Abstract: Postpartum depression (PPD) is a common complication following childbirth experienced by 15% of all new mothers. Despite its prevalence and adverse consequences for women and their children, the causes of PPD remain unclear. To date, research investigating the factors contributing to PPD has largely focused on hormonal fluctuations, although increasing attention has been given to the potential role of the immune system. Importantly, immune mediators have only been examined peripherally in depressed mothers and thus little is known about how the brain's immune system is modified in PPD. To address this gap, we used an animal model of PPD based on a well-known risk factor, gestational stress, and evaluated the maternal neuroimmune system focusing on the medial prefrontal cortex (mPFC), a key mood-related brain region implicated in PPD. Pregnant Sprague-Dawley rats were subjected to chronic variable stress from gestation days (GD)7-20 or were unstressed and then sacrificed either one day before (GD21) or one week after (postpartum day 8, PD8) delivery. Brain tissue was collected for qPCR to assess mRNA expression of the pro-inflammatory cytokines interleukin (IL)-1β, interferon (IFN)γ, and tumor necrosis factor alpha (TNFα) as well as the growth factor, insulin-like growth factor (IGF)1. Additionally, CD68, integrin alpha M (ITGAM), complement

component 3 (C3) and complement component 1 (C1q), markers associated with microglial phagocytosis of synaptic elements, were analyzed. Our results show increased expression of IL-1β and IFNγ in the mPFC of gestationally stressed mothers on GD21, suggesting a shift to a pro-inflammatory state. In addition, expression of ITGAM and C1q were increased on GD21 which may further suggest that stress leads to microglia-mediated synaptic remodeling. There was no effect of gestational stress on PD8 for any marker analyzed. Together, these data suggest that gestational stress impacts the maternal neuroimmune system which may contribute to the development of PPD.

Disclosures: C. Post: None. **A. Castaneda:** None. **P. Banta:** None. **L. Nelson:** None. **A. Saulsbery:** None. **K. Lenz:** None. **B. Leuner:** None.

Poster

595. Neuroimmunology: Behavioral Effects

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 595.04/ZZ24

Topic: F.05. Neuroimmunology

Support: NIH grant R01MH106553

Title: Examining the impact of a two-hit model of neuroinflammation on social behavior in male and female juvenile rats

Authors: *A. TURANO, M. S. WOOD, N. A. HAAS, J. M. SCHWARZ
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Abstract: Autism is characterized by impaired social interactions, inadequate verbal and nonverbal communication, restricted interests, and stereotyped behaviors but, the biological causes of these symptoms remain inconclusive. In addition to genetic factors, epidemiological data indicate that environmental factors also contribute to the risk of autism. Neonatal exposure to infectious pathogens is one of these environmental factors, suggesting that activation of the neonatal immune system may contribute to autism pathology. Microglia, the resident immune cells of the brain, perform functions crucial for normal brain development and behavior. According to a "two-hit model of neuroinflammation," neonatal neuroimmune activation causes persistent deficits in microglial functioning, resulting in an exaggerated immune response and significant behavioral deficits following subsequent immune activation later in life. Importantly, males are more likely than females to be diagnosed with autism. During early development, males and females exhibit different microglial phenotypes, possibly leaving males more susceptible to the negative outcomes associated with early-life neuroinflammation. Our goal was to better understand the impact of the two-hit model of neuroinflammation on the development and expression of social behaviors in male and female rats. We first piloted behavioral

paradigms to characterize the development of social behavior in juvenile rats, and then applied the two-hit model of neuroinflammation to determine how immune activation may affect the expression of these social behaviors. We concurrently measured cytokine expression in the male and female juvenile brain. These experiments may help to elucidate when and how a specific environmental risk factor contributes to behavioral outcomes associated with autism**.**

Disclosures: A. Turano: None. **M.S. Wood:** None. **N.A. Haas:** None. **J.M. Schwarz:** None.

Poster

595. Neuroimmunology: Behavioral Effects

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 595.05/ZZ25

Topic: F.05. Neuroimmunology

Support: NIH

Title: The impact of exercise in an enriched environment on Parkinson's disease pathology

Authors: *U. ROY[1], M. GIL[2], G. A. DE ERAUSQUIN[3]
[1]Univ. of Texas Rio Grande Valley, Brownsville, TX; [2]Dept. of Psychological Sci., The Univ. of Texas Rio Grande Valley, Brownsville, TX; [3]Neurol. and Psychiatry, UTRGV Sch. of Med., Harlingen, TX

Abstract: Parkinson’s disease (PD) is a neurodegenerative disorder affecting 4.6 million people worldwide with a projected increase to reach 9.3 million by 2030. Many research studies have shown that exercise in PD helps alleviate some of the clinical symptoms. However, underlying molecular mechanisms about the effect of exercise on the neurodegeneration is poorly understood. A few studies have indicated that physical exercise helps prevent the loss of dopaminergic neurons, which is a hallmark of PD pathology. Nonetheless, these studies didn’t include the environmental factors that might contribute to the neuronal pathology in PD. In this regard, our current work has established that enriched environment (EE) has a neuroprotective effect in PD utilizing an animal model. Briefly, EE for *in vivo* study is referred to as an enriched animal cage containing tubes, shelves, ramps, stairs, and miscellaneous ‘toys’. This set-up is changed twice a week with the aim of continuously encouraging exploration of the environment where two animals were housed together for social interaction. Based on our previous observation, we would like to explore the effect exercise in EE has on PD pathology in a mouse model. Our central hypothesis is that a voluntary exercise in EE will promote the survival of dopaminergic neuron (DAN) in substantial nigra in the brain. Preventing the loss of DAN in the brain can dramatically improve the brain pathology of PD patients. Furthermore, we will also monitor the expression of BDNF, EGF, and DJ1 proteins in the brain which are considered to be hallmark proteins for PD pathology. The proposed work was done with the transgenic mouse

model expressing the mutant A53T human alpha-synuclein protein (A53T mutant). The A53T-mutant mice were exposed to the EE in the laboratory setting as per our previous publication. Their performance-based test was on a running wheel test for behavior performance, open field for general motor activity, and rotarod to measure the motor cognition measurement etc. Following the incubation, animal brain tissue was characterized by BDNF, EGF, DJ1 expression and survival of DAN. This study will further help us in developing novel therapeutic molecules that can be called 'enviromimetics' which can be incorporated into the therapy.

Disclosures: U. Roy: None. **M. Gil:** None. **G.A. De Erausquin:** None.

Poster

595. Neuroimmunology: Behavioral Effects

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 595.06/ZZ26

Topic: F.05. Neuroimmunology

Support: NIH Grants MH111276

Title: Interaction of light, sex and gut microbiota in a diurnal model of depression

Authors: *H. XIONG, Y. LIU, W. LIAO, L. YAN
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Abstract: Gut microbiota plays an important role in human health and has been implicated in mental illness including anxiety and depression. In animal models, it has been shown that alterations in gut microbiota influence depression- and anxiety-like behaviors, likely through modulating the GABAergic and serotoninergic pathways in the brain. However, how gut microbiota is influenced by sex and season, and the extent to which the sex differences and seasonal variation contribute to the prevalence of mood disorder remains unknown. To probe these questions, we utilized a diurnal rodent model, the Nile grass rats (*Arvicanthis niloticus*). In contrast to the commonly used animal models that are nocturnal, the grass rats are active during the day, like humans. When they are housed in a winter-like dim light condition, they show increased depression- and anxiety-like behaviors compared to those in summer-like bright light condition, thus serve as a model for human seasonal affective disorder. Using this model, we explored interactions between host-intestinal microbial communities, sex and lighting conditions. Animals were housed in 12:12 hr light/dark cycle with either dim (~50 lux) or bright light (~1000 lux) during the day (n=6/sex/condition). After 4 weeks, animals were euthanized, then gut and intestines were extracted. Feces in the cecum were collected from each animal for metagenomic analysis of microbial communities. Illumina MiSeq (pair-end 250 bp) targeting on V3_V4 hypervariable regions was used to carry out the sequencing. Fastq files from the high-throughput sequencing were analyzed using Qiime2 to generate taxonomic/phylogenetic data for

statistical analysis. The non-metric multidimensional scaling analysis (NMDS) at phylogenetic levels indicated that light has significant effects on microbial community at levels of Genus (p = 0.03), Order (p = 0.04) and Class (p = 0.04). Further analysis indicated that the abundance of *Ruminococcaceae Oscillospira* was higher under bright light compared to in dim light (5.5 *vs.* 2.2%); while *Verrucomicrobiaceae Akkermansia* was lower under bright light than in dim light (10.5 *vs*. 18.1%). Analysis of variance at Phylum level revealed significant sex differences in *Bacteroidetes* (p = 0.02), and marginal differences in *Firmicutes* (p = 0.07) and *Proteobacteria* (p = 0.06). These results suggest that seasonal fluctuation in ambient light can influence the composition of gut microbial community and the relative abundance of certain gut bacteria differs between males and females. Future studies will explore how the changes in gut microbiota contribute to the depression- and anxiety-like behaviors seen in animals housed in dim light.

Disclosures: H. Xiong: None. **Y. Liu:** None. **W. Liao:** None. **L. Yan:** None.

Poster

595. Neuroimmunology: Behavioral Effects

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 595.07/AAA1

Topic: F.05. Neuroimmunology

Support: University of Amsterdam

Title: Unique neurophysiological vulnerability of the orienting network in inflammation - Evidence from the vaccination model of inflammation

Authors: *L. J. BALTER[1], S. HIGGS[2], S. ALDRED[2], J. A. BOSCH[2], M. T. DRAYSON[2], J. J. C. S. VELDHUIJZEN VAN ZANTEN[2], J. E. RAYMOND[2], A. MAZAHERI[3]
[1]Univ. of Amsterdam, Amsterdam, Netherlands; [3]Psychology, [2]Univ. of Birmingham, Birmingham, United Kingdom

Abstract: Individuals with a psychiatric disorder such as major depression often show poorer cognitive performance including attention deficits. The underlying cause of these deficits may be related to inflammation. However, to date, there has been very little direct investigations into how immune system activation affects attentional control. The current electroencephalography (EEG) study investigated the effects of experimentally induced inflammation on three distinct attentional processes: alerting, orienting and executive control. This double-blinded placebo-controlled within-subjects study (N = 20 healthy males, mean age = 24.5, SD = 3.4) used salmonella typhoid vaccination (0.025 mg; Typhim Vi, Sanofi Pasteur) to induce transient low-grade inflammation; saline was used as placebo-control. In both conditions, participants completed the Attention Network Test while EEG was recorded. Analysis was focused on

modulation of oscillatory EEG activity in the alpha (9-12 Hz) band as well as changes of the N1 event related potential (ERP), locked to onset of cues providing temporal and/or spatial information about upcoming targets.Vaccination increased inflammation, as assessed by IL-6 levels (vaccination +3.9 pg/ml; placebo -.08 pg/ml; p < .001). Greater post-cue alpha suppression (200-350 ms) for the orienting network (double vs. spatial cue) was evident in the inflammation condition relative to the placebo condition (p = .021), suggesting that inflammation led to increased processing of the orienting cue. The N1 amplitude was not affected by condition; however, a greater inflammatory response was associated with an attenuated N1 amplitude in the inflammation condition (rs = -.750, p = .002), suggesting that early sensory processes are inhibited with greater inflammation. No behavioural differences were observed between conditions.The current results revealed a unique neurophysiological vulnerability of the orienting network with increased inflammation, as shown by changes in the modulation of post-cue alpha oscillatory activity and the early N1 ERP component.

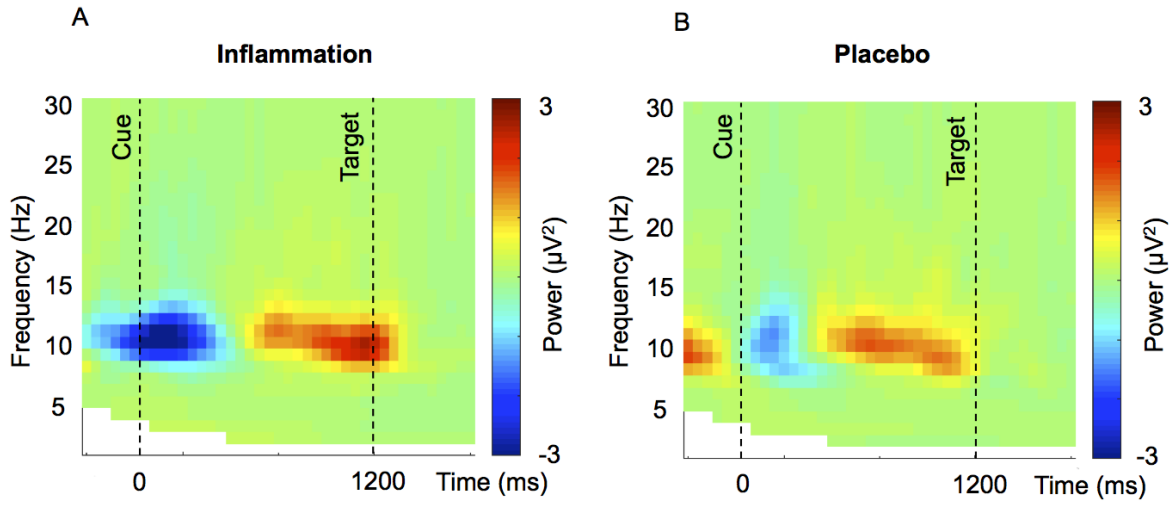

Figure 1a,b. Grand average time-frequency representations of double - spatial cue alpha power difference averaged over parietal-occipital channels for the inflammation (a) and placebo (b) condition. Dotted lines represent cue and target onset, respectively.

Disclosures: L.J. Balter: None. **S. Higgs:** None. **S. Aldred:** None. **J.A. Bosch:** None. **M.T. Drayson:** None. **J.J.C.S. Veldhuijzen van Zanten:** None. **J.E. Raymond:** None. **A. Mazaheri:** None.

Poster

595. Neuroimmunology: Behavioral Effects

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Program #/Poster #: 595.08/AAA2

Topic: F.05. Neuroimmunology

Support: Beckman Scholars award to KTG
UROP Individual Grants to TSM
UROP assistantships to KTG, EJS, TMS, CAG, KSH, and KSS

Title: Treatment with heat-killed mycobacterium vaccae enhances fear extinction in the rat fear-potentiated startle paradigm

Authors: *J. E. HASSELL, JR, K. T. NGUYEN, J. H. FOX, M. R. ARNOLD, P. H. SIEBLER, M. W. LIEB, D. SCHMIDT, E. J. SPRATT, T. M. SMITH, C. A. GATES, K. S. HOLMES, K. S. SCHNABEL, K. M. LOUPY, M. ERBER, C. A. LOWRY
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Abstract: The hygiene hypothesis or "Old Friends" hypothesis proposes that inflammatory diseases are increasing in modern urban societies, due in part to reduced exposure to microorganisms that drive immunoregulatory circuits, and a failure to terminate inappropriate inflammatory responses. Inappropriate inflammation is also emerging as a risk factor for anxiety disorders, affective disorders, and trauma-and stressor-related disorders, including posttraumatic stress disorder (PTSD), which is characterized as persistent re-experiencing of the trauma after a traumatic experience. Traumatic experiences can lead to long-lasting fear memories and fear potentiation of the acoustic startle reflex. The acoustic startle reflex is an ethologically relevant reflex and can be potentiated in both humans and rats through Pavlovian conditioning. *Mycobacterium vaccae* is a soil-derived bacterium with immunoregulatory and anti-inflammatory properties that has been demonstrated to enhance fear extinction in the fear potentiated startle paradigm when given prior to fear training. To determine if immunization with *M. vaccae* after fear conditioning also has protective effects, adult male Sprague Dawley rats underwent fear training on days -37 and -36 followed by immunizations (3x), once per week, with a heat-killed preparation of *M. vaccae* NCTC 11659 (0.1 mg, s.c., in 100 µl borate-buffered saline) or vehicle, and, then, 3 weeks following the final immunization, were tested in the fear-potentiated startle paradigm (n = 12 per group). Rats underwent fear extinction training on days 1 through 6 followed by spontaneous recovery 14 days later (day 20). Rats were euthanized on day 21 and brain tissue was sectioned for analysis of *tph2*, *htr1a*, *slc6a4*, *slc22a3*, *crhr1*, and *crhr2* mRNA expression throughout the brainstem dorsal and median raphe nuclei. Immunization with *M. vaccae* did not affect fear expression on day 1. However, *M. vaccae*-immunized rats showed enhanced between-session and within-session extinction on day 2, relative to vehicle-immunized controls. Immunization with *M. vaccae* and fear-potentiated startle altered serotonergic gene expression in a gene- and subregion-specific manner. These data are consistent with the hypothesis that immunoregulatory strategies, such as preimmunization or treatment with *M. vaccae*, have potential for both prevention and treatment of trauma- and stressor-related psychiatric disorders.

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Poster

595. Neuroimmunology: Behavioral Effects

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 595.09/AAA3

Topic: F.05. Neuroimmunology

Support: NIH Grant P20GM103442
NIH Grant 5P20GM104360-05
UND Health Challenges Seed Program Award

Title: Sensitization to a cow's milk protein results in behavioral changes and altered expression of genes associated with neuroinflammation and vascular integrity in the brain

Authors: *N. A. SMITH, D. L. GERMUNDSON, K. NAGAMOTO-COMBS
Pathology, Univ. of North Dakota Sch. of Med. and Hlth. Sci., Grand Forks, ND

Abstract: Allergic diseases are often comorbid with neuropsychiatric disorders. In particular, food hypersensitivity to cow's milk has been suspected to elicit or exacerbate behavioral symptoms in attention deficit hyperactivity and autism spectrum disorders. Besides genetic susceptibility, etiology of neuropsychiatric disorders may be attributed to epigenomic regulation of the genes that are important for neural functions. We hypothesized that peripheral allergic responses to cow's milk would lead to altered gene expression and DNA modification in the brain, ultimately affecting behavior. Using a mouse model of cow's milk allergy, we investigated transcriptional changes in the intestine and brain as well as DNA hydroxymethylation in the brain. Four-week-old male and female C57BL/6J mice were sensitized to a milk allergen, β-lactoglobulin (BLG), via five weekly oral administrations of 1 mg BLG with cholera toxin (CT) as an adjuvant. Sex-matched sham mice were given the vehicle only containing CT. In the 6th week, all mice were challenged with 50 mg BLG, and anxiety-like and repetitive behaviors were assessed by monitoring their activity on an elevated-zero maze and their grooming behavior, respectively. Transcriptional changes in 4 regions of the brain were determined using RNA sequencing and RT-qPCR. BLG-sensitized mice presented with increased anxiety-like and repetitive behaviors that were associated with elevated BLG-specific serum IgE levels. Expression of a tight junction protein, occludin, was decreased in the gut and midbrain of BLG-sensitized mice, indicating potential degradation of intestinal and blood-brain barrier integrity, respectively. Additionally, expression of the cytokine, TNFα, was induced in the hippocampus of BLG mice, suggesting the presence of an inflammatory response in this region. Furthermore, transcripts of genes implicated in neurovascular development and myelination were differentially regulated in the midbrain of BLG-sensitized mice. When changes in DNA hydroxymethylation

were examined by immunohistochemical staining of brain tissue, a significant increase in 5-hydroxymethylcytosine immunoreactivity was observed in the cerebral cortex of BLG-sensitized brain. These results demonstrated that milk allergy results in behavioral changes and neuroinflammation and suggested that regulation of associated genes may occur via epigenetic DNA modifications.

Disclosures: N.A. Smith: None. **D.L. Germundson:** None. **K. Nagamoto-Combs:** None.

Poster

595. Neuroimmunology: Behavioral Effects

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Program #/Poster #: 595.10/AAA4

Topic: F.05. Neuroimmunology

CONACYT-255317
CONACYT-573686

Title: Hypothalamic lipotoxicity leads to microglia activation and ghrelin signaling disruption in rats

Authors: *R. MALDONADO[1], M. RODRIGUEZ PADILLA[2], A. CAMACHO[3]
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Abstract: Obesity associates with chronic systemic inflammation and insulin resistance. Hypothalamic microglia activation by lipids oversupply has been shown to negatively regulate energy-sensing processes at central and peripheral sites. Here we used an *in vitro* and *in vivo* model to address whether lipid-induced toxicity correlates with an increase in inflammatory cytokine profile and changes in food intake during hypothalamic ghrelin stimulation. Primary microglia cultures and SIM-A9 cell line were incubated by 100 mM palmitic acid, palmitoleic acid, linoleic acid, stearic acid, N-Hexanoyl-D-sphingosine, or 0.1 μg/mL LPS (0111: B4) for 24h. IL-1β, IL-6 and TNF-α production were quantified by ELISA assays. *In vivo* lipotoxicity was performed by i.c.v. administration of LPS (0.1 μg/mL) or palmitic acid (32.4 mM) or artificial cerebrospinal fluid (ACSF) for 5 days following by ghrelin administration. Inflammatory activation was identified by TBK1-NFkbeta protein expression using western blot and ghrelin effects was analyzed by food intake quantification. Our results show that primary microglia and SIM-A9 stimulation by palmitic acid, palmitoleic acid or N-Hexanoyl-D-sphingosine promotes IL-1β, IL-6 and TNF-α and TNF-α release, respectively. Palmitic acid stimulation partially correlates with TBK1 activation evidenced by western blot. Also, we identified that lipotoxic stimulus by i.c.v. palmitic acid administration for 5 days does not disrupt

plasma glucose homeostasis, however, it sensitizes
ghrelin signaling pathway promoting positive food intake following ghrelin administration when compare to rats i.c.v. administered with ACSF. Food intake sensitive to palmitic acid administration correlates with inflammatory activation evidenced by NF-κB whereas a reduction in TBK1 activation in the arcuate nucleus of hypothalamus. In summary, central lipotoxic insult by i.c.v. palmitic acid administration exacerbates the orexigenic effect of ghrelin promoting food intake stimulation which potentially correlates with TBK1-NF-κB pathway activation in arcuate nucleus.

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Disclosures: R. Maldonado: None. **M. Rodriguez Padilla:** None. **A. Camacho:** None.

Poster

595. Neuroimmunology: Behavioral Effects

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Program #/Poster #: 595.11/AAA5

Topic: F.05. Neuroimmunology

Support: OSU Fellowship
5R21MH105826

Title: Microglia regulate developmental myelination, mood-related behavior and stress axis function

Authors: *L. H. NELSON[1], S. WARDEN[1], K. M. LENZ[2]
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Abstract: Microglia, the brain's resident immune cells, are important for many developmental processes. However, less is known about how microglia program behavior. We have previously shown that reversibly depleting microglia from the neonatal brain, using central infusion of liposomal clodronate, decreased anxiety, behavioral despair, and the acute stress response in adulthood (Nelson et al., 2017). To determine the brain region(s) responsible for the dampened stress response we previously observed, we assessed the number of neurons expressing cFos, a marker of neural activation, in limbic brain regions after acute restraint stress in adults. We found decreased cFos expression in the medial prefrontal cortex (mPFC), a stress and anxiety regulating brain area, in clodronate-treated rats relative to controls, suggesting there could be decreased recruitment of stress regulating brain areas following early life microglia depletion. We are currently assessing cFos staining in other brain regions that regulate anxiety and the

stress response such as the amygdala, bed nucleus of the stria terminalis (BNST) and the paraventricular nucleus of the hypothalamus (PVN). Microglia are known to regulate synaptic patterning and developmental myelination, and both of these developmental processes and microglia have been previously linked to early life programming of mood and the HPA axis (Wei et al., 2015; Singh-Taylor et al., 2015; Delpech et al., 2016). Thus we assessed gene expression of dendritic spine protein (*spinophilin)*, myelin-related proteins (*Mbp, Plp1*), and microglia-related genes that have previously been shown to support oligodendrogenesis (*Tnf, Il1b, Igf1)* (Shigemoto-Mogami et al., 2014; Hagemeyer et al., 2017). We analyzed gene expression the mPFC and amygdala at P6, P12 and P22 following microglia depletion to determine whether microglia program behavior via these underlying mechanisms. Relative to controls, microglia depleted animals showed decreases in *Mbp* and *Plp1* at P12 in the amygdala and mPFC, and these decreases persisted to P22 in the amygdala. There was no difference in *spinophilin* gene expression across conditions at any age. At P6 we found decreased *Igf1* in the prefrontal cortex and amygdala, and decreased *Tnf* in the amygdala, but there was no difference in *Il1b*. We are currently determining whether microglia regulate oligodendrocyte progenitor cell proliferation or differentiation into oligodendrocytes in stress-regulating brain areas and white matter tracts throughout the brain and if there are changes in myelination following early life stress. These studies will elucidate the role of microglia in normal and abnormal brain development.

Disclosures: L.H. Nelson: None. **S. Warden:** None. **K.M. Lenz:** None.

Poster

595. Neuroimmunology: Behavioral Effects

Location: SDCC Halls B-H

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Program #/Poster #: 595.12/AAA6

Topic: F.05. Neuroimmunology

Title: Calorie restriction only partially attenuates sickness behaviour induced by a viral mimic polyinosinic:polycytidylic acid (poly I:C)

Authors: *S. KENT, L. KIVIVALI, K. CHONG, A. KIRBY
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Abstract: Calorie restriction (CR) extends mean and maximum lifespan in a variety of animals and we have previously demonstrated that CR dose-dependently attenuates lipopolysaccharide (LPS)-induced fever and sickness behaviour. LPS is a bacterial mimetic; however, few studies have explored this phenomenon utilising a viral mimic, such as polyinosinic:polycytidylic acid (poly I:C). The current study aimed to investigate whether a 50% CR for 28 days could attenuate poly I:C-induced fever and sickness behaviour. METHOD: C57BL/6J male mice implanted with biotelemetry devices were housed at 30 ± 2 °C under a 12:12 LD cycle. In a pilot experiment

increasing doses (500, 1000, 2000, and 5000 μg/kg) of poly I:C or vehicle were administered and core body temperature (T_b) and locomotor activity measured for 24 hours. In the main experiment mice with implanted biotelemetry devices were assigned to either *ad libitum* (AL; n = 16) or CR50% (n = 16) groups for 28 days. On day 29, either 5000 μg/kg poly I:C or vehicle was injected and sickness behaviour assessed for 24 hours. RESULTS: In the pilot experiment poly I:C induced a dose-dependent increase in T_b, with the largest dose (5000 μg/kg) resulting in a 1.62 ± 0.23 °C T_b increase from baseline at 7 hours post-injection (p = .016), which was associated with reduced locomotor activity during the subsequent dark phase post-injection (p = .001). The main experiment demonstrated that CR partially attenuated poly I:C-induced fever and sickness behaviour. The AL group experienced a peak in T_b of 2.02 ± 0.22 °C 7 hours post-injection compared to a 0.94 ± 0.27 °C increase in the CR poly I:C at the same time post-injection (p = .004). Locomotor activity was reduced in the CR group only during the light phase (p = .019), most likely due to decreased food-related anticipatory behaviour whereas activity declined in the AL group only during the dark phase post-poly I:C (p = .002). The CR and AL mice demonstrated similar responses after poly I:C on other sickness behaviour measures (weight loss and reduced food intake). CONCLUSION: Poly I:C evoked a partial sickness behaviour response in CR mice, with increased T_b, reduced activity, and weight loss; however, these mice ate all of their allotted food. Given CR can fully attenuate bacterial mimic (LPS) induced sickness behaviour it appears poly I:C may initiate subtlety different pathways and that these pathways may be differentially impacted on by CR. Future research should investigate whether CR impacts on the number or activity of Toll-like receptors 3 and 4 that recognise viral and bacterial mimics.

Disclosures: S. Kent: None. **L. Kivivali:** None. **K. Chong:** None. **A. Kirby:** None.

Poster

595. Neuroimmunology: Behavioral Effects

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 595.13/AAA7

Topic: F.05. Neuroimmunology

Support: Herman Dana Foundation

Title: Platelet activation in postpartum depression

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Abstract: Supported in part by the Herman Dana Foundation.
Abstract:
Background: Altered systemic reactivity during the development of depression after delivery, may point to biomarkers and potentially implicate inflammatory involvement. Methods: Platelet indices after delivery were compared between mothers prospectively diagnosed with depression and resilient mothers. **Results:** Mean platelet volume was significantly increased immediately following delivery suggesting altered activation that accompany the triggering of postpartum depression. Pathway involvement implicate relevant molecular candidates. **Discussion:** Our findings replicate previous reports of platelet activation in depression in the context of a postpartum depressive episode.
Platelet markers may serve as biomarkers, and point to mechanistically relevant molecular targets contributing to the triggering of depression.
Key words: Post Partum Major Depressive Disorder, Blood Mononuclear cells and Gene expression

Disclosures: R. Segman: None. **T. Goltser-Dubner:** None. **T. Shimonovitz:** None. **S. Klar:** None. **L. Canneti:** None. **D. Pevzner:** None. **E. Galili-Weisstub:** None. **D. Hochner-Celnikier:** None.

Poster

595. Neuroimmunology: Behavioral Effects

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 595.14/AAA8

Topic: F.05. Neuroimmunology

Support: Von Humboldt foundation, fellowship for postdoctoral researchers (1156790)

Title: Vulnerability to inflammation-induced neuropsychiatric symptoms in obese individuals: Using the model of lipopolysaccharide administration in humans

Authors: *J. LASSELIN, K. BOY, V. WESKAMP, A. HANDKE, M. UNTEROBERDÖRSTER, A. BRINKHOFF, S. BENSON, H. ENGLER, M. SCHEDLOWSKI
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Abstract: Obesity is associated with an increase prevalence of neuropsychiatric symptoms and diseases, such as depression. Based on the facts that pro-inflammatory cytokines are able to modulate behavior and that obesity is characterized by a chronic low-grade inflammatory state, inflammation has been hypothesized to contribute to the neuropsychiatric comorbidity in obese individuals. However, a causal link between inflammation and the development of neuropsychiatric symptoms is hard to establish in humans. Here, we used an inflammatory stimulus, i.e. the intravenous injection of lipopolysaccharide (LPS), in a double-blind placebo-

controlled design to determine the vulnerability of obese individuals to inflammation-induced behavioral changes. The hypothesis was that obese individuals would show heightened behavioral response compared to normal-weight subjects for the same inflammatory stimulus, reflecting an increased sensitivity to the behavioral effects of pro-inflammatory cytokines. LPS (dose 0.8 ng/kg body weight, adjusted for blood volume in obese subjects) and placebo (saline) were intravenously injected in 14 obese healthy subjects and 23 normal-weight healthy subjects (age 18-34; 19 women/18 men) in a randomized order with 3-4 weeks wash-out. LPS administration induced, in both groups, an acute increase in blood concentrations of the pro-inflammatory cytokines interleukin (IL)-6 and tumor necrosis factor (TNF)-a, as well as in cortisol, sickness symptoms, fatigue, negative mood and state anxiety that peaked 2-3h after the administration. Obese subjects exhibited a faster recovery in IL-6 and cortisol (lower concentrations 3-6h after LPS administration) compared to normal-weight subjects. Similar pattern was observed for the behavioral changes, although this was not statistically significant. The cytokine and cortisol responses to LPS were significantly correlated with the behavioral changes, and obesity did not modulate this association. Taken together, although obesity was associated with an altered immune response to the immune challenge, this population of young and healthy obese individuals appeared to exhibit similar sensitivity to the behavioral effects of pro-inflammatory cytokines as the normal-weight subjects. Further studies will need to determine whether additional psychological and biological factors interact with the state of obesity to increase the risk for inflammation-induced neuropsychiatric symptoms.

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Poster

595. Neuroimmunology: Behavioral Effects

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Program #/Poster #: 595.15/AAA9

Topic: F.05. Neuroimmunology

Support: VA Merit Award I01BX003195-01

Title: Brain-derived neurotrophic factor polymorphism val66met expression in mice is associated with exaggerated behavioral and neuroinflammatory response to peripheral immune challenge

Authors: *A. M. GARRISON, J. C. O'CONNOR
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Abstract: Depression is a debilitating mental illness that affects millions of people worldwide and is not effectively treated with current therapies in all patients. Stress or inflammation, both

environmental factors that can precipitate depression, reduce signaling of brain-derived neurotrophic factor, a growth factor important for survival and function of neurons. Further, the expression of the high frequency human BDNF single nucleotide polymorphism Val66Met results in reduced activity-dependent release of BDNF and is a risk factor for the development of mood disorders. These observations suggest that reduced BDNF contributes to the development of depression, but the neurobiological mechanisms are unknown. We used a mouse model expressing human Val/Val or Val/Met polymorphism to investigate behavioral and neuroinflammatory responses to peripheral injection of the endotoxin lipopolysaccharide (LPS). Following 24 h after LPS injection, we measured preference for a sucrose solution, locomotor activity, and depressive response using tail suspension test. Brain hemispheres were collected for analysis of pro-inflammatory cytokine expression measured via qPCR. We found that Val/Met-expressing mice were sensitive to the LPS-induced reductions in sucrose preference and increases in pro-inflammatory cytokines. These data suggest that functional BDNF is required to maintain inflammatory homeostasis in the brain and prevent subsequent depressive-like behaviors.

Disclosures: A.M. Garrison: None. **J.C. O'Connor:** None.

Poster

595. Neuroimmunology: Behavioral Effects

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Topic: F.05. Neuroimmunology

Support: NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation.

Title: An IL-6 receptor antagonist effectively attenuates postpartum anhedonia in the female rat, but has no effect on anhedonia precipitated by sub chronic stress

Authors: N. A. HAAS, J. GOMEZ, *J. M. SCHWARZ
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Abstract: The National Institute of Mental Health has identified postpartum depression as one of several types of depression that affects 10-15% of all mothers; however, the exact underlying mechanisms that precipitate postpartum depression are still unknown. Similar to the dramatic change in hormone levels that occurs during pregnancy, the peripheral immune system is also significantly altered throughout pregnancy to protect the developing semi-allogenic fetus from being rejected by the maternal immune system (Fallon et al., 2002). We recently determined that there is also a dramatic change in the central immune system during and just after pregnancy in female rats (Sherer et al., 2017). Specifically, we observed depressive-like behaviors on the day of birth that was associated with increased IL-6 expression in the maternal brain on the day of

birth. (Posillico and Schwarz, 2016). Thus, the current study sought to determine whether blocking the function of IL-6, by infusing an IL-6 receptor antibody specifically in the postpartum brain, may prevent the anhedonia observed following birth. For comparison, we also examined whether blocking the function of IL-6 in the brain could prevent the expression of anhedonia caused by a week of forced swim in female rats. Similar to our previous findings, we measured significant anhedonia in postpartum female rats and in female rats that had a week of sub chronic stress. Treatment with an IL-6 receptor antibody into the brain effectively attenuated depressive-like behavior immediately postpartum ($p = 0.026$ vs postpartum IgG control treatment), but interestingly had no effect on the anhedonia produced by sub-chronic stress ($p = 0.790$). Analysis of cytokine expression in the brain revealed that the IL-6 receptor antibody could effectively attenuate the expression of IL-6 ($p = 0.026$) and Brain Derived Neurotrophic Factor ($p = 0.034$) in the medial prefrontal cortex of postpartum females. In contrast, the antibody had no effect on IL-6, BDNF, or other IL-6 signaling molecules in the brain following sub chronic stress. These results suggest that the molecular mechanisms that underlie the onset of anhedonia after birth and sub-chronic stress may be distinct. Moreover, the successful attenuation of postpartum anhedonia following the infusion of an IL-6 receptor antibody suggests that this antibody, or other drugs that affect IL-6 signaling in the brain, may be important potential targets for the relief for the symptoms associated with postpartum depression that may not be fully alleviated by typical antidepressants.

Disclosures: N.A. Haas: None. **J. Gomez:** None. **J.M. Schwarz:** None.

Poster

595. Neuroimmunology: Behavioral Effects

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 595.17/AAA11

Topic: F.05. Neuroimmunology

Title: Effects of chemogenetic inhibition of the ventral hippocampus on anxiety-like defensive behaviors in rats

Authors: *C. R. MAESTAS-OLGUIN[1], S. J. BOUQUIN[2], J. W. FENELLY[2], N. S. PENTKOWSKI[2]
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Abstract: Previous research in rodents and humans has implicated the ventral hippocampus in regulating anxiety. However, many rodent studies examining ventral hippocampal neuronal pathways have utilized lesion studies that create nonspecific, nonreversible alterations to the targeted area. To increase specificity, the present study sought to characterize the unique role of glutamatergic pyramidal neurons located within the ventral hippocampus in the manifestation of anxiety-like behavior during exposure to a variety of threatening stimuli. Five weeks prior to

testing, Long-Evans hooded rats received ventral hippocampal viral-vector infusions expressing either the inhibitory pAAV-CaMKIIα-hM4D-mCherry (DREADD) receptor or pAAV-CaMKIIa-EGFP (GFP). DREADD transfection allowed for the direct, noninvasive inhibition of ventral hippocampal glutamatergic neurons immediately before threat presentation. Animals were evaluated for anxiety-like behaviors including freezing, risk assessment and avoidance during testing in the elevated plus maze, light-dark exploration test and footshock-induced fear conditioning. Analysis revealed a significant effect of DREADD inhibition that was dependent on the type of threat exposure. Specifically, compared to GFP controls, DREADD-induced silencing of ventral hippocampal glutamatergic neurons reduced anxiety-like behavior in the elevated plus maze and light dark test, without affecting fear conditioning. The present results confirm that exposure to anxiety-inducing stimuli provokes activation of ventral hippocampal glutamatergic pyramidal neurons. These data add to a growing literature implicating the ventral hippocampus as a key region involved in modulating anxiety.

Disclosures: C.R. Maestas-Olguin: None. **S.J. Bouquin:** None. **J.W. Fenelly:** None. **N.S. Pentkowski:** None.

Poster

595. Neuroimmunology: Behavioral Effects

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Program #/Poster #: 595.18/AAA12

Topic: F.05. Neuroimmunology

Support: BBSRC Grant BB/N010035/1
10% financial contribution from Clasado Biosciences Ltd

Title: The effects of early-life prebiotic feeding on adult rat hippocampal function, behaviour and gut bacteria

Authors: *S. O. SPITZER[1,2], A. TKACZ[3], E. O. MANN[6], P. S. POOLE[3], D. M. BANNERMAN[4], D. C. ANTHONY[5], P. W. J. BURNET[2]
[2]Dept of Psychiatry, [3]Plant Sci., [4]Exptl. Psychology, [5]Pharmacol., [1]Univ. of Oxford, Oxford, United Kingdom; [6]DPAG, Oxford Univ., Oxford, United Kingdom

Abstract: The intake of oligosaccharide prebiotics – dietary fibres that augment the growth of beneficial gut bacteria – have neurobiological and behavioural effects in adult rodents and can improve cognitive performance. We have shown that neonatal supplementation with Bimuno galacto-oligosaccharides (BGOS) increases NMDA receptor GluN2A protein in the hippocampus of adult rats up to 8 weeks after treatment. It is important to confirm whether early-life prebiotic supplementation is able to improve and maintain brain processes in adulthood, possibly imparting resilience to age-related brain disorders.

This study tested the effects of perinatal BGOS supplementation on hippocampal electrophysiology, behaviour and the gut microbiome at various time points starting immediately after weaning up to adulthood. Suckling SD rat pups were gavaged daily with BGOS or control solution for 21 days, followed by behavioural tests or whole-cell patch-clamp recordings of hippocampal slices.
NMDA/AMPA ratio in CA1 neurons from BGOS rats were no different to controls at any age tested (P22, P56, P128+). However, decay time 1 (double exponential fit on NMDA currents) was diminished in BGOS animals (no interaction effect with age). In spontaneous synaptic events (sEPSCs), BGOS significantly reduced amplitude size (no interaction effect with age) and changed frequency depending on treatment and age (significant for treatment and interaction treatment x age).
In the elevated plus maze (EPM), perinatal BGOS supplementation had anxiolytic effects specifically at P22, when BGOS-treated rats spent more time in open arm, whilst no differences were observed across 3 time points.
To link behavioural and electrophysiology data with metagenomic profiles of gut microbiota, 16S gene was sequenced from faecal samples obtained weekly. Whilst preliminary data showed no differences in abundance of bacterial phyla, it is possible that differences occur on a smaller scale. Studies are underway to test whether the effects of transient post-weaning BGOS supplementation has more sustained effects on brain function throughout the life course.
Our data show that early-life BGOS feeding in healthy rats might not affect overall NMDA receptor expression in CA1, but may change receptor kinetics, which is sustained for at least 8 weeks after treatment. Since sEPSCs characteristics seemed to undergo age-specific changes, BGOS might induce more widespread changes on the hippocampal network long-term. The anxiolytic effects are only short-lived, which may suggest that unlike cognitive processes, changes in emotional behaviour are induced more robustly when bifidobacteria levels are augmented.

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Poster

595. Neuroimmunology: Behavioral Effects

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 595.19/AAA13

Topic: F.05. Neuroimmunology

Support: Natural and Applied Sciences Division, Hope College
Social Sciences Division, Hope College

Title: Latent infection of Herpes Simplex Virus Type I impacts exploratory behavior in mice

Authors: I. HOUGH[1], E. KAIN[1], N. SHAW[2], *G. D. GRIFFIN[3]
[1]Biol., [2]Psychology, [3]Departments of Biol. and Psychology, Hope Col., Holland, MI

Abstract: Herpes Simplex Virus Type I (HSV-1) forms a lifelong infection and affects 70% of the American population (Looker et al., 2015). Tarter and colleagues demonstrated that being seropositive for HSV-1 is associated with diminished cognitive functioning, specifically working memory (2014). To date, no study has tested the direct link between HSV-1 and working memory. In light of this gap, the goal of our study was to examine the impact HSV-1 latency has on behavior, including working memory. The BALB/c and C57/BL6 mouse strains were both used in this study. At six weeks of age, animals were inoculated with the F strain (10^5 plaque-forming units) of HSV-1 via corneal scarification. Forty-five days post infection (dpi) behavioral studies commenced. This included the open field test, a modified Morris Water Maze, alternating T-test, and a novel object recognition task. Thus far, preliminary results reveal that female C57/BL6 mice infected with HSV-1 have a longer latency to enter the center region (compared to uninfected control females; $p<0.05$) at 45 dpi. There was a trend for this when animals were tested again in the open field arena at 166 dpi. The behavior in the open field arena of female BALB/c mice was not affected by HSV-1 latency. However, infected male BALB/c mice did demonstrate a trend to have a greater latency to enter the center zone of the arena (compared to uninfected males; $p<0.1$). HSV-1 latency did not impact locomotor behaviors (total distance traveled and average velocity) of any strain or sex of mice. Analysis of the other behavioral tests is ongoing. Overall, these preliminary results highlight the importance of testing multiple mouse strains and both sexes to understand the impact of infection on adult animal behavior. Lastly, they provide evidence that HSV-1, even while not producing viral proteins, prompts long-lasting changes in animal behavior. These results lend support to the human behavior correlational studies on HSV-1 and provide a platform to better understanding central consequences of the chronic immune response to HSV-1 and similar viruses.

Disclosures: I. Hough: None. **E. Kain:** None. **N. Shaw:** None. **G.D. Griffin:** None.

Poster

595. Neuroimmunology: Behavioral Effects

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Program #/Poster #: 595.20/AAA14

Topic: F.05. Neuroimmunology

Title: Neurochemical alterations underlying traumatic memory formation in a predator exposure model of post-traumatic stress disorder (PTSD)

Authors: *D. P. KELLEY[1], K. E. VENABLE[1], P. EBENEZER[1], C. C. LEE[2], J. FRANCIS[1]
[1]Comparative Biomed. Sci., Louisiana State Univ., Baton Rouge, LA; [2]Comparative Biomed. Sci., Louisiana State Univ. Sch. of Vet. Med., Baton Rouge, LA

Abstract: Innate immune activation is associated with a multiplicity of diseases ranging from diabetes to depression and is associated with activation of the enzyme, indolamine 2,3 dioxygynase (IDO). IDO activity reduces tryptophan availability and produces deleterious tryptophan catabolites (TRYCATS) that are associated with suicide, depression, and aggression. Post-traumatic stress disorder (PTSD) is also associated with excessive innate immune activation as well as elevated rates of suicide, depression, and aggressive behavior, but neither IDO activity or the TRYCAT pathway has been explored in this disorder. Previously, we demonstrated that superoxide, total reactive oxygen species (ROS) and inflammatory factors (TLR4, NF-κB, NALP3, IL-1β, IL-18) are significantly elevated in the brain and plasma and serotonin levels are reduced in the hippocampus and frontal cortex of our predator exposure model of PTSD compared to control animals. Here, we report that IDO expression levels, IDO activity, and TRYCATS are elevated in the hippocampus and plasma ($p<.05$, $n=6$/ group) of our PTSD model. Furthermore, these factors are associated with traumatic memory, anxious behaviors, and neurotransmitter abnormalities. IDO and TRYCATS may contribute to the establishment of the traumatic memory itself through glutamatergic mechanisms as well as link ROS and inflammation to the neurotransmitter malfunction previously observed in this model. Through these mechanisms, TRYCATS may be responsible for a wide range of symptoms in PTSD and connect a preexisting inflammatory milieu with the development of PTSD.

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Poster

595. Neuroimmunology: Behavioral Effects

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 595.21/AAA15

Topic: F.05. Neuroimmunology

Title: Persistent memory deficits and neuroimmune dysfunction after immune challenge

Authors: *D. TCHESSALOVA[1], N. C. TRONSON[2]
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Abstract: The neuroimmune system is critical for maintaining normal neural plasticity and memory function, and acute inflammatory signaling results in striking behavioral changes and memory deficits. Memory impairments and cognitive decline are also observed in patients long after recovery from inflammatory insults, such as major illness or injury. In this study, we aimed

to examine whether dysregulation of memory emerges or persists long after an immune challenge, and the changes in neuroimmune mechanisms that mediate lasting alterations in neural function in females and males. We have recently established a mouse model of memory deficits emerging over the weeks and months following a subchronic peripheral immune challenge in males and females. Mice were given five injections of lipopolysaccharide (LPS: 250 μg/kg), Polyinosinic: Polycytidylic acid (Poly I:C: 6 mg/kg), or saline over 14 days. We evaluated novel object recognition memory and context fear conditioning at one or eight weeks after immune challenge. In females, memory deficits were evident at both one and eight weeks, whereas males only showed memory deficits eight weeks after the last injection. There were also sex differences in the types of memory that were impacted. Males showed disruption of object recognition memory and context fear conditioning, whereas only object recognition memory was impaired in females. To examine whether neuroimmune changes also persist after immune challenge, we assessed blood-brain barrier function using dextran labeling to detect leakage, and microglial activation using Iba-1 staining and morphological analysis. We used RNA-sequencing to identify changes in gene expression that persisted at least 12 weeks after the last injection. We observed dysregulation of immune-related gene expression primarily in males (e.g. complement genes). Together, these findings demonstrate that prior inflammatory insults induce long-lasting memory deficits in both sexes, and that different types of impairments are evident in males and females. Persistent neuroimmune mechanisms may underlie these long-lasting changes in memory. Further, these data suggest that distinct molecular mechanisms mediate memory deficits in males and females. Future work will explore the causal link between memory impairments and long-lasting neural changes following an immune challenge. These studies will help to identify possible mechanisms contributing to memory decline, memory-related disorders, and dementias in men and women following recovery from illness or surgery.

Disclosures: D. Tchessalova: None. **N.C. Tronson:** None.

Poster

596. Biological Rhythms and Sleep: Regulators

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Program #/Poster #: 596.01/AAA16

Topic: F.08. Biological Rhythms and Sleep

Support: NSF - 53-4895-0407

Title: Brain-wide imaging of *Drosophila* sleep/wake behavior at near-cellular resolution

Authors: *P. LUU[1], Y. HAN[2], A. NADTOCHIY[3], D. K. DICKMAN[4], S. E. FRASER[5], T. V. TRUONG[6]
[2]Neurosci. Grad. Program, [3]Biol. Sci., [4]Neurobio., [5]Mol. & Computat. Biol., [6]Translational Imaging Ctr., [1]USC, Los Angeles, CA

Abstract: Sleep is evolutionarily conserved across organisms from nematodes to humans, however its role and function remains only partially understood. Here we aim to quantify the sleeping brain at near-cellular resolution, with brain-wide coverage using GCaMP. We compared the sleeping duration during one photon and two photon point scanning illumination in an effort to later move onto selective volume illumination (SVI). Using SVI along with lightfield microscopy, it is possible to achieve the temporal and spatial resolution needed to capture brain-wide cellular GCaMP signal and it enables the quantification of previously unrecognized neural activity. Our second aim is to determine whether differences exist between induced sleep, sleep after sleep deprivation, and natural sleep.
In a related but parallel effort, we investigated the intrinsic brain activity of flies with a mutation in the insomniac gene during sleep deprivation. This mutation is known to cause a decrease in sleep that is independent from the canonical circadian clock pathway and that does not result in sleep rebound. By expressing GCaMP6s in insomniac positive cells, we expect to find increased neuronal activity in insomniac mutants during sleeping hours and less perturbation after sleep deprivation.

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Poster

596. Biological Rhythms and Sleep: Regulators

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH P01 NS090994
NIH R90 DA033463

Title: The bantam microRNA regulates cell proliferation and sleep in multiple mushroom body output neurons in *Drosophila melanogaster*

Authors: *K. DORFMAN, M. HOBIN, L. C. GRIFFITH
Brandeis Univ., Waltham, MA

Abstract: Post-transcriptional gene regulation by microRNA plays an important role in the regulation of sleep. Using miRNA sponge technology to inhibit miRNA function, a reverse genetics screen to identify microRNAs that regulate sleep in *Drosophila melanogaster* was previously performed. This screen identified the well-conserved microRNA encoded by the *bantam* gene as a positive regulator of sleep. This study sought to further characterize the role of bantam in sleep regulation by mapping its effects to a specific sleep circuit. Cell-type specific knock-downs of bantam were performed using the binary GAL4:UAS expression system, and

sleep was measured using the *Drosophila* Activity Monitoring (DAM) system. We mapped the effect of bantam on sleep to the Mushroom Body Output Neurons (MBONs), a class of neurons divided into 21 subtypes that serve as the output of the mushroom body circuit, and which have been previously implicated in sleep regulation. Using split-GAL4 lines to express the bantam sponge in specific subtypes of the MBONs, it was found that bantam positively regulates nighttime sleep through at least three diverse MBONs: the cholinergic γ2α'1 neurons, the GABAergic γ3 and γ3β'1 neurons, and the glutamatergic γ5β'2a neurons. The effects of bantam on neuronal morphology and cell number of the γ2α'1 and γ5β'2a neurons were assessed by co-expressing a fluorescent marker with the bantam sponge and imaging the neurons using confocal microscopy. Preliminary results indicate that flies expressing the bantam sponge in γ2α'1 and γ5β'2a neurons have a higher number of cells expressing the fluorescent marker. To determine if bantam's effects on sleep occurred through developmental processes or active adult regulation, the temperature inducible *Tubulin-GAL80* was used to knockdown bantam specifically during development or adulthood. These experiments revealed that bantam regulates different aspects of sleep in different developmental stages: expression of bantam early in development is required for normal daytime sleep, whereas expression of bantam in adulthood is necessary for normal daytime and nighttime sleep. Our results identify a role for bantam in the adult regulation of sleep in specific subtypes of MBONs, and a further role in the regulation of MBON cell number.

Disclosures: M. Hobin: None. **L.C. Griffith:** None.

Poster

596. Biological Rhythms and Sleep: Regulators

Location: SDCC Halls B-H

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Topic: F.08. Biological Rhythms and Sleep

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Title: Evolutionarily conserved regulation of sleep by epidermal growth factor receptor signaling

Authors: *D. A. LEE[1], J. LIU[1], Y. HONG[1], A. J. HILL[1], J. M. LANE[2,3], H. WANG[2,3], G. OIKONOMOU[1], U. PHAM[1], J. ENGLE[1], R. SAXENA[2,3], D. A. PROBER[1]
[1]Biol. and Biol. Engin., Caltech, Pasadena, CA; [2]Harvard Med. Sch., Boston, MA; [3]Broad Inst., Boston, MA

Abstract: Sleep is an evolutionarily conserved behavioral state whose regulation remains poorly understood. One approach towards discovering key sleep regulatory mechanisms is to identify systems that do so in both invertebrates and vertebrates. Using zebrafish, we asked whether epidermal growth factor receptor (EGFR) signaling is necessary and sufficient for vertebrate sleep, as for invertebrates. We found that overexpression of the EGFR ligand transforming growth factor alpha (TGFa) increased sleep, while loss of EGFR signaling decreased sleep. Downstream mechanisms through which EGFR signaling promotes sleep are also conserved, as TGFa-induced sleep was suppressed by inhibition of MAPK/ERK, as suggested in *Drosophila*, and by mutation of the RFamide neuropeptide VF (NPVF), similar to *C. elegans*. Additionally, we found that EGFR signaling regulates NPVF expression and NPVF neuronal activity, providing a mechanistic link between these systems. Finally, in a sleep multi-trait genome-wide association study performed using humans from the UK Biobank, we identified significant associations at genomic regions encompassing *ERBB4*, *KSR2*, and *VRK2*, genes in the EGFR signaling pathway. These signals were driven primarily by genetic associations with sleep duration and/or daytime sleepiness. Taken together, these results demonstrate that sleep regulation by EGFR signaling is conserved between invertebrates and vertebrates, and suggests an ancestral role in the regulation of human sleep.

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Poster

596. Biological Rhythms and Sleep: Regulators

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Program #/Poster #: 596.04/AAA19

Topic: F.08. Biological Rhythms and Sleep

Title: Unraveling miR-190 and its role in sleep

Authors: *E. J. RIVERA, P. GOODWIN, M. HOBIN, Z. BLEICHER, L. C. GRIFFITH
Brandeis Univ., Waltham, MA

Abstract: Sleep is a widely conserved behavior and it is known to be regulated by changes in gene expression. However, the molecular basis of the regulation of sleep remains poorly understood. Research from our lab, and elsewhere, supports the idea that microRNAs (miRs) are involved. miRs are short non-coding RNA transcripts (20-24 bp in length) that target specific mRNAs, downregulating their expression. Results from a genetic screen in which miRs were downregulated by expression of transgenes which specifically bind particular miRs (*miR-SPs*), demonstrated that miR-190 is involved in *Drosophila* sleep regulation. Pan-neuronal expression of *miR-190-SP* or mutation of the *miR-190* gene both elicited dramatic changes in *Drosophila*

sleep behavior, including decreased and fragmented total sleep, as well as deficient sleep homeostasis. Expression of *miR-190-SP* in limited numbers of cells in different brain regions using the *Gal4/UAS* system showed that disruption of miR-190 function must occur in a large number of neurons to affect *Drosophila* sleep regulation. At the molecular level, our preliminary data from RNA seq of adult heads showed that pan-neuronal expression of *miR-190-SP* induces an up or downregulation of multiple genes, including 6 genes which are intimately involved in dopamine (DA) signaling, the major pro-arousal system of the fly. Temporally-controlled expression of *miR-190-SP* demonstrated a developmental effect of miR-190 on the regulation of sleep: flies in which miR-190 function was decreased during development showed fragmented and decreased sleep whereas reduction of miR-190 only in the adult stage did not. Taken together, our data suggest that miR-190 functions during development to specify the activity of the adult arousal system.

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Poster

596. Biological Rhythms and Sleep: Regulators

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 596.05/AAA20

Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant NS098173
NIH Grant MH111276

Title: Daytime illumination modulates spatial learning through the orexinergic pathways in the diurnal Nile grass rat (arvicanthis niloticus)

Authors: *L. YAN, J. SOLER, A. NUNEZ
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Abstract: Environmental lighting conditions play a significant role in cognitive function, with the level of illumination positively correlated with cognitive performance in a diverse population of human subjects. However, the underlying neural mechanisms are not-well understood. Utilizing a diurnal rodent model, the Nile grass rat (*Arvicanthis niloticus*), our group has found that the levels of daytime illumination are associated with strength of spatial memory assessed via the Morris Water Maze (MWM) task, such that grass rats housed in a 12:12hr bright light-dark (brLD) cycle over 4 weeks showed superior MWM performance, compared to animals housed in a 12:12hr dim light-dark (dimLD) cycle. The animals in brLD condition also had higher level of hypothalamic orexin A (OXA) expression. Based on those findings, the present study tested the hypothesis that in diurnal mammals, light modulates hippocampal function via

the orexinergic system. In the first experiment, animals housed in dimLD for four weeks were then assigned to two groups to receive either OXA or vehicle solution (intranasally) 2hr prior to the MWM training session over five training days (n=8/group). The OXA treated animals exhibited faster escape latencies during the training sessions and spent more time in the target quadrant during the following probe test compared to controls, suggesting that impaired hippocampal function in dimLD was due to attenuated orexinergic output. In the next experiment, two groups of grass rats (n=12/group) received hippocampal injection of viral vector (AAV) containing either shRNA targeting orexin receptor 1 (OX1R) or scrambled (SC) shRNA into the dorsal CA1 hippocampal subregion, followed by 4 weeks of housing in brLD prior to MWM training/testing. Animals that received OX1R-shRNA showed longer escape latencies during training session compared to the SC-shRNA group, and performed at chance level during the probe trial. The results suggest that brighter illumination enhances spatial learning through the orexin-OX1R pathway to the hippocampus. These findings support the hypothesis that the orexinergic system mediates the effects of light on hippocampal-dependent learning and memory.

Disclosures: L. Yan: None. **J. Soler:** None. **A. Nunez:** None.

Poster

596. Biological Rhythms and Sleep: Regulators

Location: SDCC Halls B-H

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Program #/Poster #: 596.06/AAA21

Topic: F.08. Biological Rhythms and Sleep

Support: NIA intramural grant

Title: Intermittent fasting increases slow wave sleep duration in mice

Authors: *R. WAN, Y. LIU, M. MATTSON
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Abstract: We previously found that the intermittent fasting (IF; alternate day fasting) enhances parasympathetic tone in rats and mice, which manifests as reduced resting heart rate and blood pressure, increased heart rate variability, and enhanced cardiovascular stress resistance. Here we investigated the possible effects of IF on brain neuronal network activity and sleep duration and quality in mice. A radio telemetric device was implanted and used to monitor the cortical and hippocampal electroencephalogram (EEG) and locomotor activity in C57BL/6j mice maintained either with *ad libitum* (AL) or IF regime (Every-Other-Day fasting) for an over 5 weeks' period. The EEG activity and response to body restraint stress were examined prior to and 5 weeks after the IF regime was initiated. The results showed that: 1) IF mice increased their sleep time, particularly in 'deep sleep (slow wave sleep 2 ; SWS2), during night period on a fasting night; 2)

IF mice became more active during day period, particularly, an increased locomotive activity indicating an anticipatory food availability as the feeding time getting closer at the end of fasting day time; 3) IF reduced EEG power in lower frequencies mainly in delta (0-4 Hz), theta (4-12 Hz), sigma (12-16 Hz) and beta (16-24 Hz). The reductions occurred mostly in day period; 4) The blood glucose level and body temperature increased in response to body restraint stress. However, the magnitude of increase in blood glucose level was significantly lower when the stress response was retested on fasting and feeding days 5 weeks after mice were maintained with IF regime. Although there was a significant reduced lower spectral power in IF mice prior to stress, there was discernable effect of IF on EEG activity during or after stress.

Disclosures: R. Wan: None. **Y. Liu:** None. **M. Mattson:** None.

Poster

596. Biological Rhythms and Sleep: Regulators

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Program #/Poster #: 596.07/AAA22

Topic: F.08. Biological Rhythms and Sleep

Support: wellcome trust

Title: Neural circuits in the VTA govern vigilance state

Authors: *X. YU, N. P. FRANKS, W. WISDEN
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Abstract: We screened for novel circuits in the mouse brain that determine vigilance states. Using chemogenetic activation/inhibition combined with EEG/EMG recordings, we converged on specific neurons in the VTA. We found that activation of glutamatergic neurons, which were wake- and REM-active, produced long-lasting wakefulness. In contrast, chemogenetic activation of GABAergic VTA neurons elicited long-lasting NREM-like sleep akin to sedation. This occurred via local inhibition of glutamatergic and dopaminergic neurons in the VTA. Our findings suggest that the VTA, widely investigated for its contribution to goal- and reward-directed behaviors, contains circuitry with an unexpected role in sustaining and limiting wakefulness.

Disclosures: X. Yu: None. **N.P. Franks:** None. **W. Wisden:** None.

Poster

596. Biological Rhythms and Sleep: Regulators

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH-R01NS085477
2P01 HL095491

Title: Role of parabrachial glutamatergic signaling in the regulation of arousal

Authors: *M. A. KHANDAY[1], S. KAUR[1], C. B. SAPER[2]
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Abstract: The parabrachial nucleus (PB) of the brainstem is known to regulate cortical activation and behavioral arousal. Chemogenetic and optogenetic activation of PB increases wakefulness while cell specific lesion or chronic deletions of glutamatergic transmission in PB neurons induce non rapid eye movement sleep (NREM sleep). Based on the varied outputs of the PB, this area exerts powerful control over a wide range of neurobiological functions, including cortical arousal and therefore, it is important to understand how different subnuclei of PB affect cortical arousal. As chronic deletions of cells suffer from compensatory effects over time, it is important to address this using acute inhibition of various PB subnuclei. Therefore, in our study, we acutely modulated lateral (PBl) and medial part of PB (PBm), and investigated its effects on sleep-wakefulness. We used adult male mice which express Cre- recombinase in the glutamatergic cells, also called vesicular glutamate transporter 2 (Vglut2)-Cre. Vglut2-Cre mice (n=9) were bilateral injected stereotaxically with Adeno-associated virus (AAV) that express Ef1α::hM4Dq-mCherry into either PBm (AP = -5.2 mm, ML = ± 1.1 mm, DV = -2.6 mm), or PBl (AP = -5.3 mm, ML = ± 1.3 mm, DV = -2.4 mm) and implanted with EEG/ EMG electrodes for recording sleep. AAV was optimally expressed at 4weeks post injection, after which mice were recorded for sleep after acclimatization to the recording apparatus for a week, after intraperitoneal injection of either saline (Sal) or 0.3mg/Kg of clozapine N-oxide (CNO). Both injections were done at the early dark onset (7pm). Sleep-wake data was analyzed and compared between the Sal and CNO injection days. The injection sites for the hM4Dq were analyzed by immunohistochemistry post-hoc. We observed that out of n=9 injected mice, some had hM4 localized to PBl (n=4) and other were localized to the PBm (n=5). CNO induced inhibition of the PBm increased NREM in the dark phase by 55%; while inhibition of PBl promoted 43% increase, compared to after Sal injection. Furthermore, animals with PBl inhibition showed almost doubling of NREM 4-6h post CNO and then returned to baseline, while animals with PBm inhibition showed sustained increases of 30-90% for the entire 8-9h post CNO period. The

differential effects on NREM sleep due to inhibition of PBm and PBl could be attributed to varied PB afferent projections to arousal centers. Further, investigations are needed using cell specific markers for the PB subnuclei that can delineate the precise circuitry regulating the arousal

Disclosures: M.A. Khanday: None. **S. Kaur:** None. **C.B. Saper:** None.

Poster

596. Biological Rhythms and Sleep: Regulators

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Program #/Poster #: 596.09/AAA24

Topic: F.08. Biological Rhythms and Sleep

Support: Conacyt 243298
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Title: Adiponectin and leptin regulate VLPO activity

Authors: *O. RAMÍREZ PLASCENCIA, S. CÁRDENAS-ROMERO, L. AZUARA-ALVAREZ, A. BÁEZ-RUIZ, M. MIRANDA-MORALES, M. ATZORI, N. SADERI, R. C. SALGADO-DELGADO
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Abstract: An increasing amount of evidence indicates that overweight and obesity modify the physiological patterns of sleep and the chronotype, in both humans and rodents. Currently, there is a little understanding regarding the mechanisms of this association, with the exception that several areas within the central nervous system involved in sleep regulation, express receptors for leptin, which is a hormone secreted by the white adipose tissue and up-regulated in obesity. Leptin receptors are expressed in the Ventrolateral Preoptic nucleus (VLPO) and Median Preoptic nucleus (MnPO), two hypothalamic areas that promote sleep by inhibiting the wake-promoting nuclei [Tuberomammillary nucleus (TMN), Locus Ceoruleus (LC)] and receives circadian input by the master clock, the suprachiasmatic nucleus (SCN). The increased leptin levels in obesity are associated with the down-regulation of another adipokyne, adiponectin, which has insulin-sensitizing and anti-inflammatory properties. With the hypothesis that the increase in the leptin/adiponectin ratio might contribute to sleep disorder in obesity, in the present work, with first explore whether adiponectin receptors AdipoR1 and AdipoR2 are expressed in same brain areas as leptin. By immunohistochemistry to AdipoR1 and AdipoR2 in the rat brain, we found that these receptors are expressed in VLPO, as well as in the SCN, MnPO, TMN and LC. Given the key role of VLPO in sleep regulation, we then investigated whether the activity of this nucleus is affected by leptin and adiponectin. For that, the

electrophysiological response of VLPO neurons to leptin (10 nM) and adiponectin (200 μM) was assessed in rat brain slices (300 μm) containing the VLPO, by path-clamp in current clamp mode. Results showed that leptin increases the spontaneous activity of VLPO neurons, while adiponectin displayed inhibitory effects. These data imply that VLPO activity might be modulated by metabolic information, suggesting a new model for sleep-metabolism interaction, which might account for the sleep disturbances reported in obese subjects.

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Poster

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Program #/Poster #: 596.10/AAA25

Topic: F.08. Biological Rhythms and Sleep

Support: CONACYT 254264

Title: Sleep rebound and orexin administration change neuroglobin immunoreactivity in the rat brain

Authors: *F. A. GARCÍA-GARCÍA[1], L. RENDON[2], M. A. MELGAREJO[4], C. MORGADO-VALLE[1], G. HERNANDEZ-MARQUEZ[3]
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Abstract: Neuroglobin (Ngb) is a protein member of the globin family, expressed mainly in the central and peripheral nervous system. It is involved in the transport of oxygen, in the response to hypoxic/ischemic and oxidative stress-related insults. Recently, we showed that sleep deprivation reduces the number of Ngb positive (Ngb^+)cells in brain areas related to sleep. However, it is poorly understood whether Ngb expression depends on sleep occurrence and/or on waking promoting factors. Here, we aimed to study if sleep rebound restores the number of Ngb^+cells and if orexin-A administration affects Ngb expression in areas related with sleep-wake regulation. Male Wistar rats were sleep-deprived for 24 h using the gentle handling method.After sleep deprivation, rats were allowed a sleep rebound for 3 or 6 h. After sleep rebound, rats were euthanized, and their brains processed for Ngb immunohistochemistry. In a different group of rats, orexin-A was injected into the left lateral ventricle using a cannula. Three hours post-injection, rats were euthanized, and their brains processed for Ngb immunohistochemistry.We found that a 3-h sleep rebound is enough to restore the number of Ngb^+cells in all the analyzed

areas. A similar result was observed after 6-h sleep rebound. The injection of orexin-A increased the number of Ngb^{+}cells in areas associated with sleep-wake regulation. These results suggest that Ngb expression is sleep depend, and that orexin modulates its expression. We suggest that Ngb expression is involving in preventing cell damage due to prolonged wakefulness.

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Poster

596. Biological Rhythms and Sleep: Regulators

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Wellcome Trust (107839/Z/15/Z)
Wellcome Trust (107841/Z/15/Z)

Title: A neuronal hub binding sleep initiation and body cooling in response to a warm external stimulus

Authors: *E. HARDING, W. WISDEN, N. P. FRANKS
Imperial Col. London, London, United Kingdom

Abstract: Animals actively thermoregulate in preparation for sleeping. Mammals build nests and curl up, humans use bedding and environmental adaptation to create warm microclimates that are permissive for sleep. This strongly conserved behaviour may function to facilitate better or deeper sleep but it may also just be a matter of comfort without such function. To assess this hypothesis and evaluate whether mice sense environmental temperature to directly influence the onset of sleep we utilised a pharmacogenetics method to label and reactivate warm-sensing neurons within the preoptic hypothalamus. This method of Tet-Tagging allows us to understand the functions of neurons responding to specific stimuli, based on their expression of c-Fos. We found that within the MnPO/MPO hypothalamus distinct populations of warm-tagged neurons could induce sleep as well as body cooling, whereas an alternative population of warm-tagged MnPO/MPO neurons could induce sleep without hypothermia. We suggest the existence of a neuronal hub that uses sensory temperature cues to promote simultaneous sleep and body cooling. The efficient linking of these physiologies suggests that one function of sleep is to reduce energy expenditure.

Disclosures: E. Harding: None. **W. Wisden:** None. **N.P. Franks:** None.

Poster

596. Biological Rhythms and Sleep: Regulators

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Topic: F.08. Biological Rhythms and Sleep

Support: V.A. Merit Review I01BX00156

Title: Chemogenetic activation of corticotropin releasing factor neurons in the hypothalamic paraventricular nucleus does not disrupt the homeostatic response to acute sleep deprivation

Authors: *J.-C. HSIEH[1,2,4], S. KUMAR[1,4,5], D. J. MCGINTY[3,1], R. S. SZYMUSIAK[1,2]
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Abstract: Introduction: Our recent studies have shown that acute activation of corticotropin releasing factor (CRF) neurons in hypothalamic paraventricular nucleus (PVN) causes disruption of spontaneous sleep followed by sleep rebound, while the activity of the CRF neurons in PVN is implicated for the loss of homeostatic sleep response during chronic sleep restriction. In the present study we investigate if activation of CRF neurons in the PVN neurons during 6 hr sleep deprivation in mice disrupts suppresses the homeostatic response to sleep loss.
Methods: Male CRF-ires-Cre mice received bilateral injections of pAAV-hSyn-DIO-hM3D(Gq)-mCherry excitatory DREADD targeting the PVN. Mice were implanted with chronic EEG/EMG electrodes at the time of AAV injection. Mice were maintained 12/12 hr light dark cycle. Three weeks after AAV injections, intraperitoneal injections of vehicle or CNO (1 mg/kg) were administered at zeitgeiber time (ZT) 0, followed by 6 hours of total sleep deprivation by gentle handling. EEG slow-wave activity (SWA) of delta frequency (0.4 to 4 Hz) in NREM sleep and sleep wake measures were analyzed during the first 6 hrs of recovery sleep in the light period (ZT6-12).
Results: The mice displayed hyperactivity and heightened arousal after receiving CNO injections and showed little sign of drowsiness for the most part of the 6-hhr sleep deprivation, compared to the condition with vehicle injections, but did exhibit sleepiness and required repeated handling to maintain wakefulness during the final 1-2 hrs of sleep deprivation. During recovery period, there were no differences in time in Wake, NREM or REM, or the wake-sleep bout lengths between CNO and vehicle conditions in each of the 2-hour time blocks of undisturbed recovery sleep. The ratio of total NREM SWA or delta power during 6-hour recovery between CNO and vehicle conditions is 98.9% ± 5.3%, indicating the same homeostatic EEG response in both conditions, despite a heightened arousal caused by the activation of CRF neurons in the PVN during sleep deprivation.

Conclusion: The recovery sleep following the acute activation of CRF neurons in the PVN does not differ from the homeostatic sleep rebound following 6-hour total sleep deprivation.

Disclosures: J. Hsieh: None. **S. Kumar:** None. **D.J. McGinty:** None. **R.S. Szymusiak:** None.

Poster

596. Biological Rhythms and Sleep: Regulators

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant DA031900

Title: Dopamine terminal neurotransmission varies across sleep-wake state

Authors: *I. P. ALONSO[1], J. A. PINO[2], G. E. TORRES[2], R. A. ESPAÑA[1]
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Abstract: The dopamine transporter (DAT) is a homeostatic regulator that governs the temporal dynamics of DA neurotransmission. For example, DAT modulates extracellular DA levels across the light/dark cycle where peak extracellular DA tone is observed during the dark phase when animals are usually awake. The DAT can also undergo adaptations in response to physiological demands. For example, we recently demonstrated that DAT function varies in a diurnal fashion such that DA uptake and release are most efficient during the light phase when rats are typically asleep. What remains unclear is whether these fluctuation in DA release and uptake are associated with specific sleep-wake activity states or to other diurnal factors. To address these issues, we examined whether sleep-wake activity has an impact on DA terminal neurotransmission and which components of DA homeostasis are affected across sleep-wake activity. Rats were implanted with EEG/EMG electrodes to determine sleep-wake state (Wake, NREM, REM) immediately prior to ex vivo fast scan cyclic voltammetry detection of DA release, uptake, inhibition of DA uptake following cocaine challenge, or tissue quantification of key DA proteins. We observed a significant impact of sleep-wake state on DA terminal neurotransmission, with rats that were asleep exhibiting higher DA release and DA uptake relative to rats that were awake. Interestingly, we also observed a positive relationship between maximal DA uptake rates and the percentage of time spent in each sleep state. Further, we found that the effects of cocaine at inhibiting the DAT varied across arousal state. These results demonstrate that DA release and uptake are dynamically regulated and suggest that sleep-wake activity impacts DA neurotransmission in a manner that may influence DA-dependent processes such as cognition, drug-associated behavior, motor activity, and learning and memory.

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Poster

596. Biological Rhythms and Sleep: Regulators

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH R01HL122390

Title: Beta3-adrenergic receptor agonist-induced sleep in tumor necrosis factor alpha knockout mice

Authors: *E. SZENTIRMAI[1], A. MASSIE[2], L. KAPAS[3]
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Abstract: Introduction:
The interaction between sleep, metabolism and immune functions is well recognized. Shared central regulatory circuits and peripheral signaling and effector mechanisms, such as tumor necrosis factor alpha-α (TNFα), are likely to underpin the tight connection among these functions. The aim of the present study was to investigate the role of endogenous TNFα in spontaneous and β3-adrenergic receptor (β3-AR)-induced sleep in mice. β3-AR stimulation-induced sleep is mediated, in part, by the activation of brown adipose tissue. Since β3-AR stimulation also causes TNFα release, we investigated if the brown adipose tissue-independent component of β3-AR-induced sleep is mediated by TNFα.
Methods:
Male wild-type (WT) and TNFα knockout (KO) mice (n = 10, both genotypes) were instrumented with EEG and EMG electrodes and an intraabdominal transmitter. Spontaneous sleep-wake activity, body temperature and motor activity were recorded for three days. Baseline metabolism was recorded using indirect calorimetry three days after the completion of sleep recordings. In a separate experiment, mice were injected with i.p. saline on the control day and with 0.2 mg/kg β3-adrenergic receptor agonist (CL-316,243) on the experimental day. Sleep, body temperature and motor activity were recorded for 24 h after each treatment. Data were analyzed by using ANOVA followed by t-test.
Results:
Under baseline conditions, TNFα KO and WT mice showed normal diurnal rhythms of sleep-wake activity, body temperature and motor activity. TNFα KO mice had significantly more NREMS (WT: 269.1 ± 12.6 min vs KO: 297.4 ± 9.6 min, $p = 0.05$) and REMS (19.8 ± 2.9 min vs 35.1 ± 1.3 min, $p < 0.001$) during the dark phase than WTs. The increases in the amounts of NREMS and REMS were due to the increased number of sleep episodes in the TNF KOs. Body

temperature and motor activity in TNFα KO mice was significantly lower during the entire dark phase. TNFα KO mice had slightly lower heat production during the dark phase and lower respiratory quotients during the entire day compared to WTs. β3-AR-induced NREMS increase was slightly attenuated and body temperature increases were completely absent in the TNFα KO mice.
Conclusions:
Present results further support the role of TNFα in the regulation of sleep and metabolism.
Funding:
NIH R01HL122390
Conflicts:
None
Keywords:
Sleep, EEG, body temperature

Disclosures: E. Szentirmai: None. **A. Massie:** None. **L. Kapas:** None.

Poster

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH R01HL122390

Title: Sleep and fever caused by cell wall components of bacteria: The role of tumor necrosis factor-α

Authors: *A. R. MASSIE[1], N. MILLICAN[2], L. KAPAS[1], E. SZENTIRMAI[1]
[1]Elson S. Floyd Col. of Med., [2]Washington State Univ., Spokane, WA

Abstract: Introduction:
Muramyl peptides (MPs) and lipopolysaccharide (LPS) are components of the bacterial cell wall. Systemic injections of MPs and LPS elicit the so-called sickness syndrome, which includes fever, sleep, anorexia and behavioral withdrawal. Further, bacterial cell wall components induce the production of pro-inflammatory cytokines. Since pro-inflammatory cytokines, such as tumor necrosis factor-α (TNFα) produce similar sleep- and fever-promoting effects as MPs and LPS, it is widely assumed that symptoms of the sickness syndrome are due to the release of those cytokines. Muramyl dipeptide (MDP), the simplest MP with full immune stimulant activity, acts on NOD2 receptors, while iEDAP and LPS, components of the cell wall of gram-negative bacteria, act on NOD1 and TLR4 receptors, respectively. The aim of the present study was to investigate the role of endogenous TNFα in NOD1, NOD2 and TLR4 receptor activation-

induced sleep and febrile responses.
Methods:
Male wild-type (WT) and TNFα knockout (KO) mice (n = 10, both genotypes) were instrumented with EEG and EMG electrodes and an intraabdominal transmitter to record sleep-wake activity, body temperature and locomotion. The effects of the intraperitoneal injection of 25 mg/kg MDP, 25 mg/kg iEDAP and 0.4 µg/mouse LPS were determined in a counter-balanced order with one week between the treatments. Polygraphic recordings were scored blinded. Data were analyzed by using ANOVA followed by t-test.
Results:
MDP caused significant increases in rapid-eye-movement sleep (REMS; 6.0 ± 1.5 min/4 h), non-REMS (NREMS; 38.2 ± 8.7 min/4 h) and body temperature (0.41 ± 0.1°C) in WT animals. The effects in TNFα KO mice were significantly attenuated (NREMS: 13.3 ± 5.4 min/4 h; REMS: 1.8 ± 1.3 min/4 h; temperature: 0.30 ± 0.1°C). iEDAP did not have any significant effect on sleep and body temperature in WT animals. In TNFα KO mice, however, NREMS and body temperature were significantly elevated after iEDAP treatment (NREMS: 22.2 ± 6.5 min/4 h; temperature: 0.14 ± 0.04°C). Sleep- and fever-inducing effects of LPS were not different in the two genotypes.
Conclusions:
Endogenous TNFα is likely to play a role in sleep and fever induced by MPs, but not in the effects of LPS.
Funding:
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Conflicts:
None
Keywords:
Sleep, Microbiome, Lipopolysaccharide, Peptidoglycan, Muramyl Dipeptide, NOD1, NOD2, TLR4

Disclosures: N. Millican: None. **L. Kapas:** None. **E. Szentirmai:** None.

Poster

596. Biological Rhythms and Sleep: Regulators

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH RO1 HL122390

Title: The effects of antibiotic-induced gut-microbiome depletion on sleep in mice

Authors: *N. S. MILLICAN[1], A. R. MASSIE[1], E. SZENTIRMAI[2], L. KAPAS[3]
[2]Elson S. Floyd Col. of Med., [1]Washington State Univ., Spokane, WA; [3]WWAMI Med. Educ. Program, Washington State University, Spokane, Spokane, WA

Abstract: Introduction: Bacterial cell-wall components (BCWCs; e.g., lipopolysaccharide, peptidoglycan) and pro-inflammatory cytokines are well documented to increase sleep when injected systemically. The gut contains the body's largest reserves of both immunologically-active tissue and bacteria. Since BCWCs continuously enter the circulation from the intestines even under normal conditions, we hypothesized that translocated microbial molecules may contribute to the maintenance of sleep-wake activity. To test this, we investigated the effects of gut-microbiome depletion on sleep in mice.Methods: Male C57BL/6J mice (n = 6; 3 months old) were instrumented with electroencephalographic and electromyographic electrodes. Following surgeries, mice were individually housed in temperature controlled (29 ± 1°C), sound-attenuated chambers on a 12:12 hour light-dark cycle. Food and water were available *ad libitum*. Sleep recordings were performed after 10 days of recovery. After recording baseline sleep-wake states, mice were gavaged daily with 0.01 ml/g bodyweight of a broad-spectrum antibiotic cocktail (2.5 mg/ml ampicillin, 2.5 mg/ml metronidazole, 2.5 mg/ml neomycin, 1.0 mg/ml vancomycin—made fresh just prior to gavaging) within 30 min of dark onset for 24 days. On day 25, mice were gavaged with water. On days 26-28, to expedite gut-microbiome repopulation, mice were gavaged with fecal suspension made of feces from non-antibiotic treated mice. Somnographic recordings were scored by a blinded scorer. Paired t-tests were used to compare baseline with days 25 (maximal microbiome depletion; day 25 was used as the day of maximal microbiome depletion, rather than the final antibiotic-administration day, due to an acute effect of the antibiotics) and 26 (fecal gavage).Results: Microbiomial depletion reduced dark-phase non-rapid-eye movement sleep (NREMS) by 16.8% ($p = 0.05$) and fecal gavage returned sleep to baseline ($p = 0.44$).Conclusions: Antibiotic-induced gut-microbiome depletion significantly reduced dark-phase NREMS by ~17% and repopulation reinstituted baseline NREMS. Our findings support the hypothesis that gut-microbiome products contribute to normal sleep. At least 3 groups of microbial signals my mediate these effects: BCWCs; microbial metabolites, such as short-chain fatty acids and secondary bile acids; intestinal hormones modulated by bacteria, such as melatonin and serotonin.

Disclosures: N.S. Millican: None. **A.R. Massie:** None. **E. Szentirmai:** None. **L. Kapas:** None.

Poster

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Program #/Poster #: 596.17/BBB6

Topic: F.08. Biological Rhythms and Sleep

Support: NIH R01HL122390

Title: Role of macrophages in bacterial cell wall components-induced sleep in mice

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Abstract: Bacterial cell wall products (BCWPs), such as the peptidoglycan derivative muramyl dipeptide (MDP) and lipopolysaccharide (LPS), translocate from the intestinal lumen to the portal circulation and are present in detectable quantities in various organs, including the liver, under physiological conditions. BCWPs, acting on the pattern recognition receptors TLR4, NOD1 and NOD2, activate Kupffer cells, the resident hepatic macrophages, and elicit the production of cytokines, such as interleukin-1β and tumor necrosis factor-α. Since both BCWPs and pro-inflammatory cytokines induce sleep, we hypothesized that activated macrophages play a role in the somnogenic actions of LPS and MDP by secreting somnogenic cytokines. To test this hypothesis, we determined the effects of LPS and MDP on sleep in macrophage-depleted (MD) mice.

Male C57 mice (n = 10) were instrumented with EEG and EMG electrodes and an intraabdominal transmitter to record sleep-wake activity, body temperature and locomotion. First, the effects of the intraperitoneal (ip) injection of 25 mg/kg MDP and 0.4 µg/mouse LPS were determined in a counter-balanced order with one week between the treatments. Then, the animals received ip injection of clodronate-containing liposomes (CCL) to induce MD. 7-10 days after the CCL treatment, the sleep and thermoregulatory effects of LPS and MDP were determined again during the macrophage-depleted stage. Data were analyzed by using ANOVA followed by paired t-test.

MDP caused significant increases in rapid-eye-movement sleep (REMS; 6.0 ± 1.5 min/4 h), non-REMS (NREMS; 38.2 ± 8.7 min/4 h) and body temperature (0.41 ± 0.1°C) in mice before macrophage depletion. The sleep-inducing, but not the febrile, effects of MDP were completely abolished in MD animals. LPS significantly increased NREMS and REMS before MD treatment (NREMS: +96.8 min/8 h; REMS: +9.0 min/8 h); the effects were significantly suppressed after MD treatment (NREMS: +53.0 min/8 h; REMS: -14.6 min/8 h). Before macrophage depletion, the mice developed monophasic fever in response to LPS; in the MD stage, LPS elicited robust monophasic hypothermia.

Conclusion: Macrophages play a pivotal role in the sleep-inducing effects of BCWPs and play a role in the febrile effects of LPS, but not MDP.

Disclosures: L. Kapas: None. **N.S. Millican:** None. **E. Szentirmai:** None.

Poster

596. Biological Rhythms and Sleep: Regulators

Location: SDCC Halls B-H

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Topic: F.08. Biological Rhythms and Sleep

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Utrish Dolphinarium

Title: Fur seals suppress REM sleep for days or weeks without subsequent rebound, a finding with implications for REM sleep function

Authors: *O. LYAMIN[1,2,3], P. KOSENKO[4], S. KORNEVA[3], A. VYSSOTSKI[5], L. MUKHAMETOV[2,3], J. SIEGEL[1]
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Abstract: After deprivation of rapid eye movement (REM) sleep, land mammals increase REM sleep time, supporting the idea that REM sleep is homeostatically regulated. The semiaquatic northern fur seal (*Callorhinus ursinus*) is unique in showing both the bilateral SWS (BSWS) seen in most mammals and the unihemispheric sleep (USWS) reported in cetaceans. We recorded electroencephalogram (EEG), electromyogram, electrooculogram and electrocardiogram in freely mowing fur seals (n=4) using a datalogger during 2 days on land (baseline, B), 10-14 days in seawater, and then another 2 days on land (recovery). When in water, the average daily amount of REM sleep in seals was reduced to 3 minutes a day vs. 80 minutes when on land or in B (a 96.4+1.0% reduction) (one way ANOVA, $F_{13,33}=27.506$, $P<0.001$). The number of REM sleep episodes per day decreased to 20±3% of B ($F_{13,33}=10.754$, $P<0.001$) and the average duration of REM sleep episodes decreased to 13±1% of B ($F_{13,33}=6.508$, $P<0.001$). No REM sleep was recorded in the seals during the first 3-7 days in water. By the end of the 10th day an accumulated "loss" of the expected amounts of REM sleep averaged 765±72 minutes or 974±8% of projected daily B amounts. On the first recovery day (R1), the amounts of REM sleep were not significantly greater than during B (Tukey post hoc test, $P>0.05$, df =6). For all seals combined, the average amount above B values on R1 was only 3.2±2% of REM sleep lost in seawater. The number and duration of REM sleep episodes on R1 did not significantly differ from that in B. The amount of REM sleep on R1 did not correlate

with the amount of REM sleep lost. In contrast to REM sleep, the total amounts of slow wave sleep in seals in seawater ranged from 45-129% of B. The amount of BSWS in water was significantly smaller than that in B with a decrease from 7.5 to 0.5% of the 24-h ($F_{12,25}=22.868$, $P<0.001$). After return to B conditions the amount of BSWS on both R days doubled with respect to the average B amounts ($P<0.001$). Our data are consistent with the hypothesis that REM sleep may serve to reverse the reduced brain temperature and metabolism effects of bilateral non-REM sleep, a state which is greatly reduced when the fur seal is in the seawater, rather than REM sleep being directly homeostatically regulated.

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Poster

596. Biological Rhythms and Sleep: Regulators

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 596.19/BBB8

Topic: F.08. Biological Rhythms and Sleep

Title: The effect of ketamine on slow-wave activity (SWA) in patients with treatment resistant depression compared with healthy controls

Authors: *M. OPPENHEIMER, N. HEJAZI, B. FALODUN, W. DUNCAN, C. A. ZARATE, JR
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Abstract: Background**:** Sleep disturbance and reduced slow-wave activity (SWA) are common features of depression. Slow-wave activity (SWA) increases with duration of prior wakefulness and declines during sleep, suggesting regulation by a sleep homeostat. Ketamine is a glutamatergic drug with rapidly acting antidepressant properties associated with altered levels of SWA, with possible effects on sleep homeostasis and neural plasticity. Previous research has also demonstrated significant age and gender effects on SWA in depressed as well as healthy populations, suggesting that these factors influence sleep homeostasis, and contribute to mood disorder. The present study evaluates the effect of ketamine on SWA in patients with Treatment Resistant Depression (TRD) compared to healthy controls (HC), and examines the influence of sex and age on SWA.
Methods: Participants were TRD patients (n= 30: f= 22; BD= 9; $\bar{X}$= 41.5 y) and HCs (n= 11; $\bar{X}$= 32.9 y) who received a single infusion of ketamine (0.5 mg/kg over 40 minutes). Polysomnography was performed the night before (baseline; BL) and after ketamine (post-K) infusion. Fast Fourier transform power spectral analysis was used to analyze SWA for C3-A2 and C4-A1 EEG channels. SWA data were log-transformed prior to analysis. Linear mixed models were used for analysis.

Results: At baseline, SWA was lower in patients with TRD (232.3 ±21.4 [$\overline{X}$ picowatt ±SEM]) compared to HC (390.9 ± 92.1), [F(1,47)=5.18, p=.03]. In TRD, total night SWA increased to 287.3 ±28.6 from 232.3 ±21.4; post K vs. BL, respectively [F(1, 38)=7.24, p=.01]. The ketamine effect did not differ between TRD and HC [F(1,48.1)=1.29, p=.26], between females (325.3 ±37.14 vs. 266.4 ±30.7; post K vs. BL, respectively) and males (238.23 ±42.23 vs. 188.2 ±25.97) [F(1,37)=0.04, p=.85], or with age [F(1,37)=0.21, p=.65].
Conclusion: The increase in SWA post-ketamine infusion in patients with TRD is consistent with our earlier finding suggesting effects on homeostatic sleep mechanisms and plasticity. The current finding indicates that ketamine's effect on SWA is not influenced by age and sex. In HC, ketamine's effect is similar to TRD, but this result may be related to the small sample. Future analysis will explore the relationship between antidepressant response and change in SWA. Further analyses with larger samples are warranted.

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Poster

597. Biological Rhythms and Sleep: Systems

Location: SDCC Halls B-H

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Program #/Poster #: 597.01/BBB9

Topic: F.08. Biological Rhythms and Sleep

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Title: Roles for state-dependent corticothalamic and thalamocortical activity in visual system plasticity

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Abstract: Orientation Specific Response Potentiation (OSRP) is a form of plasticity in primary visual cortex (V1), which is initiated by waking visual experience and dependent on subsequent sleep. Our recent data suggest that presentation of a novel visual stimulus (a single oriented grating) causes immediate, instructive changes in the firing of mouse lateral geniculate nucleus (LGN) neurons - leading to increased firing rate responses to the presented stimulus orientation

(relative to other orientations). However, stimulus presentation alone does not affect V1 neurons, which show response changes only after a period of subsequent sleep. During post-stimulus Non Rapid Eye Movement (NREM) sleep, LGN neurons' overall spike-field coherence (SFC) with V1 delta (0.5-4 Hz) and spindle (7-15 Hz) oscillations increased, with neurons most responsive to the presented stimulus showing greater SFC. Furthermore, visual response changes in V1 correlated with changes in the synchrony of thalamocortical oscillations, specifically during NREM sleep. Thus, we hypothesize that state-specific features of thalamocortical communication, like NREM-specific oscillatory activity, are crucial for OSRP. To address this hypothesis, we first tested the role of layer 6 corticothalamic (CT) V1 neurons in coherent firing within the LGN-V1 network. Optogenetic interference with CT feedback to LGN during post-stimulus NREM sleep (but not REM or wake) disrupts coherent oscillations between LGN and V1, and also blocks sleep-dependent response changes in V1. We conclude that NREM oscillations relay information regarding prior sensory experience between the thalamus and cortex to promote cortical plasticity. Current studies are aimed at determining the role of cortically-projecting thalamic relay neurons in OSRP, by disrupting LGN activity in a state-dependent manner.

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Poster

597. Biological Rhythms and Sleep: Systems

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Topic: F.08. Biological Rhythms and Sleep

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National Institute Of Neurological Disorders And Stroke of the National Institutes of Health - Award Number K02NS093014

Title: Role of activity across cortex and striatum during sleep in motor skill learning

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Abstract: Motor skill learning describes the transition from the naïve execution of variable movements to a fluid, fast, and consistent motor action. Intriguingly, this process is known require both "online" training and "offline" sleep periods. Such sleep-dependent improvements during motor learning mirror offline improvements in declarative learning and memory tasks.

The neural basis of offline improvements in these tasks is often linked to coordinated activity across relevant brain regions. While motor skill learning is known to require contributions from a distributed motor network, the network basis of sleep-dependent motor improvements has not been explored.
Here, we recorded neural activity, including single unit activity and local field potentials (LFP), across primary motor cortex (M1) and dorsolateral striatum (DLS) as rats learn a reach-to-grasp skill. Evolving activity across M1 and DLS has been implicated in the refinement and binding of movements during motor skill learning. By monitoring neural activity during both online training periods and offline sleep periods, we examined how patterns of activity across M1 and DLS during sleep play a role in motor skill learning.
We report that evolving activity across M1 and DLS is correlated with motor skill learning. Coordinated activity emerges across M1 and DLS during offline periods that is linked to increases in measures of functional connectivity across M1 and DLS. This work has relevance to the neural basis of how motor skills are learned and our understanding of sleep-dependent motor improvements.

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Poster

597. Biological Rhythms and Sleep: Systems

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Program #/Poster #: 597.03/BBB11

Topic: F.08. Biological Rhythms and Sleep

Support: NIMH R01 60-670

Title: Role of the OLM interneurons during sleep-dependent memory consolidation

Authors: *M. A. FRAZER, G. POE
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Abstract: While it has long been established that sleep is critical for memory consolidation, the specific mechanisms by which this occurs are still largely unknown. Hippocampal population dynamics at each sleep stage facilitate the flow of information through the consolidation process, with interneurons acting as important regulators of oscillations and dynamics in the system. OLM interneurons are one subtype of hippocampal neurons that have been shown ito gate the information flow between entorhinal cortical and CA3 inputs, and are required for hippocampal learning tasks. Using freely behaving calcium imaging, we have observed sleep-state dependent changes in the activity of this cell population, as well as investigated the consequences of altering its activity during sleep, allowing us to elucidate changes to hippocampal information flow into the hippocampus at different sleep states and its importance to learning and memory.

Disclosures: M.A. Frazer: None. **G. Poe:** None.

Poster

597. Biological Rhythms and Sleep: Systems

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Topic: F.08. Biological Rhythms and Sleep

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Title: Differential suppression of locus coeruleus activity in REM during low estrogen phases compared with males may contribute to memory processing differences

Authors: *Y. CABRERA[1], J. JIMENEZ[1], J. HOLLOWAY[1], C. V. CHEN[2], I. LIBERZON[3], G. R. POE[1]
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Abstract: The locus coeruleus (LC), a brainstem structure recognized as the major producer of norepinephrine (NE) in the brain, plays a vital role in the alteration of arousal states. Increased LC firing during times of stress induces greater NE release at cortical synapses and contributes to the “fight or flight” response. This produces an adaptive behavioral response and strengths memory formation. LC firing decreases to almost nothing during REM sleep. Compared to men, women are more commonly diagnosed with psychiatric conditions like depression and PTSD, conditions that may be due to dysfunction in LC activity. Sexual dimorphism in LC structure and variable NE activity through the estrous cycle might play a role in these effects. Since estrogen influences LC firing and availability of NE in synapses, we hypothesized that low estrogen phases will be associated with altered responses to stressors. In addition, normal LC quiescence during REM may be dysregulated, causing errors in memory. This study addresses the role of estrogen presence in the activity of LC through the sleep/wake cycle and over the estrus cycle. I hypothesized that higher LC activity during low estrogen phases in times of physiologically advantageous quiescence (i.e. REM) produces an increase in maladaptive behaviors. Male (n=7) and female rats (high estrogen phase n=7, low estrogen phase n=7) were instrumented with tetrodes in the LC, and EEG and EMG wires to track electrophysiological activity through the estrous cycle. Presence of NE in synapses of memory circuits does not allow for normal depotentiation required for reorganization of memory components. Any presence can thus contribute to memory problems after trauma.

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Poster

597. Biological Rhythms and Sleep: Systems

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH NS098813

Title: Sex differences in murine cataplexy following conditional hypocretin degeneration

Authors: *M. D. SCHWARTZ[1], C. WONG[1], T. DATTOLO[1], S. R. MORAIRTY[1], A. YAMANAKA[2], T. S. KILDUFF[1]
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Abstract: The hypothalamic hypocretin/orexin (Hcrt) neurons are critical for the regulation of sleep/wake, metabolism and reward. In humans, degeneration of Hcrt neurons results in the sleep disorder narcolepsy. Several recent studies report an increased incidence of narcolepsy in women compared to men, along with shorter sleep latency and an earlier age of onset for excessive daytime sleepiness in women. Differences between male and female rodents have been found in Hcrt peptide and receptor expression, as well as in the response of the Hcrt system to stress and reward seeking, suggesting that sex differences in Hcrt regulation and/or signaling may underlie observed gender differences in narcolepsy symptoms in humans. In the present study, we determined whether the expression of narcoleptic symptoms is modulated by sex in *orexin-tTA;TetO diptheria toxin* (DTA) mice in which Hcrt degeneration is conditionally triggered by removal of dietary doxycycline (Dox). Male and female DTA mice maintained on ad lib Dox chow were prepared for telemetric EEG/EMG recording at ~14 weeks of age. Following recovery, undisturbed 24 h baseline EEG recordings were conducted weekly starting at ~18 weeks of age prior to Dox removal and then for 7 weeks during the Dox(-) condition. Mice were housed with running wheels from the first week of Dox(-) onwards. By week 3 of Dox(-), both males and females exhibited unambiguous cataplexy, with the majority of episodes occurring in the dark phase for both sexes. Cataplexy time and bout number were consistent with levels of cataplexy observed in our previous studies of male DTA mice. In males, cataplexy levels remained stable from week 3 to week 6. By contrast, cataplexy time and bout number in females decreased from week 3 to week 6 to approximately half that seen in males. By week 6 of Dox(-), wake time was also significantly increased in the late dark phase and early light phase, with females having fewer wake bouts across the light and dark phases, suggesting reduced sleep fragmentation and improved wake consolidation in females compared to males. These observations suggest that female mice compensate for the loss of Hcrt neurons over time through

an unknown mechanism. Such compensatory mechanisms, if they exist in humans, could contribute to observed delays in diagnosing narcolepsy in women compared to men.

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Poster

597. Biological Rhythms and Sleep: Systems

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Topic: F.08. Biological Rhythms and Sleep

Support: Wellcome Trust/Royal Society (Sir Henry Dale Fellowship) 107672/Z/15/Z

Title: Hippocampal ripples initiate cortical-hippocampal communication

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Abstract: One of the most important functions of sleep is the consolidation of memories. Not only does sleep provide shelter from external sensory input, but it has been proposed that sleep actively promotes memory formation by facilitating reprocessing of information acquired during wakefulness. In particular, according to the active systems consolidation framework, newly encoded information is initially stored in hippocampus and reactivated during subsequent sleep, leading to a strengthening of corresponding memory traces in the neocortex for long-term storage. More importantly, this process relies on an interplay of three cardinal sleep rhythms, cortical slow oscillations, thalamo-cortical sleep spindles and hippocampal sharp-wave ripples. However, how exactly do these rhythms mediate the hypothesised 'information transfer' from hippocampal to neocortical sites? Cross-area communication strongly relies on temporal co-occurrence of activation. Here we analysed whole-night sleep recordings from eleven pre-surgical epilepsy patients with depth electrodes implanted in medial temporal lobe and cortical sites. Focusing on intracranial recordings from hippocampus, entorhinal cortex and lateral temporal cortex as well as scalp EEG recordings, we set out to unravel the interregional dynamics between slow oscillations, spindles and ripples. While our findings corroborate a top-down migration of slow oscillations from cortical to medial temporal sites, we further found that hippocampal sharp-wave ripples trigger upward directed hippocampal-cortical communication, mediating the information transfer thought to underlie memory consolidation.

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Poster

597. Biological Rhythms and Sleep: Systems

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Topic: F.08. Biological Rhythms and Sleep

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Title: Cross-dynamical delay differential analysis reveals information flow during hippocampal ripples

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Abstract: High-frequency hippocampal ripples mark the time when hippocampal cells replay sequences from waking during slow wave sleep. Evidence in rodents is consistent with ripples sending information to the cortex to permit memory traces to be transferred during consolidation. However, hippocampo-cortical interactions during ripples are poorly characterized in humans. Cross-dynamical Delay Differential Analysis (CD-DDA) is a new tool to study causal connections between time series signals. Based on embedding theory from nonlinear dynamics, the classical formulation of Delay Differential Analysis (DDA) relates the differential and delay embeddings of a single time series in a functional form to uncover dynamical differences in the data. The features obtained from DDA provide a powerful basis for time-domain classification of data. In CD-DDA, we investigate causal interactions between two time series. Here, we apply this technique to intracranial recordings from patients undergoing treatment for epilepsy. By applying CD-DDA to recordings from electrodes placed in both hippocampus and remote cortical areas, we can uncover distinct patterns of directional information flow around the times when ripples occur. For many such channel pairings, there is a marked increase in cortex-to-hippocampus information flow around the time of the ripple, and this is followed by a longer period (hundreds of milliseconds) of hippocampus-to-cortex information flow. This same pattern seems to be characteristic of connections between hippocampus and a range of other cortical areas considered during ripples. Further analysis of data from additional brain areas in more subjects could help to characterize information flow in the brain more broadly.

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Poster

597. Biological Rhythms and Sleep: Systems

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Program #/Poster #: 597.08/CCC2

Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant NS088482

Title: Role of glutamate produced by melanin-concentrating hormone neurons in sleep-wake regulation

Authors: F. NAGANUMA[1], S. BANDARU[1], G. ABSI[1], M. CHEE[2], *R. VETRIVELAN[3]
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Abstract: Melanin-concentrating hormone (MCH) neurons located in the lateral hypothalamus (LH) play a key role in the regulation of rapid eye movement sleep (REMs). Optogenetic and chemogenetic activations of MCH neurons increase REMs whereas the ablation of MCH neurons affects the diurnal variation of REMs. MCH neurons also contain several other neuropeptides and neurotransmitters, many of which may participate in REMs regulation. We hypothesized that glutamate, which is present in almost all (>98%) MCH neurons, is involved in REMs regulation. We tested this hypothesis by deleting the vesicular glutamate transporter (Vglut2), which is necessary for synaptic glutamate release, from MCH neurons in mice and studying the consequent changes in sleep-wake amounts and architecture. Specific deletion of Vglut2 from MCH neurons was achieved by crossing MCH-Cre mice that express Cre recombinase (Cre) specifically in MCH neurons with Vglut2$^{flox/flox}$ mice that express lox-P modified alleles of *Vglut2*. Daily (24-h) percentages of wake, NREMs or REMs in these mice missing Vglut2 in MCH neurons (MCH-Vglut2KO mice) were not significantly different from control mice. However, the diurnal variation of REMs was significantly higher in MCH-Vglut2KO mice (142.0±11.92% of controls). These data indicate that glutamate in MCH neurons may be necessary for normal expression of diurnal rhythms in REMs in mice. We next chemogenetically activated MCH neurons in MCH-Vglut2KO mice and tested if MCH neurons can still promote REMs. We found that chemoactivation of MCH neurons in MCH-Vglut2KO mice increased REMs by 158% during the first 3 hours. These data indicate that MCH neurons can promote REMs even in the absence of glutamate.

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Poster

597. Biological Rhythms and Sleep: Systems

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Topic: F.08. Biological Rhythms and Sleep

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Title: An *in vitro* study of parahypoglossal cholinergic inputs to hypoglossal motor neurons in adult mice

Authors: *L. ZHU, E. ARRIGONI
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Abstract: Introduction. In REM sleep, the genioglossus (GG) muscle undergoes a dramatic suppression of activity. A current hypothesis is that the loss of GG activity during REM sleep is mediated by a combination of 1) monoaminergic disfacilitation and 2) a cholinergic inhibition of hypoglossal motor neurons. Strikingly, blockade of cholinergic receptors in the hypoglossal motor nucleus fully restores REM sleep tonic and inspiratory-modulated components of GG activity (Grace et al., 2013), suggesting that the cholinergic signal is largely responsible for the REM sleep suppression of GG activity. Respiratory rhythm generator neurons of the pre-Bötzinger complex drive the activation of hypoglossal motor neurons through glutamatergic premotor neurons in the parahypoglossal region (PH). Previously, we have shown that carbachol, an agonist of acetylcholine, inhibits PH glutamatergic input to hypoglossal motoneurons through a presynaptic mechanism. However, the sources of cholinergic inputs are not well understood. In this study, we investigate the PH cholinergic input to hypoglossal motor neurons.
Methods. We stereotaxically injected the PH region of ChAT-*cre* mice with a cre-dependent AAV-ChR2-mCherry to expressed channelrhodopsin2 (ChR2) in PH cholinergic neurons. We then performed whole-cell recordings in hypoglossal neurons while photostimulating PH cholinergic inputs expressing ChR2.
Results: Photostimulation of the cholinergic PH input evoked excitatory postsynaptic currents (EPSCs) in hypoglossal motor neurons. These photo-evoked EPSCs were maintained in TTX(1μM) and 4-AP(1mM), indicating monosynaptic connectivity. Bath application of scopolamine (muscarinic receptor blocker) and/or a cocktail of nicotinic receptors failed to block the photo-evoked EPSCs in hypoglossal neurons. The photo-evoked EPSCs were abolished with bath application AMPA-receptor blocker DNQX (20μM).
Conclusions: Our results suggest that 1) PH cholinergic neurons directly innervate hypoglossal motor neurons. 2) PH cholinergic neurons primarily release glutamate and excite hypoglossal motor neurons. 3) PH cholinergic inputs are unlikely responsible for the suppression of hypoglossal motor neurons during REM sleep.

Disclosures: L. Zhu: None. **E. Arrigoni:** None.

Poster

597. Biological Rhythms and Sleep: Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 597.10/CCC4

Topic: F.08. Biological Rhythms and Sleep

Title: Lateral parabrachial neurons innervate arousal-promoting regions in the rat brainstem via orexin neurons in the hypothalamus

Authors: *Y. ARIMA, S. YOKOTA, M. FUJITANI
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Abstract: Orexin (ORX) is a small hypothalamic neuropeptide, which has a critical role in the regulation of sleep-wakefulness. ORX neurons are specifically localized in the tuberal hypothalamus, including the perifornical area, lateral hypothalamus, and dorsomedial hypothalamic nucleus and project to arousal-promoting brain regions. We recently showed that the glutamatergic lateral parabrachial nucleus (LPB) neurons innervated hypothalamic ORX neurons. These findings indicated that LPB neurons could regulate sleep and wakefulness by the projections to arousal promoting brain regions via ORX neurons. To show this, we examined this potential projection by a combination of antero- and retrograde tract-tracing techniques in male Wistar rats. We injected the anterograde tracer, biotinylated dextranamine (BDA), into the LPB and the retrograde tracer, cholera toxin B subunit (CTb), into the ventral tegmental area (VTA), pedunculopontine tegmental nucleus (PPT), laterodorsal tegmental area (LDT), locus coeruleus (LC), or dorsal raphe nucleus (DR). By immunohistochemical analysis, we observed the prominent overlapping distributions of BDA-labeled fibers and CTb-labeled ORX positive neurons in the lateral part of the dorsomedial nucleus and dorsal perifornical areas. In these areas, we further observed that BDA-labeled axons showed synaptic bouton like morphology and also synaptophysin immunoreactivity were in contiguity with CTb-labeled ORX-immunoreactive neurons. From these results, we concluded that LPB neurons form functional synapses with ORX neurons that project to the VTA, PPT, LDT, LC, and DR. These results strongly suggest that LPB neurons could promote arousal via ORX neurons in the hypothalamus.

Disclosures: Y. Arima: A. Employment/Salary (full or part-time):; Shimane univercity. **S. Yokota:** A. Employment/Salary (full or part-time):; Shimane University. **M. Fujitani:** A. Employment/Salary (full or part-time):; Shimane university.

Poster

597. Biological Rhythms and Sleep: Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 597.11/CCC5

Topic: F.08. Biological Rhythms and Sleep

Support: CONACYT grant No. 245243 to JRE
CONACYT grant No. 245333 to CC
CA Neuroendocrinología BUAP-CA-288
CDO is fellowship from CONACYT

Title: Orexin agonist modified the sleep-wake pattern in narcoleptic taiep rat

Authors: *C. CORTES[1], C. DE OVANDO[2], S. RUGERIO[2], A. UGARTE[2], J. R. EGUIBAR[3]
[2]Inst. of Physiol., [3]Vice-rectory of Res. and Postgraduate Studies, [1]B. Univ. Autonoma de Puebla, Puebla, Mexico

Abstract: The homeostasis of sleep-awake cycle is modulate by orexins. The myelin mutant *taiep* rats had immobility episodes (IEs) with a rapid eye movement (REM) sleep pattern, because they had desynchronized cortical activity with theta rhythm in the hippocampus and significant reduction on the electromyography (EMG) amplitude in nuchal muscles. They also showed a disorganized sleep-wake pattern with higher transitions between them. In base of that we propose *taiep* rats as an adequate model of narcolepsy-cataplexy. The aim of this study was to analyze the effects of the intracerebroventricular (i.c.v.) administration of orexin agonist [Ala11, D Leu15] -Orexin B in male *taiep* rats at 9 months old. The subjects (Ss) were maintained under standard conditions with a 12/12 light-dark cycle (lights on at 0700) and free access to rodent food pellets and purified water. All Ss were anesthetized by i.p. injection of ketamine/xylazine mixture and implanted electrodes for EEG, EMG and EOG recordings under stereotaxic coordinates. We analyzed the effects of i.c.v. administration of orexin B agonist (1, 3, 10 nM) and evaluate sleep-wake cycle and the frequency and mean duration of IEs during continuous electrographic recordings along 8h after administration. All procedures followed the NIH rules and the protocol was approved by BUAP-IACUC. Our results showed that the administration of orexin B significantly increased progressively the awake phase with the doses administered ($P<0.05$). Additionally, the orexin agonist decreased the frequency and mean duration of IES ($P<0.05$), as well as total amount of REM sleep being more pronounced in the first 4h after administration. We conclude that the myelin mutant *taiep* rats is an adequate model of narcolepsy-cataplexy with a good response to central administration of orexin B agonist suggesting an impairment in orexinergic transmission.

Disclosures: C. Cortes: None. **C. De ovando:** None. **S. Rugerio:** None. **A. Ugarte:** None. **J.R. Eguibar:** None.

Poster

597. Biological Rhythms and Sleep: Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 597.12/CCC6

Topic: F.08. Biological Rhythms and Sleep

Support: R01 NS078410

Title: The response of nitrergic neurons in the dorsal raphe nucleus to acute sleep loss

Authors: *I. S. NICHOLS, E. CHIEM, C. VAN, A. TUCKER, F. NAJJAR, K. PAUL
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Abstract: Nitric Oxide (NO) is active in many of the neurons and brain regions that regulate sleep and its responses to the environment. In the dorsal raphe nucleus (DRN) of the brainstem, alterations in NO synthase (NOS) activation are associated with decreased REM sleep amount. While mounting evidence shows that NO is an important molecule for sleep regulatory mechanisms, the role of NO in the ability to recover from sleep loss is largely unexplored. It has been revealed that inhibition of NOS activity in DRN neurons decreases REM sleep amount in rats. Since inhibition of DRN NOS activity decreases sleep, we explored whether inhibition of sleep had effects on NOS neurons in the DRN. In order to determine the effects of sleep loss on DRN NOS, male and female mice in a 12:12 light:dark cycle were sleep restricted for 24 hrs. Sleep restricted mice were singly housed in slowly rotating wheels (1 revolution/min). Controls were allowed *ad libitum* sleep under the same conditions. We also examined NOS in cholinergic neurons of the basal forebrain, since those neurons are important regulators of the ability to recover from sleep loss. Coronal sections were obtained from the BF and DRN and processed with NADPH-d (a marker for NOS activity). The sections were imaged using Evos Fl cell imaging system, neurons displaying NADPH were counted, and optical density was obtained on ImageJ. Mice that underwent sleep restriction exhibited no differences ($p > .05$) in NADPH levels, in either the DRN or the basal forebrain, from controls. These data suggest that NOS neurons in the DRN are not responsive to acute sleep restriction.

Disclosures: I.S. Nichols: None. **E. Chiem:** None. **C. Van:** None. **A. Tucker:** None. **F. Najjar:** None. **K. Paul:** None.

Poster

597. Biological Rhythms and Sleep: Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 597.13/CCC7

Topic: F.08. Biological Rhythms and Sleep

Support: SNF156156
ERC 725850

Title: Thalamic dual control of sleep and wakefulness

Authors: *A. R. ADAMANTIDIS, Dr[1], M. BANDARABADI[3], C. G. HERRERA[4], T. GENT[2]
[1]Dept of Neurol., [2]Univ. of Bern, Bern, Switzerland; [3]Neurol., Hosp. Univ. of Bern, Bern, Switzerland; [4]Dept of Neurol., Inselspital Univ. of Bern, Bern, Switzerland

Abstract: Slow-waves (0.5 - 4 Hz) predominate in the cortical electroencephalogram during non-rapid eye movement sleep (NREM) in mammals. They reflect the synchronisation of large neuronal ensembles alternating between active (UP) and quiescent (DOWN) states and propagate along the neocortex. However, the thalamic contribution to cortical UP-states and sleep modulation remains unclear. Using multisite tetrode recordings in freely behaving mice, we show that spontaneous centromedial thalamus (CMT) neuronal firing is phase advanced to global cortical UP-states, as well as NREM-to-Wake transitions but not temporally-locked to sensory thalamic neuronal firing. Optogenetic tonic activation of CMT neurones induce rapid NREM-to-Wake transitions, whereas burst activation mimics UP-states in the cingulate cortex enhanced brain-wide synchrony of cortical slow-waves during sleep, through a thalamic relay located in the antero-dorsal thalamus. Finally, we demonstrate that both CMT and AD relay neurones are necessary for slow-wave traveling and sleep recovery following a period of extended wakefulness. These findings suggest that CMT neuronal firing patterns alone can modulate brain-wide cortical activity during sleep and provides dual control of sleep-wake states.

Disclosures: A.R. Adamantidis: None. **M. Bandarabadi:** None. **C.G. Herrera:** None. **T. Gent:** None.

Poster

597. Biological Rhythms and Sleep: Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 597.14/CCC8

Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant NS092383

Title: An intracellular study of GABAergic processes in the control of activity of neurons in the pontine reticular formation of the cat

Authors: M. XI[1], S. J. FUNG[1], S. SAMPOGNA[1], *M. H. CHASE[1,2]
[1]Websciences Intl., Los Angeles, CA; [2]UCLA Sch. of Med., Los Angeles, CA

Abstract: Our previous experimental data have provided compelling evidence that GABAergic processes in the nucleus pontis oralis (NPO) play a critically important role in the generation and maintenance of wakefulness (W) as well as active (REM) sleep (AS). These data emanate from our behavior studies in chronic cats in which the microinjection into the NPO of GABA and its agonists induces prolonged periods of W. On the other hand, the injection of GABA antagonists into the NPO results in the rapid induction of AS and an increase in this behavioral state. However, the neuronal mechanisms of GABAergic actions in the NPO to promote W and suppress AS are undetermined. Consequently, the present study was designed to explore the cellular mechanisms of GABA actions on NPO neurons that generate AS (AS-generator neurons), and provide evidence that the effects of GABA are due to a direct inhibitory action on NPO AS-generator neurons. Accordingly, the effects of the juxtacellular application of GABA and bicuculline, a $GABA_A$ antagonist, on the activity of putative AS-generator neurons which were recorded intracellularly in the NPO were examined in chloralose-anesthetized cats. The juxtacellular application of GABA hyperpolarized the membrane potential of NPO neurons, and significantly decreased the amplitude of spontaneous EPSPs and the frequency of discharge of these cells; in contrast, the juxtacellular microejection of bicuculline depolarized NPO neurons and significantly increased the amplitude of spontaneous EPSPs and the frequency of their discharge. Some of these recorded NPO neurons were intracellularly marked with neurobiotin, and identified morphologically upon immunostaining. They were medium to large, multipolar cells with diameters >20 μm, which resemble glutamatergic AS-generator cells that have been previously identified in the NPO. The present results demonstrate, at a single cellular level of analysis, that inhibitory GABAergic inputs are capable of controlling the activity and discharge frequency of AS-generator neurons in the NPO, and indicate that these NPO neurons are under tonic GABAergic inhibitory control during W. Therefore, we believe that the pontine GABAergic mechanism functions in such a way that wakefulness is induced and maintained due to the activation of GABAergic process, which results in the suppression of discharge of AS-Generator neurons in the NPO.

Disclosures: M. Xi: None. **S.J. Fung:** None. **S. Sampogna:** None. **M.H. Chase:** None.

Poster

597. Biological Rhythms and Sleep: Systems

Location: SDCC Halls B-H

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Program #/Poster #: 597.15/CCC9

Topic: F.08. Biological Rhythms and Sleep

Support: NSERC Grant
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CIHR Grant MOP - 136967

Title: Spatio-temporal organization of sleep spindles and slow waves in naturally sleeping cats

Authors: *O. BUKHTIYAROVA[1,2], S. CHAUVETTE[2], S. SOLTANI[1,2], I. TIMOFEEV[1,2]
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Abstract: Sleep spindles are brain oscillations in the frequency range of 8-16 Hz that play a role in synaptic plasticity, learning and memory consolidation. During deep sleep, spindles co-occur with slow waves (SW). Several studies separate spindles on slow (8-12 Hz) and fast (12-16 Hz) and demonstrate their difference in spatial distribution, relation to slow-wave activity and impact on global brain functions.
The aim of this study was to investigate the presence of fast and slow spindles, their features and relationship to slow waves in local field potential (LFP) of sleeping cats.
LFP recordings were performed during sleep in 7 adult cats (6 males, 1 female), 47 cortical channels total. We developed a method of automatic detection of sleep spindles based on continuous wavelet transform and Gaussian Mixture Model clustering, followed by exclusion of non-rhythmic events in post-processing. Shortest spindle had 3 cycles. SW were detected with feed-forward artificial neural network that was trained to recognize their specific pattern.
During slow-wave-sleep, spindles were detected in all investigated cortical areas. They occurred 5-20 times per minute and lasted on average 477±98 ms with the most frequent and the longest spindles in medial prefrontal cortex and the least frequent and the shortest ones in ectosylvian gyrus. Both ‘fast’ and ‘slow’ spindles could be found in each channel as a continuum of frequencies, but not as separate sets. However, frontal and fronto-lateral areas had larger number of faster spindles, and medial and posterior cortical areas had more of slower spindles. The probability of spindle onset was significantly higher in 100-300 ms period following the peak of a SW, in particular in suprasylvian gyrus, but it was less pronounced in somatosensory areas, marginal, and ectosylvian gyri. Termination of spindles more likely occurred 200-100 ms before SW peak. There was no significant difference in frequency of spindles that followed or preceded SW. Most of detected spindles were local. In male cats, if spindles were global, they tended to propagate.

We did not find a clear separation between fast and slow spindles. Our results point to cortical area-dependent specific control of spindle generation.

Disclosures: O. Bukhtiyarova: None. **S. Chauvette:** None. **S. Soltani:** None. **I. Timofeev:** None.

Poster

597. Biological Rhythms and Sleep: Systems

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Program #/Poster #: 597.16/CCC10

Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant 5T32MH020002-17
NIH Grant 1R21MH112019-01A1
Kavli Foundation Grant KSIP-2017-002

Title: Spatiotemporal dynamics in human infant sleep spindles

Authors: L. MULLER[1], S. E. PETERS[2], A. A. BENASICH[3], *T. J. SEJNOWSKI[1]
[1]Salk Inst., La Jolla, CA; [2]Ctr. for Mol. and Behavioral Neurosci., Rutgers Univ. - Newark, Newark, NJ; [3]Neurosci., Rutgers University-Newark, Newark, NJ

Abstract: During sleep, the thalamus generates a characteristic pattern of transient, 11-15 Hz sleep spindle oscillations, which synchronize the cortex through large-scale thalamocortical loops. In previous work (Muller et al., *eLife* 5, 2016), we found that sleep spindles, rather than being either perfectly synchronized across the cortex or highly localized, often organize into a neural traveling wave (nTW), rotating globally across the cortex from temporal to parietal and to frontal lobe. In adult humans, these waves travel with a peak speed between 2-5 m/s, consistent with the conduction speeds of the short- and long-range association fibers in cortex. The specific spatiotemporal patterning of neuronal spiking activity during these waves has important implications for the process of memory consolidation during sleep. In this exploratory work, we test the hypothesis that global spindle waves are present in 3 to 7-month-old, typically developing infants, using high resolution (124 electrodes) scalp recordings during a daytime nap. This developmental period is characterized by a high rate of active cortical myelination which follows a spatiotemporal trajectory from occipital and parietal cortical regions to temporal and frontal regions over the course of several months. We hypothesize that the developing topography and spatiotemporal dynamics of infant sleep spindles are associated with myelinating fibers in cortex. We analyze EEG recordings of infant sleep and compare sleep spindle activity between 3.5-4 months and 6.5-7 months-of-age. The thin cranium at these ages allows EEG to capture neural activity with relatively high spatial resolution, because of reduced signal blurring by the skull. By applying a phase-based method for detecting traveling waves in noisy

multichannel data, we quantify the relative proportion of nTW and non-nTW patterns across these age groups. We interpret the results of the analysis at different ages with a computational model of cortical synchronization under axonal time delays. Preliminary analyses suggest that spindle nTWs appear in the infant by 6.5-7 months of age and their appearance may coincide with the development of cortical white matter tracts. These results will be compared with our earlier reported findings that sleep spindle power decreases over centro-parietal regions and increases over frontal regions between these two age groups, and that this change is correlated with measures of expressive and receptive language (Peters and Benasich, *Cog Neuro Soc Conf*, 2017). [LM and SP contributed equally to this work]

Disclosures: L. Muller: None. **S.E. Peters:** None. **A.A. Benasich:** None. **T.J. Sejnowski:** None.

Poster

597. Biological Rhythms and Sleep: Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 597.17/CCC11

Topic: F.08. Biological Rhythms and Sleep

Title: Characterizing the functional role of global and local sleep slow oscillations using ECoG

Authors: *N. NATRAJ, E. F. CHANG, K. GANGULY
Univ. of California, San Francisco, San Francisco, CA

Abstract: Slow oscillations, with a peak power at ~0.8Hz, are one of the primary brain rhythms observed during sleep. Slow oscillations represent synchronized down (hyperpolarized) and up (depolarized) states of neuronal activity (Steriade and Amzica 1998), originate primarily over frontal areas, are distinct from delta or slow waves (1-4Hz) and occur most frequently in NREM sleep stage 3 and stage 4 (Massimini 2004). Slow oscillations are important for memory consolidation (Marshall et al. 2006) as they group faster rhythms such as sleep spindles (8-16Hz) (Klinzing et al. 2016) and hippocampal ripples (>70Hz) (Staresina et al. 2015). Typically, slow oscillations in humans have been studied using electroencephalography (EEG) and are characterized as unitary events of global, cortex-wide neural synchrony. However electrocorticography (ECoG) data, that offers much higher spatial resolution, has shown slow oscillations are largely regional and local events (Nir et al. 2011) distinct from the cortex-wide global EEG slow oscillation. As such, the functional role of global and local sleep slow oscillation on memory related information processing, such as the nesting of sleep spindles, remain unclear. To address this issue, we analyzed over-night, multi-site electrocorticography (ECoG) data from 5 patients undergoing neurosurgical evaluation for epilepsy. Slow oscillations at individual channels were detected using an adaptive amplitude threshold (greater than at least 85% of all down-state to up-state peak amplitudes, if not higher), duration (900-3000ms cycle

duration) and sleep stage (NREM sleep). The local or global specificity of any given slow oscillation at any channel and time in sleep was quantified by its co-occurrence with slow oscillations across all other channels within a 500ms window. Results showed that the specificity of slow oscillations occupied a continuum; while the vast majority of slow oscillations at any given ECoG channel were local, global and more regional slow oscillations were also observed. Moreover, local slow oscillations tended to have smaller peak to peak amplitudes while global slow oscillations tended to have larger peak to peak amplitudes. Global slow oscillations largely occurred in stage 3 and 4 of NREM sleep while local and regional sleep oscillations were observed in all sleep stages. Preliminary analyses also showed that global slow oscillations tended to preferentially nest ongoing sleep spindles when compared to local sleep slow oscillations. Together, our findings shed new light on the functional role of slow oscillations based on its local or global specificity.

Disclosures: N. Natraj: None. **E.F. Chang:** None. **K. Ganguly:** None.

Poster

597. Biological Rhythms and Sleep: Systems

Location: SDCC Halls B-H

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Program #/Poster #: 597.18/CCC12

Topic: F.08. Biological Rhythms and Sleep

Support: DARPA HR0011-17-2-0025
NIH RO1 NS12542
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Cyberonix

Title: Vagal-evoked cortical potentials in monkeys follow a circadian pattern

Authors: *I. REMBADO[1], D. SU[3], A. LEVARI[4], L. SHUPE[5], E. E. FETZ[2], S. ZANOS[6]
[1]Dept. of Physiol. & Biophysics,, [2]Physiol. and Biophysics, Univ. of Washington, Seattle, WA; [3]Neurolog. Surgery, Univ. of Washington, Seattle, WA; [4]Leadership and Strategic Thinking, Univ. of Washington, Seattle, WA; [5]Dept. of Physiol. & Biophysics, Univ. of Washington, Seattle, WV; [6]Bioelectronic Med., Feinstein Inst. for Med. Res., Roslyn, NY

Abstract: Left vagus nerve stimulation (VNS) is used as therapy for epilepsy and major depression and has been tested clinically in the treatment of headaches, sleep disorders and in augmentation of rehabilitation with physical therapy after stroke [1, 2]. VNS produces afferent volleys that generate vagal evoked potentials (VEPs), recorded at different brain sites. The clinical significance of VEPs remains unclear but they are modulated by several VNS parameters, including current intensity and frequency of stimulation [3]. It is unknown whether they are also modulated by ongoing brain activity, which is known to change during the course

of day-night cycles. We sought to determine whether VEPs in macaque monkeys are modulated by time of day and night, during many hours of free behavior. In two male macaque monkeys epidural and intracortical electrodes were implanted in prefrontal, sensorimotor and parietal cortical areas and a bipolar cuff electrode was implanted on the left cervical VN. We used an updated version of Neurochip2 [4] to deliver trains of stimuli to the VN while simultaneously recording cortical activity continuously for 12-15 hours, while the animals were freely behaving in their cages. We tested several different pulsing frequencies (from 5 to 300 Hz), and 2 different pulse counts in a train (5 and 10 pulses). VEP responses in different cortical sites were compiled by averaging of corresponding cortical recordings triggered by the last stimulus in the train. The magnitude of the VEP responses was quantified by calculating the root-mean square (RMS) of the VEP between 15 and 400 ms after the last stimulus in the train. Both animals showed larger magnitude of VEP responses with stimulation at higher pulsing frequencies. In both animals, the VEP response was significantly modulated with time of day: VEP magnitude was minimal during early morning hours and maximal during late evening hours. VEPs in prefrontal sites showed a stronger modulation compared to sensorimotor and parietal sites. This study shows that VEPs change in a cyclical, circadian manner, with the time of day during which VNS is delivered. We are investigating whether ongoing cortical activity, which correlates with different brain and behavioral states, mediates this effect. These findings could have implications for experimental studies on the effects of VNS on brain function, for clinical trials studying VNS in brain diseases and for optimizing currently approved therapies involving VNS. [1] Henry, Neurology 59: S3-14, 2002; [2] Dawson et al, Stroke 47: 143-50, 2016; [3] Hagen et al, J Clin Neurophysiol 31: 143-48, 2014; [4] Zanos et al, IEEE TNSRE 19: 427-35, 2011

Disclosures: I. Rembado: None. **D. Su:** None. **A. Levari:** None. **L. Shupe:** None. **E.E. Fetz:** None. **S. Zanos:** None.

Poster

597. Biological Rhythms and Sleep: Systems

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Program #/Poster #: 597.19/CCC13

Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant K01-ES026839

Title: Quantitative analysis of polysomnograms can stratify risk of adverse cardiovascular events in older adults with sleep disordered breathing

Authors: *S. V. GLISKE
Sleep Disorders Clinic, Dept. of Neurol., Univ. of Michigan, Ann Arbor, MI

Abstract: Objective. At the population level, sleep disordered breathing (SDB) is known to have adverse effects on cardiovascular health. However, more information is needed to understand how severity of SDB modulates this risk at the individual patient level. The objective of this study was to identify quantitative sleep metrics which stratify risk of adverse cardiovascular events better than the existing measure (apnea hypopnea index (AHI)) in older adults with sleep apnea .
Methods. Subjects were selected from the Sleep Heart Health Study, with the inclusion criteria of 1) having a high quality EEG recording, and 2) being a non-smoker. This yielded 1,036 subjects. EEG data was quantified using a linear combination of the mean power spectrum per sleep stage. ECG data was quantified using a linear combination of 108 features, assessing variability in R-wave amplitude and inter-beat timing. Cox proportional hazard models for time to first adverse cardiac event were computed to assess utility of various combinations of predictors.
Results. After accounting for age, gender, and BMI, inclusion of AHI resulted in negligible improvement in the model ($p = 0.7$). However, inclusion of either of our novel, quantitative metrics did improve over the age, gender, BMI model with high statistical significance (EEG, $p<10^{-9}$; ECG, $p<10^{-11}$).
Conclusions. Quantitative analysis of EEG and ECG portions of polysomnograms provides additional information about the relationship between SDB and adverse cardiovascular events which is not captured by the standard apnea severity index (AHI).

Disclosures: S.V. Gliske: None.

Poster

597. Biological Rhythms and Sleep: Systems

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Program #/Poster #: 597.20/CCC14

Topic: F.08. Biological Rhythms and Sleep

Support: Sagol School of Neuroscience, Tel Aviv University (M.G.S.)
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Title: Intracranial electrical closed loop stimulation locked to hippocampal sleep-slow-oscillations in humans

Authors: *M. GEVA-SAGIV[1,4], E. A. MANKIN[1], D. ELIASHIV[2], N. TCHEMODANOV[1], Y. NIR[4,5,6], I. FRIED[1,3]

[1]Neurosurg., [2]Dept. of Neurol., [3]Dept. of Psychiatry and Biobehavioral Sci., Univ. of California, Los Angeles, CA; [4]Sagol Sch. of Neurosci., [5]Dept. of Physiol. and Pharmacol., Tel Aviv Univ., Tel Aviv, Israel; [6]Functional Neurophysiol. and Sleep Res. Lab., Tel-Aviv Sourasky Med. Ctr., Tel Aviv, Israel

Abstract: Slow waves (SWs, <4Hz) are the most prominent field potential oscillations during NREM sleep and may provide a temporal frame for a cortical-hippocampal dialogue that promotes memory consolidation. At present, there is a formidable gap between invasive mechanistic studies in animals linking temporal coordination between the hippocampus and cortex to memory consolidation and non-invasive human studies. Building upon animal models in which precisely timed electrical stimulation reinforced the endogenous coordination between hippocampal sharp wave-ripples, cortical slow wave up-states and sleep spindles, we set out to implement a real-time closed-loop (RTCL) system that could trigger electrical stimulation during sleep in the neocortex of humans timed precisely relative to sleep signatures recorded in the hippocampus. Upon informed consent, 12 patients with pharmacoresistant epilepsy, who had been implanted with intracranial electrodes for clinical monitoring in preparation for a possible surgical cure at UCLA, participated in recordings and intracranial electrical stimulation during 8 daytime naps and 6 overnight sleep sessions. Depth electrodes recorded detailed spiking activity (>500 unit clusters), local field potentials (8 microwires per electrode), and intracranial EEG (average of 9 electrodes per patient, 7-8 contacts per electrode) across multiple brain regions (including medial, temporal and extra-temporal cortical regions) during sleep. We developed a RTCL system that monitored sleep activity in the medial temporal lobe (MTL) and triggered brief (50ms) electrical stimulation in the neocortex, locked to a specific phase of MTL slow waves. Here, we demonstrate the ability of the RTCL loop to deliver electric stimulations robustly locked to MTL SWs up-states and find that stimulation locked to up-states enhanced subsequent slow wave activity. We present analysis of both the immediate (msec) and delayed (minutes) effects of such phase-locked stimulation on spindle power, as well as on single-unit entrainment to slow-wave phase. These effects are separately examined near the stimulating channel, in the MTL, as well as in additional brain areas. Additionally, we investigate the effects of RTCL stimulation on the coupling between SWs, spindles and hippocampal ripples, as well as on sleep-dependent memory consolidation.

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Poster

597. Biological Rhythms and Sleep: Systems

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 597.21/DDD1

Topic: F.08. Biological Rhythms and Sleep

Support: EU grant H2020, grant agreement 720270-Human Brain Project SGA1
"Sinergia" CRSII3_160803/1

Title: Bistability and complexity within the sleeping brain: Simultaneous intracranial eeg and high-density scalp eeg recordings

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Abstract: The clinical evaluation of disorders of consciousness (DOCs) in severely brain-injured patients relies on their ability to connect to the surrounding environment and demonstrate their subjective experience through motor behavior. To overcome this clinical problem, it has been recently developed a theory-driven, objective measure of the level of consciousness (Perturbational Complexity Index - PCI) calculated as the algorithmic complexity of the spatiotemporal pattern of the cortical responses obtained by perturbing the cortex with transcranial magnetic stimulation (TMS) (Casali et al. Sci Tr Med 2014). In awake healthy subjects, the EEG response to TMS (TEP) show multiple components possibly reflecting recurrent and causal interactions among different cortical areas (Sarasso Clin EEG Neurosci 2014) and results in high values of PCI. On the contrary, in vegetative state patients as well as in anesthesia and during the deepest stages of sleep (non-REM sleep), TEPs results in a positive-negative deflection highly resembling sleep slow-waves associated to low values of PCI. It is well known that spontaneous sleep slow-waves emerge from the bistable dynamics given by the alternation of neuronal intense firing (up-states) and silence (down-states) (Steriade J Neurosci 1993). It has been suggested, by means of intracranial electrical stimulation and recordings, that neuronal bistability could be responsible for loss of complexity in non-REM sleep (Pigorini et al. NeuroImage 2015). However, a direct link between bistability and loss of complexity is still missing. To this aim, the present work combines intracortical single pulse electrical stimulation (SPES) in humans undergoing pre-surgical evaluation, simultaneous intracortical recordings and scalp high-density electroencephalography (hd-EEG, 256 channels). Preliminary results show that during wakefulness the complex spatiotemporal dynamics observable at the scalp level are sustained by recurrent, causal interactions among different cortical areas. During non-REM sleep, when consciousness fades, the occurrence of cortical down-states after a transient activation (i.e. bistability) prevents the emergence of deterministic interactions leading to low PCI at the scalp level. Although very preliminary, these results draw a first link between local bistable dynamics characterizing cortical neurons during sleep and loss of complexity, a theoretical requirement for consciousness. Future studies should investigate whether sleep-like mechanism may account for the collapse of thalamo-cortical complexity detected by perturbations in pathological conditions such as in the DOCs patients.

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Poster

597. Biological Rhythms and Sleep: Systems

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 597.22/DDD2

Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant R01-MH-099645
NIH Grant R01-EB-009282
US Office of Naval Research Grant N00014-13-1-0672
National Science Foundation Graduate Research Fellowships Program

Title: Detecting causal interactions between brain regions during human sleep spindles

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Abstract: During non-REM sleep, the brain generates sleep spindles which are large amplitude oscillations that have complex spatiotemporal structure. Sleep spindles are ~1 s, 10-16 Hz oscillations that are thought to originate in the thalamus, however extensive corticothalamic feedback have been proposed to trigger, synchronize, and terminate spindle occurrence. The coordination of sleep spindles with slower (down state) and faster (hippocampal ripples) rhythms is believed to be important for sleep dependent memory consolidation. Here, we assess whether sleep spindles reflect periods of causal interactions between two brain regions. To detect causality between two time series, we developed cross-dynamical delay differential analysis (CD-DDA). We believe this nonlinear measure is complementary to traditional measures of directed influence, such as granger causality. We validated our measure on two simulated datasets, a Rössler system driving a Lorenz system, and one population of Izhikevich neurons driving another population. For our main analysis, we used data from three patients (2 women, 1 man, age: 40.7 ± 8.1) with intractable epilepsy implanted with depth sEEG electrodes in the cortex and thalamus. Here we analyze one night of sleep, from stages 2 and 3, and 64 corticothalamic pairs across all subjects. Spindles were detected on thalamic channels using a previously published protocol, and analyses were restricted to -500 ms to +500ms relative to the spindle onset in the thalamus. When applying CD-DDA, 53/64 corticothalamic pairs showed

significant cortical to thalamic directionality compared to baseline, and 56/64 showed significant thalamus to cortex directionality. Across trials, most channels showed asymmetrical causality after spindle onset. For example, 10/22 centroparietal sites and 25/42 frontal sites showed greater cortex to thalamus influence than thalamus to cortex after spindle onset. Thus compared to non-spindle times, spindle epochs reflect periods of information flow between brain regions. Future work will explore patterns of control across spindles at a given cortical site, as well as how association of spindles with down states or ripples affects causal interactions between brain regions.

Disclosures: C.E. Gonzalez: None. **A.L. Sampson:** None. **R. Kim:** None. **C. Lainscseck:** None. **R. Mak-McCully:** None. **H. Bastuji:** None. **P. Chauvel:** None. **M. Rey:** None. **E. Halgren:** None. **T.J. Sejnowski:** None.

Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 598.01/DDD3

Topic: F.10. Food Intake and Energy Balance

Title: Effect of sigma-1 receptor antagonist PD144418 on motivational aspects of feeding behaviors in male and female rats

Authors: *M. TAPIA, J. R. LEE, D. K. MILLER, M. J. WILL
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Abstract: A contributing factor to the obesity epidemic is the lack of balance between energy intake and energy expenditure. An increase in energy intake can lead to an increase in food consumption therefore, it is important to find ways to decrease food intake. Accumulating evidence regarding the sigma-1 receptor (σ1R) suggests its involvement in rewarding and motivational processes, through the effects vary based upon the ligand studied. While σ1R antagonist BD1047 did not alter reinforced behavior, BD1063 dose dependently reduced operant binge-like eating. In addition, pretreatment with BD1063 significantly reduced palatable, but not normal, chow intake in mice. PD144418 [1,2,3,6-tetrahydro-5-[3-(4-methylphenyl)-5-isoxazolyl]-1-propylpyridine] has been characterized as a potent and selective σ1 ligand, exhibiting a high affinity and selectivity for σ1Rs. In rodent behavioral studies, PD144418 has been found to produce a dose-dependent attenuation of locomotor activity of cocaine and by itself does not suppress basal locomotor activity in mice. However, nothing is known about its effects on motivation related to food. Therefore, two behavioral tasks were used to examine PD144418's effect on motivation and food consumption: 1) a progressive ratio (PR) operant task to examine motivation for food and 2) a free feeding paradigm, where no operant task was required to earn food. Male and female rats (n=8/group) were first trained on a fixed ratio (FR)

schedule of reinforcement. Following FR training, rats were tested under a PR schedule of reinforcement. 15-minutes prior to testing, each rat received a single dose of PD144418 (0 or 10 µmol/kg, ip). Pretreatment with PD144418 (0 or 10 µmol/kg, ip) on the consumption of freely available sucrose pellets was also examined. To determine the effects of acute (24-hr) food deprivation on the motivational effort to work for sucrose pellets, home-cage chow availability was altered in the final experiment. Findings revealed that when rats are pretreated with a 10umol/kg dose of PD144418, there is a significant reduction in their motivational effort to work for chow and sucrose pellets under a PR schedule of reinforcement but not of consumption when chow and sucrose pellets are freely available. Moreover, when homeostatic aspects of feeding were altered via acute food deprivation, pre-treatment with PD144418 suppressed the effects of acute food deprivation on the motivational effort to work for sucrose pellets but did not alter consumption under acute food deprivation when sucrose pellets were freely available. These effects were moderated by sex.

Disclosures: M. Tapia: None. **J.R. Lee:** None. **D.K. Miller:** None. **M.J. Will:** None.

Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 598.02/DDD4

Topic: F.10. Food Intake and Energy Balance

Title: Mice lacking PTP1B in astrocyte protect against obesity induced by a high fat diet

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Abstract: There are several lines of evidence that astrocyte regulates energy metabolism via leptin and insulin signaling, and also, the inflammatory signaling in astrocyte is required for hypothalamic inflammation induced by a high fat diet (HFD). Protein tyrosine phosphatase 1B (PTP1B) is a ubiquitously expressed protein tyrosine phosphatase which has been shown to negatively regulate both insulin and leptin signaling, and the expression of PTP1B is increased by inflammatory mediator such as TNFα. Recent studies utilizing mice with tissue-specific knock-out of PTP1B found the brain to be the primary site for PTP1B regulation of body weight. However, the specific sites mediating this effect in the brain are completely unknown. Especially, the role of PTP1B in astrocyte in the regulation of energy metabolism still remains unclear. In the present study, we investigated the role of PTP1B in astrocyte under HFD conditions. We generated astrocyte specific PTP1B deficient mice (KO) by crossing PTP1B

loxP/loxP mice with GFAP-Cre heterozygous mice. To assess whether energy balance is affected by PTP1B deficiency in astrocyte, we examined body weights in mice placed on either HFD or a chow diet at weaning. Body weights of KO mice were significantly lower than those of WT mice on HFD after 15 and 18 weeks of age (male and female, respectively). In contrast, on a chow diet, male and female mice showed no significant differences in body weight between genotypes. These results suggest that deficiency of PTP1B in astrocyte protects against obesity induced by HFD. Our data are also consistent with several recent studies showing an important role for astrocyte in energy metabolism and implicate PTP1B as a potentially important component of astrocyte in the regulation of energy balance under HFD conditions.

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Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 598.03/DDD5

Topic: F.10. Food Intake and Energy Balance

Title: High-fat feeding causes microglial activation and inflammation in ventral tegmental area in mice

Authors: *A. MIZOGUCHI[1], R. BANNO[1,2], H. YAGINUMA[1], K. TAKI[1], M. SUGIYAMA[1], T. TSUNEKAWA[1], H. TAKAGI[1], Y. ITO[1], H. ARIMA[1]
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Abstract: The feeding behavior is regulated not only by the hypothalamus but also the limbic system. In the limbic system, dopaminergic neurons in the ventral tegmental area (VTA) project to the nucleus accumbens (NAc). This neurocircuit processes information related to food reward, such as hedonic values of food, and is activated by consuming palatable food and provides emotional satiety. Recent studies reveal interactions between this system and diet induced obesity, which is one of the major health issue in many countries. One recent study shows that the inflammation in NAc is induced by a high fat diet (HFD) in mice, accounting for the dysfunction of reward system and heightened food cravings in saturated fat and sugar. In turn, it is widely known that HFD is responsible for hypothalamic inflammation via microglial activation, which is accompanied by leptin resistance in the arcuate nucleus becoming the cause of obesity. However, it is still unclear whether HFD causes inflammation in VTA in the reward system.
To clarify this, we placed 10-week-old male C57BL/6J mice on a chow diet or HFD for 3 days, 7

days and 28 days, respectively. Mice VTA were delivered immediately after they were perfused by 1×PBS for 5minutes under anesthesia, and their mRNA expressions of inflammatory cytokines (TNFα, IL1β and IL6) and microglial activation markers (Iba1, CD11b, Emr1 and CD68) were analyzed by quantitative real-time PCR. We found that in the group on HFD, there were significantly elevated mRNA expressions of IL1β for 3 days, TNFα, IL1β, IL6 and Iba1 for 7 days, and TNFα, IL1β, Iba1, CD11b and Emr1 for 28 days compared to the group on a chow diet, respectively.
These results suggest that the inflammation in VTA is induced by HFD, and there is a possible mechanism in which the inflammation is occurred via the activation of microglia in VTA, providing an insight into the pathophysiology of obesity caused by the dysfunction of reward system under HFD conditions.

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Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

Location: SDCC Halls B-H

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Program #/Poster #: 598.04/DDD6

Topic: F.10. Food Intake and Energy Balance

Support: MH093650
MH091945
DA030425
DA044664

Title: Reward sensitivity deficits in rats following intermittent access to a palatable diet

Authors: *C. F. MOORE, V. SABINO, P. COTTONE
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Abstract: Eating disorders and forms of obesity are associated with brain reward dysfunction. In this study we investigated the sensitivity of the brain reward system of subjects undergoing chronic diet cycling by testing the effects of *d*-Amphetamine, a dopamine releaser. For this purpose, a group of male Wistar rats was provided a regular chow diet 7 days a week (*Chow/Chow*), whereas a second group of rats was provided chow for 5 days a week, followed by a 2-day access to a highly palatable sucrose diet (*Chow/Palatable*). Following 5 weeks of diet alternation, we investigated *d*-Amphetamine sensitivity during access to the palatable diet (*'P Phase'*) as well as during withdrawal from it (*'C Phase'*). We measured the effect of *d*-Amphetamine on locomotor activity and brain stimulation reward (BSR), home-cage self-

administration of *d*-Amphetamine, and *d*-Amphetamine-induced conditioned place preference. In addition, we used quantitative polymerase chain reaction (qPCR) to investigate diet-induced molecular neuroadaptations. Palatable diet cycling resulted in hypophagia of the standard chow, overeating of palatable food upon renewed access, and compulsive-like eating. During the *P*, but not the *C phase*, diet cycled rats showed decreased sensitivity to both the locomotor stimulating and the threshold-reducing effects of *d*-Amphetamine. The rewarding effects of *d*-Amphetamine were also reduced in *Chow/Palatable* rats during the P Phase, shown by blunted place conditioning. In addition, during access to the palatable diet, *Chow/Palatable* rats showed increased self-administration of *d*-Amphetamine in the home cage, as compared to controls. Furthermore, we found that intermittent access to a palatable diet altered expression of dopamine signaling targets. These results indicate that diet cycled rats show a phase-dependent deficit in the brain reward system, as revealed by a decreased sensitivity and reward to *d*-Amphetamine, as well as increased self-administration of *d*-Amphetamine when the highly palatable food access is renewed following withdrawal from the diet. In summary these results suggest that, in pathological eaters, brain reward dysfunction may be dependent upon the feeding state of the individuals.

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Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 598.05/DDD7

Topic: F.10. Food Intake and Energy Balance

Support: 1Z1AES102805

Title: A locus coeruleus to lateral hypothalamus circuit for suppression of feeding

Authors: *N. R. SCIOLINO[1], C. M. MAZZONE[1], N. W. PLUMMER[1], J. AMIN[1], K. G. SMITH[1], C. A. MCGEE[1], C. X. YANG[1], M. J. KRASHES[2], A. V. KRAVITZ[2], M. R. BRUCHAS[3], J. D. CUSHMAN[1], G. CUI[1], P. JENSEN[1]
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Abstract: Clinical evidence implicates altered norepinephrine (NE) signaling in overeating and excessive weight gain. Although modulators of NE signaling are currently the most effective drugs for weight loss, they result in adverse side-effects due to their broad actions throughout the nervous system. Thus, there is a critical need to identify specific NE circuits that suppress feeding without other effects. Towards this goal, we used chemogenetics in combination with fiber photometry to reveal that activation of NE-locus coeruleus (LC) neurons results in

suppressed feeding and weight loss. This key finding, along with evidence that feeding is also suppressed by delivery of NE agonists into the lateral hypothalamus (LHA), suggests that increased NE-LC activity suppresses feeding through select inputs to the LHA. To test this hypothesis, we used optogenetics to activate the LC-LHA circuit in our knock-in mouse line that expresses cre recombinase under control of the noradrenergic dopamine beta-hydroxylase (*Dbh*) promoter. We injected the LC of *Dbh*^{cre} mice *with a cre-responsive virus expressing* channelrhodopsin-2 (ChR2) or eYFP control, *and then* implanted optical probes over the LHA. We found that photostimulation (10 Hz) of the LC-LHA circuit rapidly suppressed feeding in ChR2 mice relative to controls**.** To rule out the possibility that this effect was due to changes in anxiety, mice were tested in the elevated plus maze and real-time place aversion test. In both tests, photostimulation had no effect on anxiety-like behavior in ChR2 mice relative to eYFP controls, demonstrating the LC-LHA circuit regulates feeding independent of anxiety. To ascertain if NE signaling from LC neurons is required to suppress feeding, we used our *Dbh* conditional knockout allele in combination with *En1*^{cre} (LC-Dbh mutants) to disrupt NE synthesis selectively in LC neurons. LC-Dbh mutants and littermate controls were pretreated with vehicle or the alpha-2 adrenoceptor antagonist yohimbine (3 mg/kg i.p.), which is known to activate LC neurons. We found that yohimbine suppressed feeding in littermate controls but had no effect in LC-Dbh mutants. Collectively, these findings reveal a novel role for LC neurons in the suppression of feeding that is mediated by release of NE in the LHA. The findings suggest that targeting specific NE neural pathways may yield improved weight loss therapies without anxiety side-effects.

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Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 598.06/DDD8

Topic: F.10. Food Intake and Energy Balance

Support: Shire

Title: Effects of lisdexamfetamine on instrumental and consummatory behaviors supported by foods with varying degrees of palatability: Exploration of a binge eating model

Authors: *R. PRESBY[1], R. A. ROTOLO[1], J.-H. YANG[1], M. CORREA[2], J. D. SALAMONE[1]
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Abstract: It is widely recognized that overconsumption of high-sugar or high-fat diets is associated with multiple conditions, including obesity, hypertension, and type 2 diabetes. Furthermore, binge eating disorder (BED) affects approximately 2% of the US adult population, and occurs more frequently in females. Thus, it has become important to develop animal models of palatable food consumption that may have relevance for BED and other conditions associated with intake of highly palatable foods. Operant behavior tasks that involve food reinforcement have been used in order to allow animals choices between high value rewards that are obtained by a high degree of effort vs. low-effort/lower value options. The catecholamine uptake blocker lisdexamfetamine (LDX) has been approved for the treatment of BED. The present experiments studied the effect of LDX on both food intake and food-reinforced behavior, as assessed in singly housed, female Wistar rats. Three groups of rats received different food exposure conditions in the home cage randomly spread over several weeks: the chocolate exposure group (CE) was exposed to brief access of chocolate and additional lab chow (n=15), a lab chow exposure (LChE) group was given additional access to lab chow (n=8), and a third group was given empty food dishes (n=7). In tests of food intake under non-restricted conditions, injections of LDX (0.1875-1.5 mg/kg) significantly reduced intake of both chocolate and chow in the CE group. In the LChE group, there was a trend towards reducing chow intake induced by LDX. All rats were trained on the Progressive Ratio (PROG)/chow feeding choice task, in which they had the option of working for high carbohydrate chocolate flavored pellets by lever pressing (high value reward/high effort) or approaching and consuming a concurrently available lab chow (low effort/low value reward). The LChE group and the empty food dish group were combined to create one control group (n=15). There was a significant overall dose related suppressive effect of LDX on lever pressing but no group difference, and no dose x group interaction. A significant reduction in lever pressing was seen at the 3 highest doses of LDX. LDX significantly decreased chow intake in the CE group at the 3 highest doses, but not in the control group. In conclusion, LDX appears to affect both food intake and food-reinforced operant behavior across all groups, with larger effects seen in the group exposed to chocolate.

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Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 598.07/DDD9

Topic: F.10. Food Intake and Energy Balance

Support: NIH Grant MH112105

Title: The endocannabinoid AEA amplifies food preferences in *C. elegans*

Authors: S. FAUMONT, S. LEVICHEV-CONNOLLY, R. BERNER, *S. R. LOCKERY
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Abstract: The endocannabinoid system, comprised of the endocannabinoids AEA (N-arachidonoyl-ethanolamine) and 2-AG (2-Arachidonoylglycerol), their receptors, CB1 and CB2, and their metabolic enzymes, is believed to integrate internal energy state and external food cues to modulate feeding. For example, cannabinoids, acting on CB1, can increase preference for rich, "tasty" food, a response called hedonic amplification. In mammals, cannabinoids can increase sensitivity to odors and sweet tastes, which may underlie amplification. We are developing *C. elegans,* an omnivorous bacterivore, as a model in which to investigate the neurophysiology of hedonic amplification. We found that exposure to AEA, an endogenous cannabinoid in *C. elegans*, increases the worms' preference for preferred, high quality bacteria over less preferred, low quality bacteria, mimicking hedonic amplification in mammals. Furthermore, AEA acts bidirectionally, increasing consumption of high quality bacteria while decreasing consumption of low quality bacteria. We also found that deletion of the CB1 homolog, *npr-19,* eliminates hedonic amplification in *C. elegans*. Amplification was rescued by expression of wild type *npr-19* or human CB1 driven by the endogenous *npr-19* promoter, establishing a humanized worm for cannabinoid signaling studies. Deletion of the olfactory neuron AWC, which directs chemotaxis to food, abolished hedonic amplification measured in terms of food attraction. Consistent with this finding, calcium imaging revealed that AEA bidirectional modulates AWC, increasing and decreasing its responses to high and low quality food, respectively. We are testing the hypothesis that AEA acts directly on AWC to modulate food preferences in hedonic amplification.

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Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 598.08/DDD10

Topic: F.10. Food Intake and Energy Balance

Title: Effects of maternal docosahexaenoic acid (DHA) supplementation on lipid peroxidation products in offspring mouse

Authors: *T. WOO[1], B. YANG[4], R. LI[2], K. FRITSCHE[3], G. Y. SUN[5], M. GREENLIEF[4], D. Q. BEVERSDORF[6]
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Abstract: The brain and retina are known to comprise of high levels of docosahexaenoic acid (DHA). Recent studies have demonstrated beneficial effects of dietary supplementation of DHA in the form of fish oil, including alleviating autism-associated behaviors in a gene/stress mouse model, but the mechanism(s) of action are not fully understood. Recent studies have focused on 4-hydroxy hexenal (4-HHE) and 4-hydroxy nonenal (4-HNE) which are peroxidation products of DHA and arachidonic acid (ARA), respectively. In this study, we determined the levels of these alkenals in heart, plasma, and brain tissue of weanling pups after being nursed by mothers given a DHA-supplemented diet. In the heart tissue, pups with a maternal DHA diet resulted in a 4.3-fold increase in 4-HHE. In the plasma, the maternal DHA diet induced a 1.7-fold increase in 4-HHE and a significant decrease in 4-HNE. Analysis of brain tissue indicated a significant increase of 4-HHE levels in cerebral cortex and hippocampus, but not in striatum and cerebellum, suggesting differences in lipid peroxidation activities within brain regions. Consistent with the results of lipid peroxidation products, analysis of fatty acids revealed a significant increase in DHA and decrease in ARA levels in offspring plasma, heart and brain regions, albeit to different extent. Taken together, this study demonstrates how maternal supplementation of DHA can influence fatty acid concentrations and their lipid peroxidation products in brain and body organs. It is possible that these changes in fatty acids and peroxidation products underscore the redox homeostasis and behavioral outcomes during the developmental period. Now we are using the SERT-KO/S mouse model to examine whether maternal stress alters lipid peroxidation activity in the brain and whether DHA supplement can mitigate these changes.

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Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 598.09/DDD11

Topic: F.10. Food Intake and Energy Balance

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UVA Brain Institute 2017 Pilot Grant
Presidential Fellowship for Collaborative Neuroscience
University of Virginia start up funds

Title: Non-canonical dopamine circuit causes metabolic disorganization and obesity

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Abstract: Across the globe, widespread availability of energy-dense, rewarding foods is increasing the prevalence of obesity. In addition to inducing overconsumption, hypercaloric diets disorganize circadian feeding pattern, switching food intake from a meal-centered schedule to one based on frequent snacking. This out of phase consummatory behavior results in weight gain and metabolic disease. However, the mechanism of how palatable foods disrupt daily rhythms of feeding and metabolism is unknown. Since midbrain dopamine is released in response to rewarding sensory cues such as food rich in sugar and fat, we explored the role of dopamine circuitry in mediating energy-dense diet induced perturbation of feeding schedule. Here, we demonstrate that energy-dense foods modulate dopaminergic signaling within the central circadian pacemaker, disrupt daily rhythm of feeding and cause metabolic desynchrony in peripheral tissues. In addition to a delayed rate of photic entrainment (Grippo *et al.*, 2017 *Current Biology*), we observe that D1 dopamine receptor (Drd1) null mice are resistant to diet induced obesity and metabolic syndrome. Genetic rescue of Drd1 expression specifically within the suprachiasmatic nucleus (SCN) of these mice restores rest phase consumption, weight gain and obesogenic symptoms on an energy dense, high-fat diet. This work identifies SCN-Drd1-dependent signaling as a promising therapeutic target for the prevention of obesity.

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Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

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Program #/Poster #: 598.10/DDD12

Topic: F.10. Food Intake and Energy Balance

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PSC/CUNY Grant 60102-00 48

Title: Acquisition and expression of sucrose conditioned flavor preferences following dopamine D1, opioid and NMDA receptor antagonism in C57BL/6 mice

Authors: B. ISKHAKOV, G. FAZILOV, M. SHENOUDA, A. BURAS, D. BHATTACHARJEE, P. DOHNALOVA, J. ISKHAKOVA, F. BOURIE, *R. J. BODNAR
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Abstract: In addition to the abilities of sucrose and saccharin to induce intake in non-deprived rodents, conditioned flavor preferences (CFP) are elicited by sucrose relative to saccharin in rats and inbred mice. Both acquisition (learning) and expression (maintenance) of sucrose-CFP can be modified by pharmacological receptor antagonism that is further subject to murine genetic variance. Thus, muscarinic cholinergic receptor antagonism with scopolamine eliminated the acquisition (learning) of sucrose-CFP in BALB/c mice, reduced its magnitude in SWR mice, but failed to affect the response in C57BL/6 mice. This pattern differed from the greater potency of scopolamine to reduce sucrose and saccharin intake in C57BL/6 and BALB/c mice relative to SWR mice. The three strains also display differential sensitivity to dopamine D1 and opioid receptor antagonists in reducing sucrose or saccharin intake. Whereas dopamine D1 receptor antagonism eliminates acquisition of sucrose-CFP in SWR, but not BALB/c mice, opioid receptor antagonism eliminates this response in SWR, but not BALB/c mice. N-methyl-D-aspartate (NMDA) receptor antagonism is more potent in eliminating acquisition of sucrose-CFP in BALB/c relative to SWR inbred mice. The present study examined whether naltrexone, SCH23390 or MK-801 altered acquisition and expression of sucrose-CFP in C57BL/6 mice. In acquisition experiments, male food-restricted C57BL/6 mice were treated with vehicle, naltrexone, SCH23390 or MK-801 30 min prior to each of ten daily sessions in which they alternately consumed a flavored (CS+, e.g., cherry) 16% sucrose solution and a differently-flavored (CS-, e.g., grape) 0.05% saccharin solution followed by six two-bottle CS choice tests mixed in 0.2% saccharin without injections. SCH23390 and MK-801, but not naltrexone eliminated sucrose-CFP acquisition in C57BL/6 mice. In expression experiments, C57BL/6 mice underwent the ten training sessions without injections followed by two-bottle CS choice tests 30 min following vehicle, naltrexone, SCH23390 or MK-801. SCH23390 more effectively reduced the magnitude of sucrose-CFP expression than naltrexone or MK-801 in C57BL/6 mice. Thus,

dopamine D1 and NMDA receptor signaling is essential for learning of sucrose-CFP in C57BL/6 mice. This pattern of antagonist effects differed from BALB/c and SWR strains, further indicative of murine genetic variance.

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Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

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Program #/Poster #: 598.11/DDD13

Topic: F.10. Food Intake and Energy Balance

Support: UNAM, DGAPA IN 217117

Title: Blockade of dopamine D2 receptors in the nucleus accumbens prevents the behavioral changes induced by intermittent access to sucrose

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Abstract: Excessive consumption of highly-palatable food is frequently found in patients with binge eating disorder (BED), bulimia nervosa (BN), and in some obese patients. Specifically, in individuals diagnosed with BED and BN, bingeing is a key eating disorder feature, even in absence of energy restriction. According to the experimental evidence, alterations in the mesolimbic dopaminergic transmission produced by non-homeostatic feeding behavior may be associated with changes in the reward system analogous to those produced by drugs of abuse. Although it is known that changes in dopaminergic transmission mediated by D2 receptors in the nucleus accumbens shell (NAcS) are related to binge-eating symptoms, it has not been evaluated whether these receptors may be a potential target for the treatment of eating pathology with binge-eating. Correspondingly, the aim of the present study was to evaluate whether sugar bingeing induced by intermittent access to a sucrose solution produced changes in the structure of feeding behavior and if blocking D2 receptors in the NAcS prevented these changes. We used the intermittent access to a 10% sucrose solution (2 h/day for 4 weeks) model to induce sugar bingeing in Sprague Dawley female rats. After 28 days, experimental subjects consumed in a 2-hour period more than 50% of the caloric intake consumed by the subjects with *ad libitum* access to the sweetened solution without any increase in body weight or fat accumulation. Once established the binge-like behavior, we characterized the structure of feeding behavior

(microstructural analysis) and evaluated the motivation for palatable food (breakpoints). We found that feeding episodes had short latencies, high frequencies, as well as short durations and inter-episode intervals. Furthermore, we observed that the intermittent access to sucrose protocol did not increase breakpoints, as occurred in subjects with *ad libitum* access to the sucrose solution. Finally, we evaluated the effects of D2 receptor blockade in the NAcS, and found that raclopride (18nM) administration blocked the increase of sucrose consumption, as well as the changes in the frequency and duration of episodes induced by intermittent access to the sucrose solution. In summary, our results suggest that alterations in behavioral patterns associated with binge-eating behavior depend in part on the dopaminergic transmission in the NAcS and that the antagonism of D2 receptors should be considered as a plausible therapeutic tool for feeding pathology with binge-eating.

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Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

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Topic: F.10. Food Intake and Energy Balance

Support: Howard Hughes Medical Institute
Jane Coffin Childs Fund Postdoctoral Fellowship

Title: Dopamine neuromodulation of host-seeking behavior in the female mosquito

Authors: *T. R. SORRELLS, L. B. VOSSHALL
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Abstract: Dopamine circuits integrate sensory information, internal state, and experience to control goal-directed behavior in widely diverged species. It is unclear how these circuits are repurposed during evolution to control the different behavioral drives in different species. Mosquitoes, along with several other independent insect taxa, have evolved to seek out and bite human hosts to acquire protein for egg development. Host cues such as human body odor, carbon dioxide, and heat synergize to increase mosquito arousal and initiate search and feeding behaviors. Although mosquitoes also feed on plant nectar for energy, this source of food is insufficient for reproduction and its acquisition is behaviorally distinct from blood-feeding. We are studying the control of host-seeking drive in the *Aedes aegypti*mosquito through genetic manipulation of dopamine circuits in the brain. Blood-feeding behavior specifically requires one of the four dopamine receptors present in the mosquito genome. The expression patterns of these receptors are substantially diverged from those of the vinegar fly Drosophila melanogaster,

suggesting that evolutionary changes in this neuromodulatory system may have contributed to the distinct behavioral drives of these two insects. We are currently developing additional driver and effector lines to further dissect the role of dopamine circuits in the behavior of this important human disease vector.

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Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

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Topic: F.10. Food Intake and Energy Balance

Support: PRIN 2012JTX3KL_002

Title: Central effects of the satiety signal oleoylethanolamide in an animal model of frustration stress-induced binge eating disorder

Authors: *C. A. GALLELLI[1], A. ROMANO[1], M. V. MICIONI DI BONAVENTURA[2], J. B. KOCZWARA[1], M. E. GIUSEPPONI[2], T. CASSANO[3], C. CIFANI[2], S. GAETANI[1]
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Abstract: Binge-eating disorder (BED), characterized by compulsive and uncontrollable overeating of highly palatable food (HPF), has been associated to altered dopamine (DA) and serotonin (5-HT) brain signalling. The satiety signal oleoylethanolamide (OEA) has emerged as a potential novel pharmacological tool for controlling aberrant eating patterns, by restoring a normal brain dopaminergic response, when it is deregulated by an excessive dietary fat intake. Based on these premises in this study we investigated in a rat model of BED the effects of OEA: 1) on Fos expression and tissue monoamine (DA, 5-HT, Noradrenaline) concentrations in brain areas controlling feeding and reward; 2) on the modulation of DA release within the shell of the nucleus accumbens (AcbSh). In our model, female rats with a history of intermittent food restriction and HPF consumption showed binge-like food intake after the exposure to a "frustration stress" consisting of the sight of unreachable HPF (BED rats). Control rats were exposed to the same experimental manipulations except for food restriction and did not show any binge eating behaviour. OEA was administered (10 mg/kg i.p.) to two different sets of both BED and control rats. A first set was sacrificed 2 hours after OEA administration; their brains were partly sliced into 20 μm coronal sections (immunostained for Fos), and partly microdissected for monoamine determination by HPLC.The second set of rats was subjected to in vivo microdialysis of the AcbSh, collecting dialysates every 15 min, and was first intraperitoneally

treated with OEA (10 mg/kg) and then challenged with a subcutaneous dose of amphetamine (0.5 mg/kg). DA dialysate levels were analysed by HPLC. OEA administration was able to restore a "normal" brain activity, by reducing the stress-induced Fos increase in brain areas regulating feeding and the dopaminergic signalling. Moreover, we found that OEA treatment decreased DA efflux in the AcbSh, following either stress exposure or amphetamine challenge. At tissue level, we found that OEA also reduced DA concentration within the Acb of BED rats. As far as the serotonergic system, we found that OEA is able to enhance 5-HT transmission in most of the brain areas analysed, selectively in bingeing rats. Overall, these results further enrich our current knowledge on the central effects of OEA and support, for the first time, the hypothesis that OEA might represent a novel potential pharmacological target for the treatment of BED.

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Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

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Topic: F.10. Food Intake and Energy Balance

Support: ANR GRANT
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Title: Common neural underpinnings between anorexia, memory and addiction

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Abstract: In neurons of the nucleus accumbens, activation of a cAMP signaling is a means of transforming an immediate reduction of drugs' rewarding effect into a durable dependence, mimicking a form of learning. After recruiting cAMP-response element binding protein (CREB)-binding protein, the resultant phosphorylated CREB (pCREB) favors the expression of some genes (FosB, ΔFosB, and CART: cocaine- and amphetamine-regulated transcript) to the detriment of others (methyltransferase G9a of histone), from where come changes in neuron morphology. Serotonin (5-HT, 5-hydroxytryptamine) volume transmission trough many receptors act on cAMP signaling and thus modulate the activity of the reward neural pathways. Our previous studies show that stimulation of Gs-coupled serotonin 4 receptors (5-HT_4Rs) triggers activation of cAMP/PKA/CART/FosB/ΔFosB signaling pathway, which serve to induce anorexia-like behavior. Here, we examine how cAMP in the NAc impacts food intake. We found

that elevated levels in cAMP induced by local infusion of BIMU8, a 5-HT_4Rs agonist, into the NAc were more prominent when BIMU8 was co-infused with St-Ht31 peptide that blocks AKAP (A-kinase anchoring protein) / PKA binding. Results includes that the levels of CART peptide, known to promote anorexia and addiction, were more elevated following St-Ht31/BIMU8 co-treatment than in mice infused only with BIMU8 into the NAc. Finally, mice with highest increased levels of cAMP and CART induced by the blockade of AKAP/PKA binding in the NAc display the highest restrictive food intake following food deprivation, supporting the view that anorexia becomes persistent through similar mechanisms underlying habituation towards learning and memory.

Disclosures: V. Compan: None. **G. Conductier:** None.

Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

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Title: Impact of nutrition on risk decision making

Authors: *L. LIU[1], S. STRANG[1], S. O. ARTIGAS[1], A. ULRICH[2], J. TARDU[2], O. UHL[3], B. KOLETZKO[3], S. M. SCHMID[2,4], S. Q. PARK[1]
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Abstract: Abstract: Previous research has provided evidence for nutrition-driven modulation on social decision-making processes. Macronutrient composition of a food modulates different biochemical processes, leading to dissociable influence on decision making. Specifically, a higher protein-carbohydrate ratio food alters blood tyrosine levels, presumably leading to an elevation in neurotransmitter dopamine. This is crucial, since many studies using pharmacological or genetic approaches have shown that an enhancement in brain dopamine leads to risk-proneness. In this study, we investigate whether a balanced one-shot Western-style meal with different protein-carbohydrate ratio is sufficient to impact risk decision making. **Methods** Thirty-five male subjects (mean age = 23.40 y, SD = 3.31; mean BMI = 22.96, SD = 1.75) were

investigated in two different days with a gap of 7-9 days. In each session, they received either a high- (25%/50%) or a low- (10%/80%) protein/carbohydrate ratio breakfast. We monitored plasma tyrosine and the individual monetary risk decision making behavior. Also, individual differences in the behavioral inhibition system and the behavioral approach system (the BIS/BAS scale) were assessed. **Results** The plasma tyrosine level was significantly enhanced, whereas the plasma tryptophan level was decreased in the high protein/carbohydrate session when compared with the low protein/carbohydrate session. Furthermore, participants showed significantly more risk-seeking behavior in the high vs. low protein/carbohydrate session. Strikingly, this session difference in risk-seeking behavior was significantly correlated with the individual differences in the BAS-fun seeking scale, suggesting that the risk decision making behaviors in individuals with high level of behavioral approach may be more susceptible to the balance between protein and carbohydrate intake. **Conclusions** Our results demonstrate how risk decisions can be impacted by the macronutrient composition of a daily meal, by unveiling its underlying metabolic processes.

Disclosures: L. Liu: None. **S. Strang:** None. **S.O. Artigas:** None. **A. Ulrich:** None. **J. Tardu:** None. **O. Uhl:** None. **B. Koletzko:** None. **S.M. Schmid:** None. **S.Q. Park:** None.

Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

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Topic: F.10. Food Intake and Energy Balance

Support: ICMR/DHR

Title: Involvement of transient receptor potential vanilloid (trpv) type channels in olanzapine-induced hyperphagia and weight gain

Authors: *R. SINGH[1], Y. BANSAL[1], T. SOGA[2], I. PARHAR[2], A. KUHAD[1]
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Abstract: Background: Despite the clinical benefits, atypical antipsychotics (AAPs) exerts troublesome adverse effects particularly hyperphagia, weight gain, dyslipidemia, insulin resistance, metabolic and cardiac complications. Recent evidence shows the role of TRPV channels in reward-seeking and feeding behavior through modulation of mesolimbic dopaminergic pathways. **Aim**: With this background present study was designed to investigate the role TRPV channels on hyperphagia and weight gain induced by olanzapine in mice. **Material and methods:** Induction of schizophrenia-like behaviors in mice (n=10) was done with MK-801 (0.1 mg/kg, i.p.) for five days. 6th day onwards animals were being treated with

olanzapine (6 mg/kg p.o.) and control (CMC 0.25%, p.o.) for 4 weeks. Weekly assessment of feed, water intake, body weight was done and on last day of fourth week fasting glucose and oral glucose tolerance test was done. Quantification of TRPV1 and TRPV3 gene expression in nucleus accumbens (NAc), hypothalamus and ventral tegmental area (VTA) were done. **Results**: MK-801 induced a schizophrenia-like behavioral alteration in mice which were significantly reversed by olanzapine treatment. Acute treatment of olanzapine-induced hyperphagia and while significant weight gain was observed as compared to control. A significant increase in TRPV3 gene expression in NAc and hypothalamus were observed while TRPV1 expressions were significantly in hypothalamus and VTA. These results indicate the role of TRPV channels in mesolimbic system (NAc and VTA) which may contribute to active reward circuitry leading to hyperphagia and weight gain. **Conclusion**: Our primary results indicated that TRPV1 and TRPV3 channels may involve in antipsychotics induced hyperphagia, weight gain and their modulators could be a therapeutic alternative in the management of hyperphagia, weight gain, food addiction, and eating disorders.

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Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

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Topic: F.10. Food Intake and Energy Balance

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Title: Nutritional lipids and glial remodeling

Authors: *C. ROVERE[1,2], C. CANSELL[2], O. LE THUC[2], K. STOBBE[2], C.-A. MOSSER[3], F. BRAU[2], N. DEVAUX[2], C. LEBEAUPIN[2], E. AUDINAT[4], J.-L. NAHON[2], N. BLONDEAU[2]
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Abstract: Energy balance is finely regulated by the central nervous system: it integrates peripheral signals reflecting the energy status of the organism and in turn adapts food intake and energy expenditure in order to maintain a stable weight throughout adult life. The hypothalamus

(HT) is one of the cerebral structures having a major role in the integration of those signals. Several studies show that obesity induced by a high fat diet (HFD) leads to inflammation at the level of HT which could cause obesity. Moreover, lipids contained in HFD might be directly responsible for the onset of the inflammatory response. At cellular level, this inflammation is in part characterized by an activation of microglia cells and astrocytes in the HT. In rodent, recent studies show that hypothalamic proliferation of microglia cells and astrocytes is observed in the first 24 hours of consumption of HFD, well before the development of obesity, and seems to be reversible. We therefore assume that early glial activation would be an adaptive mechanism involved in the physiological regulation of energy balance and that overexposure to nutritional lipids could deregulate this inflammatory response and lead to obesity. In our study we observed an increase in the expression of the astrocytes and microglial cells markers (GFAP and Iba1 respectively) in the HT after 1 h of HFD consumption. Moreover we observed morphological modifications of microglial cells in the HT after 3h of HFD consumption. This remodeling is associated with differential activation of specific inflammatory markers and hypothalamic peptides involved in energy balance regulation. Our results suggest that inflammation induced by HFD consumption is a very early phenomenon which might be involved in the central regulation of energy balance. In the future, this glial remodeling will modulate using pharmacogenetic tools in order to establish the cascade of molecular and cellular events at the origin of CNS perturbations associated with obesity.

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Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

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Program #/Poster #: 598.18/DDD20

Topic: F.10. Food Intake and Energy Balance

Title: The effect of naloxone in the consumption of a high carbohydrate diet at weaning and its repercussion on the intake of hypercaloric diet in adult male rats

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Abstract: Evidence suggests that infancy may be a critical period for the exposure to hypercaloric food (rich in both fat and carbs). The hypercaloric food tends to have high palatability, which make it highly preferred. The overconsumption of this food may produce aberrant patterns of eating resulting in health problems.

At preadolescence, some of the neurotransmission systems are maturing (such as the opioid system), and due to the implication of opioids in the hedonic perception of stimuli, alterations of this pathway may affect the appetitive and consummatory aspects of alimentary behavior. On this basis, the effects of the opioid antagonist, Naloxone (NA) on the consumption of high-carbohydrates food during preadolescence and its repercussion on the consumption of hypercaloric food in adulthood were studied.
Four groups of male Wistar rats were exposed to different pharmacologic treatments on infancy during 18 days, starting at 23 postnatal day (PND); 15 min after injection the rats were exposed to a food rich in carbohydrates (CHO).
Groups:
NALCHO: NA prior CHO exposure.
CHONAL: NA after CHO exposure.
VEHCHO: Vehicle (VA) Administration prior CHO exposure.
CHOVEH: VA after CHO exposure.
At 70 days, as adults, base line (BL) of only standard food (STD) was measured, and at 75 PND, all groups (n=11) received a hypercaloric diet (HCD, rich in carbs and fat) during 4 weeks. At 103-108 PND a post-treatment (PT) period with only standard food (STD) was measured and then re-exposed (RE) to the HCD at 108-113. Food intake and body weight were registered.
At weaning, NALCHO show higher intake of calories from STD than VEHCHO and CHOVEH, and ate fewer calories from CHO than the other 3 groups. The overall (STD+CHO) calories consumed weren't affected.
At adulthood, after the hypercaloric diet was removed, we found a negative contrast effect. Intake of STD significantly decreased in PT respect of BL. The CHOVEH ate fewer STD calories than all the other 3 groups in PT. The NALCHO ate more calories than the vehicle groups, and CHONAL more than VEHCHO in RE. CHONAL gained more body weight than VEHCHO and CHOVEH, and NALCHO more than VEHCHO in BL and RE. Overall body weight gain was less in PT than in the HCD exposure. The NAL groups gained more weight than Vehicle groups in RE.
Results suggest that the blockade of the opioid system at weaning can alter the consumption of a high-in-carbohydrates food, depending on the administration occurs either, before or after having this food. Besides, this condition at infancy has repercussion in adulthood, regardless of the opioid blockade contingencies.

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Poster

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Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 598.19/DDD21

Topic: F.10. Food Intake and Energy Balance

Support: R00AA021782

Title: An animal model of binge-like eating using short vs long access self-administration

Authors: *G. R. CURTIS, L. SANZALONE, N. MACK, J. R. BARSON
Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Binge eating is a defining feature of binge-eating disorder and can lead to the development of obesity, which itself can cause numerous health problems. This behavior is characterized by the consumption in a discrete time period of an abnormally large amount of food, particularly palatable food high in sugar and fat, even in the absence of hunger. As few animal studies have successfully modeled this behavior, the goal of the present experiment was to develop a paradigm for reliably inducing binge-like eating. Adolescent female Long-Evans rats (N = 16), with *ad libitum* access to chow and water in the home cage, were trained 5 days per week in operant chambers to lever-press on a fixed ratio 1 (FR1) schedule of reinforcement for the highly caloric and palatable Chocolate Ensure Plus®. Half of these animals self-administered Ensure in short-access sessions of 30 minutes per day, while the other half were given long-access sessions of 6 hours per day (n = 8/group). Once their responding was stable, the short-access group consumed significantly more calories of Ensure than the long-access group during the initial 30-minute period of daily access, eating as much as 15% vs 8% of their total daily calories during this time. Notably, despite their greater overall Ensure intake, the long-access group had a similar body weight to the short-access groups, with both groups weighing significantly more than home cage control animals consuming only chow and water (N = 12). This indicates that excessive weight gain may be induced by binge-like intake of palatable food as well as general overconsumption of palatable food. In a separate cohort of adolescent female Long-Evans rats (N = 16), given short-access sessions for Ensure, we found that intake was statistically indistinguishable whether access was given for 3, 5, or 7 days per week. These results demonstrate that short, 30-minute access to Ensure leads to over-eating of palatable food, over-eating not driven by hunger, over-eating that occurs in a discrete period of time, and excessive weight gain. As such, we believe that this model reflects critical aspects of binge eating and can be thus used to identify underlying neural substrates of this behavior.

Disclosures: G.R. Curtis: None. **L. Sanzalone:** None. **N. Mack:** None. **J.R. Barson:** None.

Poster

598. Non-Peptide Regulation of Food Intake and Energy Balance

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 598.20/DDD22

Topic: F.10. Food Intake and Energy Balance

Support: Ministry of Science & Technology, Israel, grant number 3-13608

Title: High-fat diet induced obesity and weight loss - searching for epigenetic mechanisms / markers

Authors: M. COHEN-OR[1], Y. GERBERG[2], T. KISLIOUK[4], N. MEIRI[4], *A. WELLER[3]
[1]Fac. of Life Sci. and the Gonda Brain Res. Ctr., Bar-Ilan Univ., Ramat-Gan, Israel; [2]Psychology, [3]Bar-Ilan Univ., Ramat Gan, Israel; [4]Inst. of Animal Science, ARO, The Volcani Ctr., Bet-Dagan, Israel

Abstract: **Background**: The hypothalamic arcuate nucleus (ARC) has an important role in energy regulation, including body weight regulation. This occurs by a balance between anorexigenic (proopiomelanocortin [POMC] and Cocaine and amphetamine-regulated transcript [CART]) and orexigenic (NPY, AgRP) neuropeptides. The cleaved product of Pomc αMSH is secreted and binds to receptors such as the Melanocortin 4 receptor (Mc4r) in hypothalamic nuclei such as the paraventricular nucleus (PVN). Binding of neuropeptides to this receptor transmits a signal of hunger/satiety in accordance to the binding peptide. Our goal is to investigate whether obesity caused by consuming a diet rich in calories and fat can be reversed by caloric restriction and what are the epigenetic mechanisms related to this process. **Results**: Both high fat diet (HFD) and caloric restriction (CR) affected body weight in rats. HFD fed rats weighed more than chow fed rats on postnatal day (PND) 90. 40% caloric restriction (HF-CR40), applied from PND 90-120 reduced their body weight only by 10-15%. The CR did not correct the weight to the level of the control group. Mc4R mRNA pattern was opposite to the body weight - the chow-chow group showed the highest expression while Mc4R expression in HF-CR40 group remained similar to that of the HF group. We found that the promoter of Mc4R is completely unmethylated whereas the coding sequence is hypermethylated. Analysis of CpG methylation at the Mc4R coding regions revealed a difference in the methylation state between the groups; the HF-HF and the chow-chow groups showed a similar methylation profile while the CR group showed higher methylation levels. Examination of the CREB transcription factor binding levels showed negative correlation with the methylation levels - higher binding levels were found in the HF-HF and chow-chow groups. Additionally, we found differences in the DNMT expression levels between the groups. **Conclusion**: We suggest that the methylation profile in a constant diet keeps to a lower level compared to a state of an attempt to reduce body weight via caloric restriction - in this case, the body will counteract this process and try to keep to a weight set point by increasing the methylation levels, thus reducing CREB transcription factor the binding levels and diminishing Mc4R expression.

Disclosures: M. Cohen-Or: None. **Y. Gerberg:** None. **T. Kisliouk:** None. **N. Meiri:** None. **A. Weller:** None.

Poster

599. Appetitive and Incentive Learning and Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 599.01/DDD23

Topic: G.01. Appetitive and Aversive Learning

Title: Effects of neonatal limited nesting stress on anxiety-like behavior and impulsivity in adult rats

Authors: *A. C. TALK, C. JEREMY, J. SHERREY, S. FISHER, A. HAMLIN, E. KYONKA
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Abstract: Growing up within a deprived childhood has been linked to impulsive decision making. However, it is not clear that a causal relationship between early life deprivation and later impulsivity exists. A third factor, such as genetics, could account for impulsivity as well as material deprivation in offspring. We conducted a series of experiments in which stress in the form of limited nesting material was randomly assigned to neonatal rats. We then assessed impulsivity, as well as anxiety-like behaviors, of the male rats after they grew into adulthood. Our goal was to establish whether a causal relationship exists between early life limited nesting stress and later impulsivity. In a series of three experiments, male Wistar rats were cross-fostered and randomly assigned to either a control condition or to being reared with limited access to nesting material during postnatal days (PND) 2-9. The rats were weaned on PND 22. From PND 60 to 150, the rats were tested on an elevated plus maze and an operant delay-discounting procedure (experiment 1), an elevated plus maze and an operant probability-discounting procedure (experiment 2), or an elevated plus maze and a dark-light box procedure (experiment 3). Across the three experiments, the deprived rats spent more time in the open arms of the plus maze than controls. Similarly, in the light-dark box, the deprived rats had a reduced latency to enter the lit side of the box, and spent more time in the light than controls. There were no statistically reliable differences between deprived and control rats on impulsivity measures during the delay-discounting procedure or the probability-discounting procedure. We are currently conducting histological analysis of the rats in these studies to determine whether changes in dendritic arborization or spine density has occurred in the frontal cortex of the deprived rats. Our results thus do not support a causal link between depriving rat pups of nesting material and later impulsivity. However, the results do support a causal link between early life deprivation and later anxiety. In our study, limited nesting material stress reduced anxiety-like behaviors in adulthood.

Disclosures: A.C. Talk: None. **C. Jeremy:** None. **J. Sherrey:** None. **S. Fisher:** None. **A. Hamlin:** None. **E. Kyonka:** None.

Poster

599. Appetitive and Incentive Learning and Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 599.02/DDD24

Topic: G.01. Appetitive and Aversive Learning

Support: Korea Research Foundation

Title: Activation of leptin receptor-expressing neurons in lateral hypothalamus enhances food-seeking without altering food intake in mice

Authors: *Y. LEE, D.-S. HA, M. KIM, H. SONG, C. NAMKOONG, D. CHUN, H. CHOI
Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: The symptom of eating disorders that is difficult to treat is a dissociation between food-seeking behavior and metabolic needs. The lateral hypothalamus (LHA) regulates various motivated behaviors including food intake, and among many neuronal populations inside, LHA GABAergic neurons are known to be involved in modulation of food reward and consumption. Previous studies showed that activation of LHA GABAergic neurons enhance food intake and compulsive behaviors in mice. However, specified behavioral phenotypes and functions of the subset of LHA GABAergic neurons are unclear. Thus, our research aimed to identify the food-related behavioral phenotypes that are regulated by leptin receptor-expressing neurons in LHA. We performed food-seeking test, operant chamber test, overall chow/sucrose/saccharin consumption, preference test and marble burying test. Interestingly, through behavior assays, we found that chemogenetic activation of LHA leptin receptor neurons only increased ‘food-seeking’ behavior without altering food intake. However, activation of LHA GABAergic neurons increased both food intake and compulsive behaviors without affecting food-seeking. These results suggest that food-seeking is independent from food intake, and LHA leptin receptor neurons are specifically involved in food-seeking behavior that can be targeted to treat eating disorders.

Disclosures: Y. Lee: None. **D. Ha:** None. **M. Kim:** None. **H. Song:** None. **C. NamKoong:** None. **D. Chun:** None. **H. Choi:** None.

Poster

599. Appetitive and Incentive Learning and Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 599.03/EEE1

Topic: G.01. Appetitive and Aversive Learning

Support: NIDDK grant R01DK085721

Title: The effects of novelty on food consumption in male and female rats

Authors: *E. GREINER[1], G. D. PETROVICH[2]
[2]Psychology, [1]Boston Col., Chestnut Hill, MA

Abstract: Novel foods and novel environments impact consumption, but research into how the two interact, and whether there are sex differences, is lacking. Here, we sought to determine if exposure to a novel context enhances food neophobia—defined as a lower intake of a novel food compared to familiar—and whether the effect is sex dependent. We also wanted to establish whether the effects of novelty on food consumption in either sex were mediated by anxiety and thus could be attenuated by administration of sub-anesthetic doses of ketamine. Male and female Long Evans rats were tested for consumption in either their home cage or in a novel context (n=8 per group) and were given two foods, one familiar (rat chow) and one novel (Test Diet pellets; TD). They received 8 testing sessions on separate days and were acutely deprived of food for 20 hours prior to each. During Test 1 and 2, males and females tested at home had a significant preference for the familiar (rat chow) over novel (TD) food ($p=0.001$, both), while rats tested in a novel context ate similar, small amounts of each food. Total consumption was lower in the novel context groups compared to home cage tested groups for both sexes ($p=0.002$) but females tested in the novel context ate the least. In Test 3, male and female rats tested at home consumed equal amounts of the two foods and by Test 8 were showing a significant preference for TD (males, $p=0.03$; females, $p=0.016$). Males tested in the novel context showed higher consumption of TD by Test 4 ($p=0.014$) whereas females showed equal consumption of both foods during all tests. Further analysis of total consumption in Test 6, 7, and 8 showed that males tested in a novel context ate significantly more than females (T6, $p=0.009$; T7, $p=0.008$; T8, $p=0.003$). These results indicate that rats in a familiar context, regardless of sex, and males in a novel context habituate to novelty faster than females in a novel context. On-going experiments are examining if the sustained, suppressed consumption that females tested in the novel context show throughout testing is mediated by heightened anxiety and could be alleviated by anxiolytic ketamine.

Disclosures: E. Greiner: None. **G.D. Petrovich:** None.

Poster

599. Appetitive and Incentive Learning and Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 599.04/EEE2

Topic: G.01. Appetitive and Aversive Learning

Support: NIH, NIDDK Grant R01DK085721

Title: Context-induced renewal of responding to food cues: The effect of context pre-exposure in male and female rats

Authors: *D. LAFFERTY, G. D. PETROVICH
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Abstract: The environments in which we consume food can later influence eating behavior. Environmental food cues can stimulate food seeking, but how we respond depends on context. Context-mediated renewal is a well-suited paradigm to investigate environmental control of responding to food cues. Recent work found that males demonstrate robust context-mediated renewal of responding to a food cue after extinction but females do not. These results were established in rats with an "ABA renewal" paradigm in which a cue-food association is acquired in one context, extinction of the conditioned responding occurs in a different context, and the renewal of responding is induced by the acquisition context. The goal of the current study was to determine if context-mediated renewal could be strengthened, particularly in females, by pre-exposure to both contexts prior to training. First, food restricted adult male and female rats (N=32) experienced pre-exposure to the behavioral contexts (experimental groups) or remained in their homecage (control groups). Then all rats underwent Pavlovian conditioning in which they were presented with a tone cue (conditioned stimulus, CS) followed by delivery of palatable food pellets (unconditioned stimulus, US). Acquisition of the CS-US association occurred in a distinct context that varied in olfactory, visual, and tactile features from the context used for extinction training. By the end of acquisition training (5 sessions, each with 8 CS-US pairings) all groups showed similar robust conditioned responding (approach to the foodcup) during the CS. After extinction training (2 sessions, each with 8 CSs) all groups decreased responding during the CS. Rats were tested for renewal of responding with CS-only presentations in each context, on separate days counterbalanced for order. Here we compared responding during the CS (elevation above baseline) in the acquisition vs. extinction contexts. Analyses revealed the only group to demonstrate robust renewal were males that did not receive pre-exposure, as indicated by their higher responding during CSs when tested in the acquisition compared to extinction contexts ($t(1,14)=2.69$, $p<.01$). Additionally, the groups that had pre-exposure had higher baseline (preCS) responding in the extinction context ($t(1,30)=2.38$, $p<.05$) while all groups had similar preCS responding in the acquisition context ($p>.05$). These results

demonstrated sex differences similar to the patterns found in previous research and that pre-exposure to behavioral contexts did not improve renewal in either sex. Current work is investigating whether US delivery during pre-exposure is important for the lack of effect on renewal.

Disclosures: D. Lafferty: None. **G.D. Petrovich:** None.

Poster

599. Appetitive and Incentive Learning and Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 599.05/EEE3

Topic: G.01. Appetitive and Aversive Learning

Support: CIHR

Title: The pharmacological stressor yohimbine, but not U50,488, increases responding for conditioned reinforcers previously paired with alcohol or sucrose

Authors: *R. I. TABBARA[1,4], A. RAHBARNIA[1,4], A. D. LÊ[2,3,5], P. J. FLETCHER[1,4,3]
[1]Psychology, [2]Pharmacol. and Toxicology, [3]Psychiatry, Univ. of Toronto, Toronto, ON, Canada; [4]Biopsychology, [5]Neurobio. of alcohol, Campbell Family Mental Hlth. Res. Institute, Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada

Abstract: Stressful life events can induce craving and relapse to alcohol. In laboratory animal models, pharmacological stressors, such as the alpha-2 adrenoreceptor antagonist yohimbine and the kappa-opioid receptor agonist U50,488, can impact ethanol (EtOH) consumption and reinstate extinguished EtOH-seeking. Although alcohol has primary reinforcing properties, environmental stimuli repeatedly associated with alcohol can acquire incentive value and motivate non-abstinent and abstinent alcoholics to drink. It is unknown whether stress can potentiate these motivational properties of alcohol-paired stimuli. This work examined the effects of the pharmacological stressors yohimbine (alpha-2 adrenoreceptor antagonist) and U50,488 (kappa-opioid receptor agonist) on responding for conditioned reinforcement; a test that assesses the reinforcing properties of reward-related cues in animal models. This work also examined whether their effects on responding interact with the nature of the reward delivered (alcohol vs. sucrose). Male Long-Evans rats were mildly-food deprived and received access to EtOH solutions for 1 hr/day at increasing concentrations (3% w/v for 6 days, 6% w/v for 6 days, 12% w/v for 12 days) or a sucrose solution (21.7% for 2 days) in drinking cages. Next, they underwent 35 sessions of Pavlovian conditioning consisting of 12 trials, where a 5-sec tone-light conditioned stimulus (CS) was paired with 0.15 ml of 12% EtOH (w/v) or 21.7% sucrose. Tests of responding for conditioned reinforcement were then conducted, during which presentation of the CS alone (now acting as a conditioned reinforcer; CRf) was contingent upon pressing one of

two levers (CRf lever). Pressing the other lever had no programmed consequences (NCRf lever). To determine the effects of yohimbine and U50,488 on responding for conditioned reinforcement, rats received an injection of yohimbine (1.25, 2.5 mg/kg i.p.) or U50,488 (1.25, 2.5 mg/kg s.c.) 30 min prior to testing. Both doses of yohimbine selectively potentiated responding for a CRf previously paired with EtOH or sucrose; an effect that was maintained over several tests. However, neither doses of U50,488 affected responding. Results suggest that the ability of yohimbine to potentiate responding for a reward-related cue is not unique to alcohol-paired stimuli, and is probably not related to its stress-like effects.

Disclosures: R.I. Tabbara: None. **A. Rahbarnia:** None. **A.D. Lê:** None. **P.J. Fletcher:** None.

Poster

599. Appetitive and Incentive Learning and Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 599.06/EEE4

Topic: G.01. Appetitive and Aversive Learning

Support: NIDA K08-DA-037912

Title: Cannabinoid agonist effects on pavlovian conditioned approach behavior

Authors: *A. GHEIDI[1], B. N. FROELICH[2], C. J. FITZPATRICK[2], R. L. ATKINSON[2], C. N. BARCELO[2], J. D. MORROW[1]
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Abstract: Pavlovian conditioned approach (PCA) can be used to measure motivational salience attribution by repeatedly pairing a neutral conditioned cue (e.g. a retractable lever) with the response-independent delivery of a reinforcer (e.g. a food pellet). Under these conditions, animals will begin to either sign-track, i.e. approach and contact the neutral cue, or goal-track, i.e. approach the location of impending reinforcer delivery. Sign-tracking indicates an attribution of motivational salience to the cue, and is correlated with vulnerability to both addiction- and PTSD-like behaviors in rats. Sign-tracking is highly dopamine-dependent, and cannabinoids are known to regulate dopaminergic neurotransmission. We therefore investigated the effects of a cannabinoid agonist on acquisition of sign- and goal-tracking behavior. Rats received vehicle or the nonselective cannabinoid agonist CP 55,940 in a dose of 10 μg/kg, 50 μg/kg, or 100 μg/kg followed by PCA training for 7 days. Training sessions consisted of a retractable lever presentation followed immediately by food delivery into a magazine, repeated 25 times. Then 5 days of crossover training was conducted with drug rats switched to receiving vehicle and vehicle rats receiving 100 μg/kg of drug. CP 55,940 dose-dependently reduced sign-tracking and increased goal-tracking. During the crossover phase, the drug rats showed decreased goal tracking while the vehicle rats showed deceased sign tracking. To determine whether sign and

goal trackers have constitutive differences in the cannabinoid system, a different group of rats (previously characterized as sign or goal trackers) were sacrificed and their brains prepared for in *situ* hybridization using radioactive S^{35}. Riboprobes complementary to the mRNA of cannabinoid receptor 1 (CB1) and fatty acid amide hydrolase (FAAH) were visualized with Kodak film. Quantification of mRNA expression is ongoing. We conclude that cannabinoid agonism decreases acquisition of sign tracking and promotes acquisition of goal tracking behavior. This was the opposite of our original hypothesis and may be due to disrupted timing of dopaminergic activity, which in turn would interfere with sign-tracking behavior.

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Poster

599. Appetitive and Incentive Learning and Memory II

Location: SDCC Halls B-H

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Program #/Poster #: 599.07/EEE5

Topic: G.01. Appetitive and Aversive Learning

Support: U of M Grant U032826
NSF GRFP
DoD NDSEG
NIDA Grant K08 DA037912-01
NIDA Grant T32 DA007281

Title: Are cues pursued for their own sake, or because they lead to rewards?

Authors: *C. E. MARÍA-RÍOS[1], C. J. FITZPATRICK[1], T. GEARY[2], J. D. MORROW[1,2]
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Abstract: When a neutral stimulus is repeatedly paired with an appetitive reward, two different types of conditioned approach responses may develop: a sign-tracking response directed toward the neutral cue, or a goal-tracking response directed toward the location of impending reward delivery. Sign-tracking responses have been postulated to result from habitual processes that are insensitive to outcome devaluation, while goal-tracking may develop from a more explicit cognitive representation of the associated outcome. However, Pavlovian responses are typically sensitive to outcome devaluation, and the published literature has been inconsistent on the sensitivity of sign-tracking to devaluation. We therefore tested sign- and goal-tracking before and after devaluation of a food reward using lithium chloride and found that sign-tracking was sensitive to outcome devaluation, while goal-tracking was not. We also confirmed that both responses are Pavlovian because they can be learned under negative contingency conditions. Although both sign- and goal-tracking responses are likely dependent on the acquired incentive

and predictive values of the cues, the increased motivational value sign-trackers attribute to the cue, suggests that sign-tracking behavior is mostly guided by the incentive value, while goal-tracking relies more on the predictive value. This interpretation is supported by the findings for outcome devaluation where only the incentive value of the reward was altered, and only sign-tracking behavior was affected. To further explore this idea, we sought to test the effects of a blocking paradigm on sign- and goal-trackers. Blocking is thought to occur because learning is driven by prediction errors. Accordingly, once an unconditioned stimulus is completely predicted by a cue, no further learning will occur. If a second cue is then added simultaneously with the pre-trained cue but does not give any new information about the outcome, the pre-trained cue should block learning about the second cue. Under these training conditions, we found that goal-trackers showed complete blocking of the new added cue, while sign-trackers blocked, but to a lesser extent. This supports the previously stated hypothesis that sign- and goal-tracking follow different rules of reinforcement learning.

Disclosures: C.E. María-Ríos: None. **C.J. Fitzpatrick:** None. **T. Geary:** None. **J.D. Morrow:** None.

Poster

599. Appetitive and Incentive Learning and Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 599.08/EEE6

Topic: G.01. Appetitive and Aversive Learning

Title: Incentive salience attribution predicts task-irrelevant attention biases in human sign- and goal- trackers

Authors: *M. DIBARTOLO[1], K. M. FRASER[1], V. NICHOLAS[2], P. H. JANAK[1,2], S. M. COURTNEY[1,2,3]
[1]Psychological & Brain Sci., [2]Neurosci., Johns Hopkins Univ., Baltimore, MD; [3]Kirby Res. Ctr., Kennedy Krieger Inst., Baltimore, MD

Abstract: Individuals differ in their attribution of motivational value, also referred to as incentive salience, to reward-paired cues. In rats, individuals that are prone to attribute high levels of incentive salience to cues are more impulsive and more prone to cue-driven reinstatement of drug self-administration. However, little to no evidence to date has been found indicating whether humans demonstrate individual differences in incentive salience attribution comparable to that found in animal models. To investigate this, we developed a novel paradigm to examine sign- and goal-tracking behavior in humans. We tested healthy young adults (N = 45, 34 female) on an eye-tracking-based autoshaping task. In this task, one continuously present stimulus served as a "goal," and after a varying amount of time a separate "sign" stimulus appeared for five seconds at a different location, followed immediately by delivery of monetary

reward at the goal location. Both stimuli were interactable - movement of the gaze into the cue location produced both auditory and visual feedback, whereas goal gaze interactions only produced visual feedback. Autoshaping occurred over 4 blocks of 10 trials, immediately followed by a centralized discrimination task (CDT) implemented to assess attentional biases. During CDT trials, previous sign and goal stimuli were occasionally presented as distractors for a brief period of time (125 ms) immediately prior to responses. Eye movements were tracked throughout both tasks. We used a standard Pavlovian conditioned approach index to classify individuals as sign-trackers and goal-trackers based on their behavior during autoshaping. Upon sign stimulus presentations, sign-trackers rapidly interacted with this cue and fixated in the region of the cue for the duration of its presentation, whereas goal-trackers instead spent the duration fixated in the goal region. We found that sign-tracking behavior predicted subsequent attentional biases, even when the sign-cue was task-irrelevant. Interestingly, the phenotype into which people were sorted was well matched with self-report of enjoyment of interacting with the sign or goal. These data indicate that humans differ in their propensity to attribute reward-paired cues with incentive salience and that the degree to which they do so has implications for the capture of attention by previously rewarded cues, providing a framework for the investigation of these neural processes in humans.

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Poster

599. Appetitive and Incentive Learning and Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 599.09/EEE7

Topic: F.04. Stress and the Brain

Support: NIMH K23MH092648
NCRR UL1 RR024986
NCATS 2UL1 TR000433

Title: Insular cortex and sympathetic nervous system responses to motivational salience

Authors: *K. G. WARTHEN[1], A. BOYSE-PEACOR[2], B. SANFORD[3], B. J. MICKEY[2]
[1]Bioengineering, Univ. of Utah, Salt Lake City, UT; [2]Dept. of Psychiatry, Univ. of Utah Sch. of Med., Salt Lake City, UT; [3]Univ. of Michigan, Ann Arbor, MI

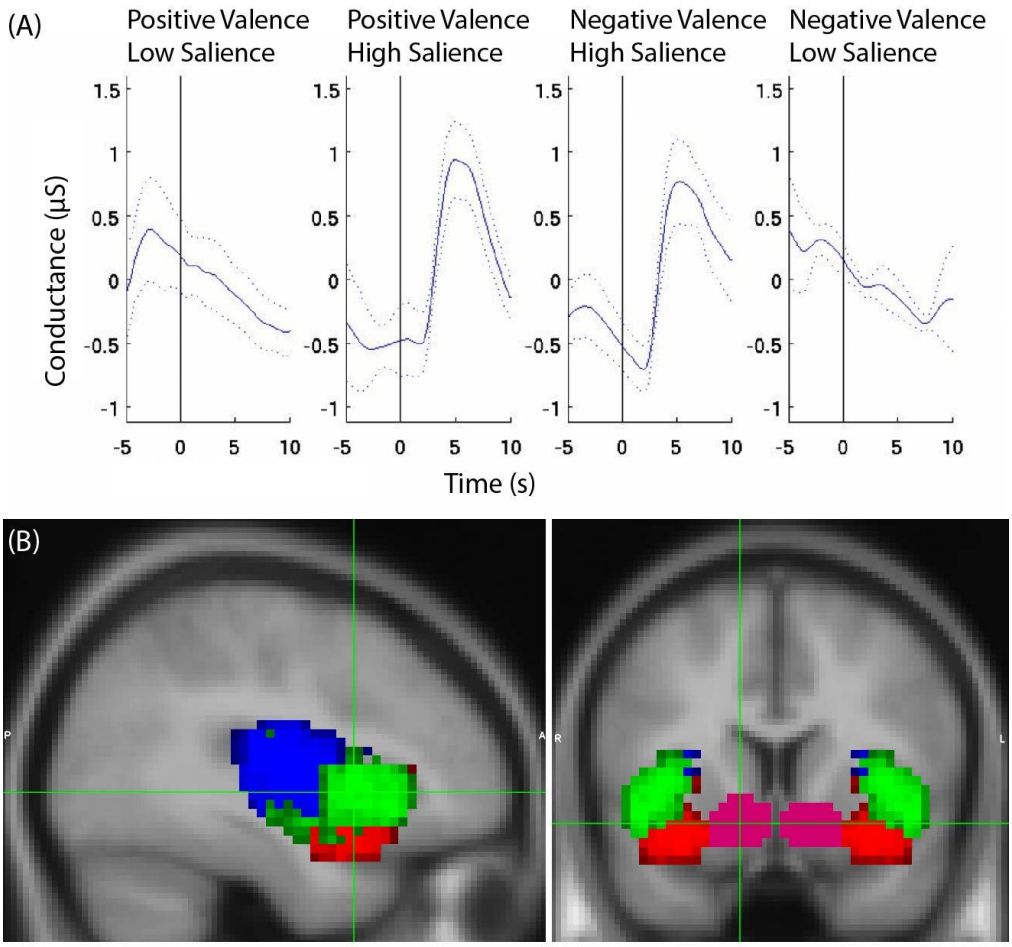
Abstract: Peripheral sympathetic nervous system (SNS) activity has been widely used in psychological research as a proxy for central nervous system activation, but how the peripheral SNS is controlled centrally is not well understood in humans. Furthermore, individual differences in SNS activity, which are thought to influence risk for a variety of health problems,

have not been well described. We hypothesized that SNS activity would be associated with neural responses in nucleus accumbens (NAc) and anterior insula (AI).

We characterized phasic SNS activation by measuring electrodermal responses (Fig. 1A) from 212 young adults (both sexes) as they performed a monetary incentive task. Forty-two of these subjects performed the same task during functional magnetic resonance imaging (fMRI). Stimuli presented during the task varied by salience (high versus low) and valence (win versus loss). Linear mixed models were used to identify associations of SNS activity with responses in four regions of interest: NAc, ventral AI, dorsal AI, and posterior insula (Fig. 1B).

Task performance and subjective arousal ratings predicted SNS activation, while subjective affect ratings did not. SNS responses to high-salience stimuli were positively associated with fMRI responses in the NAc and the ventral and dorsal AI, but not the posterior insula, after controlling for performance, subjective ratings, age, and sex.

These findings suggest that individual variation in human NAc and AI function underlie individual differences in SNS activation during motivated behavior. These effects appear to be specific to anterior versus posterior insula, and specific to salience versus valence. Modulation of excessive SNS activity might be achievable by targeting NAc and AI function in humans.



Panel (A) shows electrodermal responses by condition for an individual subject. Panel (B) shows parasagittal and coronal sections of the regions of interest used in analysis, where purple is the nucleus accumbens, blue is the posterior insula, red is the anterior ventral insula, and green is the anterior dorsal insula.

Disclosures: K.G. Warthen: None. **A. Boyse-Peacor:** None. **B. Sanford:** None. **B.J. Mickey:** None.

Poster

599. Appetitive and Incentive Learning and Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 599.10/EEE8

Topic: G.01. Appetitive and Aversive Learning

Support: FUKL Grant

Title: Individual differences in the effects of chronic nicotine on autoshaping learning

Authors: *L. A. ORTEGA MURILLO[1], M. LAMPREA[2], J. CIFUENTES[1], E. OCAMPO[1], L. GARCIA[1], C. NOVOA[2], J. SOLANO[2]
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Abstract: Current research has focused on learning and motivational processes for nicotine that are proposed to be critical for the development and maintenance of tobacco addiction. In particular, nicotine administration has been associated with the enhancement of nonassociative incentive and reinforcing properties of naturally rewarding stimuli. In the present study, the role of chronic nicotine was assessed on the acquisition phase of an autoshaping task with a natural unconditioned stimulus (food pellets). Chronic nicotine (3.6 mg/kg/day; dose reported as free base), or vehicle, were administered using mini-osmotic pumps that were inserted subcutaneously under anesthesia. Parallel to previous literature, chronic nicotine transiently enhanced lever-pressing performance. However, such effects were primarily found for rats with higher levels of lever pressing responses. Chronic nicotine also modulated goal-tracking behavior, although following differential profiles for rats with high or low levels of lever pressing behavior. In addition, chronic nicotine resulted in differential response bias profiles between rats with high or low levels of lever pressing. Together, these findings suggests a differential effect of chronic nicotine on autoshaping acquisition depending upon individual differences in the levels of acquisition of the autoshaping task. Future studies on such individual differences may help understand to the mechanisms underlying the complex and varied incentive and reinforcing effects of nicotine on natural stimuli.

Disclosures: L.A. Ortega Murillo: None. **M. Lamprea:** None. **J. Cifuentes:** None. **E. Ocampo:** None. **L. Garcia:** None. **C. Novoa:** None. **J. Solano:** None.

Poster

599. Appetitive and Incentive Learning and Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 599.11/EEE9

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant AA024112

Title: Systemic and intracerebroventricular nicotine administration increases goal-tracking during Pavlovian conditioned approach paradigms in Long-Evans rats

Authors: *H. A. PEARSON, P. J. MEYER
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Abstract: There is substantial individual variability in the response to reward-predictive stimuli ("cues"). For example, when a discrete cue (e.g. a lever) is presented during a Pavlovian conditioned approach (PavCA) paradigm, Sprague-Dawley rats will either approach the discrete cue ("sign-tracking") or the reward delivery location ("goal-tracking"), and sign- or goal-tracking phenotypes are associated with relapse to drug-seeking in the presence of discrete drug-cues or drug-paired contexts, respectively. Previously, nicotine has been shown to increase sign-tracking but not goal-tracking to a food cue in these Sprague-Dawley rats. However, because PavCA phenotypes are influenced by genetic structure (Fitzpatrick et al., 2013), the effect of nicotine on these phenotypes may be as well. In order to investigate this hypothesis, we administered a nicotine or saline injection to Long-Evans rats prior to testing in PavCA paradigms during which a lever predicted either a food or ethanol reward. In experiment 1, male (n = 16) and female (n = 16) Long-Evans rats were administered a nicotine (0.4 mg/kg S.C.) or saline injection 15 minutes prior to testing in a PavCA paradigm during which a lever predicted the delivery of a banana pellet (25 presentations per session for 11 sessions). In experiment 2, male, Long-Evans rats (n = 40) with or without prior chronic intermittent access to ethanol were administered a nicotine (0.4 mg/kg S.C.) or saline injection 15 minutes prior to testing in a PavCA paradigm during which a lever predicted the receipt of 0.2 ml ethanol (15% v/v; 12 presentations per session for 27 sessions). Finally, in experiment 3, in order to determine whether central administration of nicotine would affect goal-tracking similarly to systemic administration, male, Long-Evans rats (n = 15) were administered bilateral, intracerebroventricular injections of nicotine (8ug/ml; 1ul per side) or saline into the lateral ventricles directly prior testing in the PavCA paradigm described in experiment 1 (9 sessions). Surprisingly, nicotine-treated rats increased goal-tracking, but not sign-tracking, relative to saline-treated controls in all three experiments. Our findings indicate that nicotine enhances goal-directed behavior for both food and ethanol cues, and that this effect is centrally mediated. Therefore, nicotine can increase both sign- and goal- tracking, and this likely depends on genetic background. Thus, nicotine may be able to promote relapse differently among individuals and effective relapse prevention may require individualized treatments.

Disclosures: H.A. Pearson: None. **P.J. Meyer:** None.

Poster

599. Appetitive and Incentive Learning and Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 599.12/DP13/EEE10

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant H212100-G

Title: Acid sensing ion channel-1a in pavlovian reward conditioning

Authors: *A. GHOBBEH[1], R. J. TAUGHER[1], R. FAN[1], R. T. LALUMIERE[2], J. A. WEMMIE[1]
[1]Psychiatry, [2]Dept. of Psychological and Brain Sci., The Univ. of Iowa, Iowa City, IA

Abstract: Pavlovian fear conditioning has been shown to depend on acid-sensing ion channel 1A (ASIC1A), however it is unknown whether conditioning to rewarding stimuli also depends on ASIC1A. Here we sought to test the hypothesis that ASIC1A contributes to Pavlovian conditioning to a non-drug reward by assessing several conditioning paradigms in which the relationship between the conditional stimulus (CS) and the unconditional stimulus (US) was varied. We found significant effects of ASIC1A disruption which depended on the paradigm. When the CS preceded the US, and signaled an upcoming food reward, *Asic1a*$^{-/-}$ mice exhibited striking deficits in conditioned responses compared to *Asic1a*$^{+/+}$ mice. Alternatively, when the CS was co-initiated with the US and signaled immediate reward availability, the *Asic1a*$^{-/-}$ mice exhibited an increase in conditioned responses compared to *Asic1a*$^{+/+}$ mice, which contrasted sharply with the deficits in the first experiment. The altered behaviors associated with ASIC1A disruption in these paradigms were likely due to differences in conditioning because neither the *Asic1a*$^{-/-}$ nor *Asic1a*$^{+/+}$ mice acquired conditioned responses when the CS and US were explicitly unpaired. Furthermore, the *Asic1a*$^{-/-}$ mice exhibited normal conditioned responding when the amount of overlap between the CS and US was altered. Taken together, these results suggest that ASIC1A plays a critical, yet complex role in Pavlovian reward conditioning. Moreover, these results suggest that the effects of ASIC1A disruption depend on the temporal relationship between the CS and US. More research will be needed to deconstruct the roles of ASIC1A in these fundamental forms of learning and memory.

Disclosures: A. Ghobbeh: None. **R.J. Taugher:** None. **R. Fan:** None. **R.T. LaLumiere:** None. **J.A. Wemmie:** None.

Poster

599. Appetitive and Incentive Learning and Memory II

Location: SDCC Halls B-H

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Program #/Poster #: 599.13/EEE11

Topic: G.01. Appetitive and Aversive Learning

Support: MOST 105-2320-B-002-067-MY2
MOST 103-2911-I-038-501
Intramural Research Program of NIDA

Title: Transient activation of VTA afferent input from the parabrachial nucleus disengages reward seeking

Authors: *H.-J. YAU[1], J.-H. TSOU[2], F.-Y. GUO[1], A. BONCI[2]
[1]Natl. Taiwan Univ., Grad. Inst. of Brain and Mind Sci., Taipei, Taiwan; [2]NIDA/NIH, Baltimore, MD

Abstract: Animals rely on gustatory stimuli to differentiate preferred food for survival from the food that causes illness. Taste helps establish food preference or aversion by associating postingestional effects of food through classical conditioning process and the neural processing of taste aspect of food starts from the gustatory system. Accumulated studies have shown that the parabrachial nucleus (PBN) is an important interface between the taste system and the feeding system. Despite that the PBN sends projections to the ventral tegmental area (VTA), a heterogeneous brain region that plays a key role in processing reward or aversion-related stimuli, it is not clear whether the gustatory/visceral system interacts with the reward/aversion pathways to regulate food-seeking or -avoiding behaviors. The research aims to investigate the functional roles of an afferent input from the PBN to the VTA. Given that PBN is an important relay of gustatory pathway, we first combined excitatory optogenetic approach with an operant food self-administration paradigm to examine the behavioral roles of PBN-to-VTA connection. The histology results show that PBN sends substantial glutamatergic projections to the VTA. Surprisingly, we found that the optical activation of PBN-to-VTA glutamatergic inputs dampens food self-administration behaviors. In addition, we found activating PBN-to-VTA input drives aversion. We propose to investigate further the regulatory mechanism of PBN regulation onto VTA circuits and explore possible application of in controlling food cravings, which leads to eating disorders and obesity.

Disclosures: H. Yau: None. **J. Tsou:** None. **F. Guo:** None. **A. Bonci:** None.

Poster

599. Appetitive and Incentive Learning and Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 599.14/EEE12

Topic: G.01. Appetitive and Aversive Learning

Support: Swedish Research Council

Title: Decoding neuroanatomy and behavioral roles of distinct subpopulations in the ventral tegmental area (VTA) to advance potential for selective treatment in substance use disorder, mood disorders and Parkinson´s disease

Authors: *Z. BIMPISIDIS, N. KÖNIG, B. VLCEK, Å. WALLÉN-MACKENZIE
Uppsala Univ., Uppsala, Sweden

Abstract: The VTA is involved in reward processing and related behaviors. Consequently, VTA dysfunction is correlated with neuropsychiatric disorders such as substance use disorder and depression as well as non-motor complications occurring after dopamine replacement therapy in Parkinson's Disease (PD). Common for VTA disorders is the lack of cure and severe side effects upon treatment. Traditionally considered mainly dopaminergic, the VTA has been exposed as a strongly heterogeneous brain area comprising of several subpopulations of dopaminergic, glutamatergic and GABAergic cells, as well as of co-releasing neurons. We propose that the neurocircuitry and functional role of distinct VTA subpopulations should be decoded in order to further understand VTA function and to achieve selective treatment of VTA disorders. We recently identified unique molecular markers that characterize distinct neuronal subpopulations within the VTA and have demonstrated that cells surviving in PD express one such specific molecular marker, the gastrin releasing peptide (GRP). We have also shown that the promoters of these markers can be experimentally exploited as drivers of Cre recombinase which is useful for circuitry- and functional analysis implementing mouse transgenics and optogenetics. To specifically target neuronal subpopulations in the VTA, we currently utilize transgenic mouse lines expressing Cre recombinase under the promotor activity of previously defined markers. Using fluorescent optogenetic constructs, we selectively tag these neurons to map their distribution within the VTA, characterize their neurotransmitter phenotypes and identify their target areas. Additionally, by behavioral optogenetics and conditional knockout strategies, we investigate the role of these cells in reward-related behaviors. So far, we have characterized two distinct subpopulations within the VTA (subVTA): subVTA1 and subVTA2, that both show distinct distribution within the VTA as well as specific projection patterns. Behavioral optogenetics show that stimulation of the subVTA2 subpopulation induces approach behavior while stimulation of the subVTA1 subpopulation has no observable behavioral effects. Eliminating the ability of subVTA1 and subVTA2 to release dopamine causes distinct behavioral responses to drugs of abuse and natural rewards. Our current findings add valuable insight in the heterogeneity and functional neuroanatomy of the VTA and provide knowledge that could potentiate the search for more selective therapeutic approaches in disorders where the VTA function is compromised, including substance use disorder, mood disorders and non-motor symptoms of PD

Disclosures: Z. Bimpisidis: None. **N. König:** None. **B. Vlcek:** None. **Å. Wallén-Mackenzie:** None.

Poster

599. Appetitive and Incentive Learning and Memory II

Location: SDCC Halls B-H

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Program #/Poster #: 599.15/EEE13

Topic: G.01. Appetitive and Aversive Learning

Support: NIDA Grant R21DA043190
Whitehall Foundation Grant 2017-12-98
SUNY Brain Network of Excellence Post-doctoral Fellow program and T32 AA007583

Title: Activation of GABA projection neurons from the ventral tegmental area to the nucleus accumbens enhances adaptive reward learning without affecting motivation in rats

Authors: *M. FEJA[1], K. T. WAKABAYASHI[1,2], M. P. K. LEIGH[1], K. A. HAUSKNECHT[2], R.-Y. SHEN[2], S. HAJ-DAHMANE[2], C. E. BASS[1,2]
[1]Pharmacol. and Toxicology, Univ. At Buffalo SUNY, Buffalo, NY; [2]Res. Inst. on Addictions, Univ. at Buffalo, Buffalo, NY

Abstract: Mesolimbic dopamine projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) are critical for cue-motivated responding. However, the VTA also contains GABA interneurons, and approximately one third of mesoaccumbal projecting neurons are GABAergic. Recently we used a novel combinatorial viral approach to target activating designer receptors exclusively activated by designer drugs (DREADDs) to VTA glutamate decarboxylase 1 (GAD1)-positive neurons in rats. We demonstrated that chemogenetic activation of VTA GABA neurons decreases motivation for reward-predictive cues. Yet activation of the dense VTA GABA projections to the NAc alone, by CNO microinfusion into the NAc, does not influence motivation for the cue. Thus, the role of mesoaccumbal GABA projections in reward-seeking processes remains unclear. In this study, we hypothesized that VTA GABA neurons projecting to the NAc are necessary for reward learning. We therefore examined the effects of VTA GABA activation under conditions of changing reward value, using a cue-dependent operant task in which the magnitude of an appetitive natural reward (i.e. sucrose) is unexpectedly altered within session. We then chemogenetically activated all VTA GABA neurons by giving the CNO systemically (0.3 mg/kg i.p.), or just the VTA GABA neuron terminals in the NAc by microinfusion of CNO. Our results show that systemic CNO, which simultaneously activates VTA GABA interneurons and GABAergic projection neurons, decreased cue responding uniformly across lower and higher than expected reward sizes. However, microinfusion of CNO into the NAc, which activates accumbal terminals of VTA GABA projection neurons, enhanced learning when the reward value was less than expected. CNO had no effect in GFP control rats. These results clearly establish that mesoaccumbal GABA neurotransmission causally contributes to reward learning independently from reward-seeking mediated by cue salience.

Disclosures: M. Feja: None. **K.T. Wakabayashi:** None. **M.P.K. Leigh:** None. **K.A. Hausknecht:** None. **R. Shen:** None. **S. Haj-Dahmane:** None. **C.E. Bass:** None.

Poster

599. Appetitive and Incentive Learning and Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 599.16/EEE14

Topic: G.01. Appetitive and Aversive Learning

Support: CRC 1080
Max Planck Society Graduate Fellowship

Title: Altered reinforcement learning dynamics in heterozygous DAT-Cre KI mice

Authors: *K. M. COSTA[1,2], J. ROEPER[1]
[1]Inst. for Neurophysiol., Goethe Univ., Frankfurt am Main, Germany; [2]Natl. Inst. for Drug Abuse, Baltimore, MD

Abstract: Transgenic mouse lines that express Cre-recombinase under the control of endogenous promoters are ubiquitous tools for the genetic manipulation of specific cell-types. However, the insertion of the Cre gene can result in aberrant gene expression and affect cell function independently of the experimental goal. This occurs in the DAT-IRES-Cre line, where Cre is expressed under the control of the dopamine transporter (DAT) gene to target midbrain dopamine neurons. Homozygotic animals of this DAT-Cre KI line have a 50% reduction in DAT protein levels in the striatum (Bäckman et al, 2006). In contrast, heterozygous DAT-Cre KI mice have only a non-significant reduction in DAT expression and thus are widely used, with the implicit assumption that they have no potential behavioral phenotype. However, we discovered that heterozygous DAT-Cre KI does affect behavior in a reinforcement learning task. DAT-cre KI (N = 20) and WT littermate (N= 17) water-restricted mice learned that an auditory cue signaled the availability of a sucrose water solution in a delivery port. Reinforced cues were presented in 10 trials in daily sessions for 11 days, after which animals underwent 6 daily extinction sessions. We found that DAT-cre KI mice showed a higher probability of responding to cues (≈12%), and shorter response latencies (≈15%) than WT controls already in the first acquisition session. Two-way ANOVA also showed significant interactions between the genotypes and the temporal progression of learning in both latency ($F_{10, 350} = 2.3$, $P < 0.05$) and response probability ($F_{10, 350} = 2.4$, $P < 0.01$). Furthermore, during extinction we observed a significant interaction between genotype and session progression for response latency ($F_{5, 175} = 2.8$, $P < 0.05$) and the time the mice spent in port during cue presentation ($F_{5, 175} = 3.18$, $P < 0.01$). We also tested these animals in open field exploration, novel object preference test, hole-board exploration and spontaneous alternation in the plus maze. These tasks are sensitive to effects in a variety of cognitive processes, including locomotor control, exploratory drive and working memory. Interestingly, we did not observe any difference between the genotypes in any of these tasks.Our results demonstrate that the DAT-cre KI alters the dynamics of reinforcement

learning, in particular by increasing responding during early acquisition. Importantly, as far as we have tested, only reinforcement learning was affected. Currently, we are investigating whether DAT-cre KI mice also respond differently to drugs of abuse.

Disclosures: K.M. Costa: None. **J. Roeper:** None.

Poster

599. Appetitive and Incentive Learning and Memory II

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 599.17/EEE15

Topic: G.01. Appetitive and Aversive Learning

Support: NSF GRFP
ARCS Scholar
CONTE

Title: Influence of stress on decision-making behavior in mice

Authors: *R. G. WILLIAMS, S. SANDBERG, P. E. M. PHILLIPS
Neurosci., Univ. of Washington, Seattle, Seattle, WA

Abstract: We have previously shown that, in the core of the nucleus accumbens, corticotropin-releasing factor (CRF) increases evoked dopamine release and produces conditioned place preference in stress-naïve animals. However, following two-day, repeated forced swim stress (rFSS), neither of these effects are present. This work demonstrates an interaction between CRF and some aspect of reward processing, at the level of the nucleus accumbens, that is sensitive to stress. To ascertain the degree to which this mechanism influences integrated reward behavior and, specifically, reward-based decision making, we used an operant concurrent-choice task where male mice could choose between [concentrarion] sucrose solution or water delivery. Following initial training, α-helical CRF (9-41) [50ng/200nL] or vehicle (1% acetic acid in saline) was administered intracranially to the nucleus accumbens core, counterbalanced over two sessions (separated by one baseline session). Next, the animals underwent rFSS, were reintroduced to the task, and were retested with α-helical CRF (9-41) and vehicle. Prior to stress, mice exhibited a significant preference for sucrose over water ($P < 0.05$), made more total nose pokes into the sucrose receptacle than the water receptacle throughout the session ($P < 0.05$), and had shorter latencies for choosing sucrose on choice trials ($P < 0.05$), although latencies were equivalent between sucrose and water trials when only one option was available ($P > 0.05$). Following administration of α-helical CRF into the nucleus accumbens core, there was a trend towards decreased sucrose preference and number of head entries into the sucrose receptacle compared to vehicle administration. Moreover, there was a significant increase in latency to choose sucrose on choice trials following α-helical CRF ($P < 0.05$) administration. These data

suggest that, in stress-naïve animals, endogenous CRF signaling potentiate decisions for more palatable rewards. Following stress, there was a decrease in sucrose preference during baseline sessions ($P < 0.05$), but α-helical CRF no longer weakened the preference.

Disclosures: R.G. Williams: None. **S. Sandberg:** None. **P.E.M. Phillips:** None.

Poster

599. Appetitive and Incentive Learning and Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 599.18/EEE16

Topic: G.01. Appetitive and Aversive Learning

Support: Grant-in-Aid for Scientific Research (C, #24530917) from the JSPS

Title: Effect of blood pressure reduction on working memory performance in spontaneously hypertensive rats: Characteristics of response latency in the delayed-matching-to-place task

Authors: *T. SATO
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Abstract: Spontaneously hypertensive rats (SHRs) were originally developed as an animal model for human hypertension. It is well known that SHRs have certain behavioral characteristics, such as increased locomotor activity; however, the locomotor activity declines with decreased blood pressure (BP) induced by intravenous administration of hypotensive drugs. This study aimed to determine the effects of BP reduction on memory function in SHRs. Our previous study revealed no differences in the number of correct responses, an index of performance accuracy in a delayed-matching-to-place (DMTP) task, between the SHR group and the Wistar-Kyoto (WKY) rat group (the normotensive control group). In this study, we further examined lever-pressing latency, an index of performance speed, in these two groups. We used the DMTP task to compare the latency to selective lever-pressing responses between 8 well-trained SHRs and 8 WKY rats. After pre-training, all rats were repeatedly tested in the DMTP task for four sessions. In each session, rats were intravenously administered hydralazine, a direct acting vasodilator, at one of the four different doses (0, 0.1, 0.3, and 0.6 mg/kg) in a randomized order. During the DMTP task, rats were reinforced with food when they made a matching response, such as pressing the same lever as the sample extended at the beginning of each trial, but a press on the other lever (an incorrect response) ended the trial without reinforcement. Each task session consisted of 100 trials. At the beginning of each trial, one lever was randomly selected as the sample lever and extended in the chamber. When a rat pressed the sample lever, the lever retracted, and the food-cup light in the rear wall illuminated. After the rat made a nose poke to the food cup twice, both levers were extended. Both levers retracted when one of them was pressed. To obtain a retention gradient, the time interval between the first nose-poke and the

end of delay interval, during which the nose-poke was not effective for the presentation of both levers, varied in five different time lengths (0, 2, 5, 10, and 20 s) in a randomized order. A three-factor analysis of variance indicated that the SHR group showed a significantly longer latency to choice responses than did the control group at different delay interval of retention and dosage of hydralazine, excluding when receiving the 0.6-mg/kg dosage. In conclusion, the results indicate that the SHRs take longer time to correctly respond (choice response latency) than do the WKY rats, although no differences were observed in the number of correct responses between these two groups. Notably, decreased BP does not improve working memory performance in SHRs.

Disclosures: T. Sato: None.

Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 600.01/EEE17

Topic: G.02. Motivation

Support: Natural Sciences and Engineering Research Council (05069-2014, JPB; CGS M, CKL)

Title: Amygdala and thalamic inputs to the nucleus accumbens similarly regulate feeding and reinforcement

Authors: *C. LAFFERTY[1], S. REED[2], J. MENDOZA[1], A. YANG[2], J. P. BRITT[1]
[1]Psychology, [2]Integrated Program in Neurosci., McGill Univ., Montreal, QC, Canada

Abstract: Excitatory inputs to the nucleus accumbens (NAc) encode features of reward-associated cues and motivational state. The specific information encoded in amygdala and thalamic inputs is unclear, but recent studies suggest these pathways have opposing influences on behaviour. To better understand NAc information processing and input-specific function, here we compare manipulations of these two pathways on behaviours highly sensitive to NAc activity. We report that amygdala and thalamic input-specific manipulations in mice produce comparable changes in behaviour on a range of tasks. Photo-inhibition of either input increases free food consumption as well as effortful reward seeking, particularly during periods of cued reward unavailability. Activation of either input abruptly terminates consummatory behaviour, and both inputs robustly support intracranial self-stimulation. These data suggest that glutamatergic drive, irrespective of source, is a main determinant of NAc behavioural control. Disruptions in NAc glutamate input both motivate unproductive reward seeking and increase feeding.

Disclosures: C. Lafferty: None. **S. Reed:** None. **J. Mendoza:** None. **A. Yang:** None. **J.P. Britt:** None.

Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 600.02/EEE18

Topic: G.02. Motivation

Support: NIH R01 MH042984
NIH R01 MH113588

Title: Elevated dorsal striatal brain response when satiated during delay discounting in women remitted from bulimia nervosa

Authors: *A. BISCHOFF-GRETHE, C. E. WIERENGA, U. F. BAILER, W. H. KAYE
UCSD, La Jolla, CA

Abstract: Introduction: Bulimia nervosa (BN) is characterized by cycles of binge-eating and compensatory (e.g., self-induced vomiting) behavior, as well as periods of dietary restraint, suggesting variable under- and over-control that may be homeostatic-state related. To examine the influence of hunger and satiety on impulsivity in BN, the current study examined limbic (habit-based) and cognitive (goal-based) frontostriatal circuitry during temporal discounting when fasted and fed.
Methods: We compared 25 remitted BN (RBN) to 23 demographically matched healthy comparison women (CW) performing a delay discounting task when hungry (after a 16 hour fast) and when satiated (after being fed 30% of daily caloric needs) using functional magnetic resonance imaging. To determine whether choice behavior differed between groups, a Group (RBN, CW) × Visit (Hungry, Satiated) × Percent Monetary Difference linear mixed effects (LME) analysis was computed in R. A similar LME examined reaction time. To model individual brain reward valuation response, a general linear model included only decision trials in which the early reward option was available immediately (i.e., “Today”). The beta regressors were then fit to a Group x Visit LME. Regions of interest included the bilateral dorsal caudate and ventral striatum.
Results: No significant group differences were found in choice behavior. While both groups responded more quickly when hungry than when fed ($p=0.004$), RBN responded more slowly overall relative to CW, $p=0.045$. There was a group x visit interaction in the bilateral dorsal caudate and left ventral striatum. In all three clusters, RBN exhibited a lower BOLD response than CW when hungry, and a greater response compared to CW when fed. CW also responded more robustly when hungry than when fed. In contrast, RBN had a higher BOLD response when fed than when hungry in the bilateral dorsal caudate but did not show significant BOLD response

differences based on hunger levels in the left ventral striatum.
Conclusion: CW used greater neural resources within the dorsal and ventral striatum when hungry than when fed, suggesting that immediate rewards were more appetitive in the hungry state, and that greater goal-directed processing was needed to for consideration of distal rewards. In contrast, RBN did not distinguish immediate rewards based upon homeostatic state in the ventral striatum and showed greater goal-directed processing when fed than when hungry. Similar to our prior findings with taste, our results suggest RBN may not sufficiently devalue immediate monetary rewards after eating, and that greater cognitive resources may be necessary to maintain control following a meal.

Disclosures: A. Bischoff-Grethe: None. **C.E. Wierenga:** None. **U.F. Bailer:** None. **W.H. Kaye:** None.

Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

Location: SDCC Halls B-H

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Program #/Poster #: 600.03/EEE19

Topic: G.02. Motivation

Support: NSERC Grant 98181
CIHR Grant 094843

Title: Projections from the nucleus accumbens shell to the ventral pallidum are involved in the control of palatable food intake in adult female rats

Authors: *S. CHOMETTON[1], G. GUÈVREMONT[1], J. SEIGNEUR[2], E. TIMOFEEVA[1], I. V. TIMOFEEV[2]
[1]CRIUCPQ, Quebec, QC, Canada; [2]CERVO, Quebec, QC, Canada

Abstract: Introduction: Palatable food is very appealing and can be consumed even when there is no metabolic need. In our modern society, palatable food is ubiquitous and easily accessible, leading to eating disorders such as binge eating, which is more prevalent in women. In the brain, the nucleus accumbens shell (AcbSh) is part of the reward system and contains mostly inhibitory GABAergic neurons. Inhibition of this brain region in male rodents induces an increase in food intake, and activation via its projection to the lateral hypothalamus (LH) attenuates food intake. However, the main target of the AcbSh is the ventral pallidum (VP), and this structure is sensitive to the hedonic aspect of food. **Objectives**: In the present study, we examined the effects of direct stimulation of the AcbSh on sucrose intake in adult female rats, and the involvement of the VP in this response. **Methods**: The consumption of 10% sucrose and lick microstructure of adult female rats were analyzed following (1) direct stimulation of the AcbSh using optogenetics, (2) pharmacological inhibition of the VP, and (3) stimulation of projections from the AcbSh to

the VP using optogenetics. The expression of *c-fos* mRNA after stimulation of the AcbSh directly, and after stimulation of axonal terminals from the AcbSh to the VP were also studied. **Results**: Direct stimulation of the AcbSh results in a decrease in sucrose intake, meal duration, and total number of licks. In these rats, the expression of *c-fos* mRNA increased in the AcbSh and decreased in the LH and VP. Similarly, a decrease in sucrose intake, meal duration, and total number of licks, was observed upon inhibition of the VP with muscimol, and also upon stimulation of axonal terminals from the AcbSh to the VP. In this last experiment, the expression of *c-fos* mRNA increased in the AcbSh and decreased in the VP, but no variation was observed in the LH compared to the control group. **Conclusion**: This study shows that, not only stimulation of the AcbSh, but also stimulation of projections from the AcbSh to the VP, results in a reduction in sucrose intake in adult female rats. These projections play a role in the regulation of palatable food intake, regardless of the already known role of the AcbSh projections to the LH.

Disclosures: S. Chometton: None. **G. Guèvremont:** None. **J. Seigneur:** None. **E. Timofeeva:** None. **I.V. Timofeev:** None.

Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

Location: SDCC Halls B-H

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Program #/Poster #: 600.04/EEE20

Topic: G.02. Motivation

Support: MH063649
DA015188

Title: Optogenetic stimulation of the orbitofrontal cortex enhances food 'liking' vs 'wanting'

Authors: *I. MORALES[1], K. C. BERRIDGE[2]
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Abstract: Optogenetic stimulation of the orbitofrontal cortex enhances food 'liking' to palatable tastes
Research in our lab has identified a number of cortical and subcortical hedonic hotspots that generate increases in hedonic impact or ‘liking’ for a sensory pleasure in brain limbic structures. These pleasure generators are small subregions (1 to 10 mm^3) in insula and orbitofrontal cortex, as well as subcortical nucleus accumbens and ventral pallidum (VP) where pharmacological microinjections of mu-opioid or orexin receptor agonists enhance by 200-300% hedonic ‘liking’ facial expressions elicited by sucrose. Here we more directly study neuronal causation of liking reactions by controlling activity of neurons in the hedonic ‘hotspot’ located in anterior-medial orbitofrontal cortex (OFC). We infected neurons with either channelrhodopsin (ChR2) or an

inactive control virus into the OFC and recorded affective orofacial reactions elicited by oral infusions of sucrose, quinine, or water. Rats were also tested on food intake (palatable and regular chow), self-stimulation, and conditioned taste aversion discrimination tasks. Our results so far suggest that optogenetic stimulation of the OFC site dependently increases 'liking' reactions to sucrose solutions without affective negative reactions to bitter quinine or neutral water. Similar hedonic enhancements have not been observed in our control virus animals. Our tests so far suggest the newly identified hedonic hotspots in cortical brain structures may amplify 'liking' of taste sensation via neuronal activation in these regions.

Disclosures: I. Morales: None. **K.C. Berridge:** None.

Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 600.05/EEE21

Topic: G.02. Motivation

Support: NHMRC grant APP1125478

Title: Optogenetic stimulation of the paraventricular nucleus reduces motivation to self-administer sucrose

Authors: *C. MITCHELL[1], J. YEOH[1], C. ADAMS[1], E. CAMPBELL[2], J. BAINS[3], G. MCNALLY[4], B. GRAHAM[1], C. DAYAS[1]
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Abstract: Corticotrophin-releasing factor (CRF) neurons of the hypothalamic paraventricular nucleus (PVN) are the principal mediators of the neuroendocrine stress response. Interestingly, recent work has shown that acute PVN CRF cell activation evokes a complex repertoire of behavioural responses that occur independent of the neuroendocrine axis. These behavioural effects appear to be mediated through the lateral hypothalamus (LH) - a central site responsible for orchestrating motivated behaviour. Here, we asked whether repeated optogenetic stimulation of PVN CRF cells could evoke a long lasting impact on behaviour reflective of a change in motivated state. To achieve this aim, male and female *CRF-Cre* mice (n=19) received stereotaxic injections of AAV5-DIO-ChR2-EYFP or EYFP control virus into the PVN followed by a unilateral fibre optic probe positioned just dorsal to the viral injection site. Mice were then trained to self-administer 10% sucrose from a FR1, FR3 and then a progressive ratio (PR) schedule of reinforcement. All mice then received optogenetic stimulation (473nm, 10Hz, 10ms pulse width, 15mW, 30s on, 30s off) for 1 hour daily for 5 consecutive days. After two days rest

mice were re-tested for motivated responding under PR conditions. Compared to controls, CRF-ChR2 animals displayed a significant decrease in PR responding for sucrose (p=0.004). One week later a subgroup of sucrose trained animals (n=8) received the same optogenetic stimulation as above and two hours later were then processed for Fos immunohistochemistry. This approach revealed a significant increase in Fos-positive cells within the PVN (p=0.0342) and the LH (p=0.0308). Together these studies demonstrate that chronic optogenetic stimulation of PVN CRF cells can produce a change in motivated responding for sucrose and we identify the LH as a likely substrate for these effects.

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Poster

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Title: Activation of glucagone-like peptide-1 receptors reduces the motivation to consume sucrose pellets during skilled reach foraging via neurotransmission in nucleus accumbens shell

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Abstract: The appetite-reducing gut-brain peptide glucagon-like peptide -1 (GLP-1) has in addition to food intake reduction been attributed to a variety of physiological functions including reward modulation. The findings that local infusion of GLP-1 analogues into the nucleus accumbens (NAc) shell, an area essential for motivation regulation, reduces the intake of high fat diet and cocaine self-administration lead us to speculate that activation of accumbal GLP-1 receptors (GLP-1R) reduces the motivation to work for reward. In order to further explore this hypothesis we evaluated the effect of GLP-1 analogues on skilled reach foraging in the Montoya staircase test. Electrophysiological field potential recordings and whole cell recordings were performed in the NAc ex vivo to further link behavioural performance with neurophysiological responses. We found that in rats with acquired skilled reach performance the GLP-1 analogues exendin-4 (Ex4) and liraglutide, but not dulaglutide, reduced the consumption of sucrose pellets compared with vehicle. Furthermore local bilateral infusion of Ex4 into NAc reduced the

consumption of sucrose pellets in rats with acquired skilled reach performance. Supporting our behavioural data, ex vivo electrophysiology revealed a suppression of evoked field potentials following administration of Ex4 and liraglutide, while dulaglutide did not affect accumbal neurotransmission. In addition, whole cell recordings showed no effect by Ex4 on the frequency or amplitude on inhibitory inputs, indicating that the effect is primarily mediated via modulation of glutamatergic neurotransmission. Furthermore previous studies report that GLP-1 analogues enhance learning, we investigated if GLP-1 analogues administered throughout the entire training period would increase skilled reach foraging in the Montoya staircase test designed to reflect consumption driven by learning. In this design, Ex4 and dulaglutide, but not liraglutide, increased the consumption of sucrose as well as the success rate compared with vehicle. This suggest that activation of GLP-1R enhances learning of motivated behaviours in this context. Collectively these data suggest that activation of accumbal GLP-1R reduces the motivation to work for a sucrose reward in rats with acquired skilled reach performance. Given that motivation is intimately associated with addiction we speculate that accumbal GLP-1R signalling may be an important regulator throughout the addiction processes, not only in regards to food but also with respect to drugs of abuse.

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Poster

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Topic: G.02. Motivation

Support: NIH Grant MH063649

Title: Mapping behaviors elicited by optogenetic stimulation of lateral hypothalamic and lateral preoptic subregions

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Abstract: Electrical stimulation of the lateral hypothalamus (LH) and lateral preoptic area (LPO) in rats is known to elicit a variety of behaviors including eating, drinking, self-stimulation, and general locomotion. However, two issues with electric stimulation are the recruitment of both cell bodies and fibers of passage near the electrode and that the neural volume recruited is

unclear. We build upon this research foundation by probing various LPO and LH subregions with optogenetic excitation in conjunction with assays measuring food intake, self stimulation, and general behaviors, followed by immunohistochemical measures of immediate early gene (IEG) "plumes" that reflect stimulated neural volumes. Laser stimulation (1-3 mW/mm^2) of LH subregions elicited intense eating in some subjects and intense self-stimulation in others, whereas LPO stimulation did not produce changes in these behaviors. IEG plume mapping of functional effects revealed that, along the rostrocaudal axis, the middle LH region is associated with eating and the posterior LH region is associated with self-stimulation. Increasing laser intensity can convert these behaviors into escape responses in middle and posterior LH subjects, and can increase locomotion in dorsal LH subjects. These data collectively provide a new LH map of behaviors with defined regions of activation in stereotaxic space.

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Poster

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Topic: G.02. Motivation

Support: NIH Grant DA015188
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Title: Optogenetic excitation of limbic corticotropin releasing factor neurons modulates motivation

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Abstract: Corticotropin releasing factor (CRF) is known as the brain's master stress molecule, but also plays a role in reward seeking. Previous pilot work from our lab suggested that optogenetic stimulation of CRF neurons in either nucleus accumbens (NAc) or central nucleus of amygdala (CeA) of CRH-Cre rats supported laser self-stimulation. Here we show that optogenetic CRF neuron stimulation can also amplify incentive motivation to earn and consume natural sucrose rewards, and narrowly focus intense motivation specifically on the particular sucrose-earning option that has been associatively paired with CRF laser stimulation. In a two-choice sucrose experiment, stimulation of CRF neurons in either CeA or NAc biased rats to specifically earn and consume laser-paired sucrose, while ignoring an alternative option to earn identical sucrose pellets delivered without laser. Additionally, CRF stimulation in NAc or CeA

enhanced motivation breakpoint or effort to work for sucrose in a progressive ratio task. By contrast, we found that stimulation of CRF neurons in bed nucleus of stria terminalis (BNST) caused rats to avoid the laser-paired sucrose option and instead choose the alternative sucrose-alone option, and suppressed motivation to work for sucrose rewards in a progressive ratio task. Together, these data suggest that stimulation of NAc or CeA CRF neurons can focus and enhance incentive motivation for natural rewards, whereas BNST CRF stimulation may suppress incentive motivation or have aversive effects.

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Poster

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Topic: G.02. Motivation

Support: SSTF Project SSTF-BA1301-07

Title: Medial preoptic circuit induces hunting-like actions to target objects and prey

Authors: *Y. JEONG, S.-G. PARK, D. KIM
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Abstract: As animals forage, they must obtain useful targets by orchestrating appropriate actions that range from searching to chasing, biting and carrying. Here, we reveal that neurons positive for the α subunit of Ca2+/calmodulin-dependent kinase II (CaMKIIα) in the medial preoptic area (MPA) that send projections to the ventral periaqueductal gray (vPAG) mediate these target-directed actions in mice. During photostimulation of the MPA-vPAG circuit, mice vigorously engaged with 3D objects and chased moving objects. When exposed to a cricket, they hunted down the prey and bit it to kill. Our study explains how the brain yields a strong motivation to acquire a target object along the continuum of hunting behavior.

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Poster

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Title: Chemogenetic stimulation of posterior ventral tegmental area-nucleus accumbens shell circuitry prolongs novelty response in rats

Authors: *J. M. ILLENBERGER, H. LI, M. N. CRANSTON, C. F. MACTUTUS, K. A. MCLAURIN, R. M. BOOZE
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Abstract: Dopamine release to the nucleus accumbens from neurons of the ventral tegmental area (VTA) is critical for orientation and response to novel stimuli. It is unclear which specific cell populations modulate responses to novelty, as there is considerable heterogeneity between cells of the anterior and posterior VTA. The current experiment used a retroDREADDs technique to stimulate neurons of the VTA which project to the nucleus accumbens shell (AcbSh) prior to testing under habituated or novel conditions. AAV-CMV-GFP/Cre was injected into the AcbSh and AAV-hSyn-DIO-hM3D(Gq)-mCherry (a presynaptic enhancer in the presence of its cognate ligand clozapine-N-oxide (CNO)) was injected into the VTA of Fisher 344/N rats to trigger human M3 muscarinic (hM3D(Gq)) receptor production specifically in neurons of the VTA projecting to the AcbSh. Following three days of habituation, animals that received viral infusions (n=10) and animals that received sham surgeries (n=2) were administered 1 mg/kg intraperitoneal saline (1 day) or CNO (4 days) and then repeatedly tested in locomotor activity chambers for 1 hour until well-habituated. To test the locomotor response to novelty without impeding the animals' ability to move freely, the white background noise present throughout habituated conditions was discontinued for a 10-minute period at the 30-minute timepoint during the 1-hour test session. Saline (1 day) or CNO (4 days) was administered again prior to testing under novel conditions to determine if stimulating hM3D(Gq) receptors increased the locomotor response. Stimulating hM3D(Gq) receptors in the VTA enhanced the locomotor response to novelty (e.g. removal of white background noise) without altering activity under habituated conditions or ability to detect a gap in white noise ($p \leq 0.04$). Confocal imaging confirmed hM3D(Gq)-mCherry production in the posterior limb of the VTA (pVTA). The current results support evidence of anterior-posterior heterogeneity in cells of the VTA and identify the pVTA-AcbSh as a circuit likely involved in the etiology of psychopathy in which responses to novel stimuli may be diminished, such as depression or drug addiction.

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Poster

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Topic: G.02. Motivation

Support: DA011289 (JAK)
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Title: Low frequency stimulation has a bidirectional effect on plasticity in periaqueductal gray and rostromedial tegmental nucleus GABAergic synapses in the ventral tegmental area

Authors: *R. ST. LAURENT[1], J. A. KAUER[2]
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Abstract: Persistent opioid-induced changes in components of the reward pathway, such as the dopamine-rich ventral tegmental area (VTA), may precede the transition to addiction. Spontaneous activity of dopamine cells in the VTA is tightly regulated by inhibitory inputs. Opioids depress inhibitory synapses on VTA dopamine cells, increasing their excitability, and morphine exposure can affect plasticity at these synapses. However, the VTA is a heterogeneous region with different subsets of neurons having distinct functional effects on behavior, and therefore, opioid-induced adaptations may also depend on the precise circuit involved. Here, we use optogenetic strategies to investigate the plastic properties, sensitivity to opioids, and relevance to behaviors associated with reward and aversion of two different presynaptic GABAergic afferents to the VTA. The periaqueductal gray (PAG) and the rostromedial tegmental nucleus (RMTg) both have dense expression of mu opioid receptors. Like the PAG, the RMTg seems to be a key region activated by aversive stimuli (Jhou et al, Neuron 2009 61:5). $RMTg_{GABA}$ to VTA synapses are strongly depressed by opioids and influence VTA firing rates. In contrast, the opioid sensitivity of PAG GABAergic inputs to the VTA and their behavioral relevance has not yet been explored. We first compared synaptic plasticity and opioid-sensitivity of PAG_{GABA} to VTA and $RMTg_{GABA}$ to VTA synapses *in vitro*. In acute midbrain slices we performed whole-cell recordings from VTA dopamine cells and measured optically-evoked inhibitory postsynaptic currents (oIPSCs). We discovered that low frequency stimulation, 1 Hz for 6 minutes, had opposite effects on these populations: PAG oIPSCs potentiated (134±13% of baseline, $p<0.05$, $n = 17$) whereas RMTg oIPSCs depressed (79.6±7.3% of baseline, $p<0.05$, $n = 15$). Furthermore, both PAG and RMTg oIPSCs were strongly depressed by bath application of 1 μM DAMGO, a mu opioid receptor agonist (PAG: 22.9±11.2% of baseline, $p<0.05$, $n = 6$; RMTg: 32.2±6.5% of baseline, $p<0.05$, $n = 5$), with PAG oIPSCs trending towards stronger

depression. In conclusion, we are the first to report that 1) low frequency stimulation induces long-term potentiation at PAG_{GABA} to VTA synapses and conversely long-term depression at $RMTg_{GABA}$ to VTA synapses, and 2) opioids profoundly depress PAG_{GABA} to VTA synapses. Future experiments will measure the behavioral output of activating PAG_{GABA} to VTA or $RMTg_{GABA}$ to VTA synapses *in vivo* using a real-time place preference procedure.

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Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

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Topic: G.02. Motivation

Support: This work was supported by NIDA/NIH.

Title: Activation of a hypothalamic-ventral tegmental circuit gates motivation

Authors: *J. N. SIEMIAN, F. L. SCHIFFINO, M. PETRELLA, J. E. SLOCOMB, S. SARSFIELD, M. L. ZUCCOLI, M. SLOMP, Y. APONTE
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Abstract: Across species, motivated states such as food seeking and consumption are essential for survival. Optimal performance of these behaviors is mediated by neuronal circuits that modulate energy balance and feeding. The lateral hypothalamus (LH) has been known for decades to play a fundamental role in regulating feeding and reward-related behaviors. However, the contribution of the diverse neuronal populations in the LH have not been thoroughly identified. Here we examine how lateral hypothalamic leptin receptor-expressing (LH^{LEPR}) neurons, a subset of GABAergic cell types in the LH, regulate motivation to obtain food as measured by responding on a progressive ratio (PR) schedule of reinforcement, a widely-used behavioral task to assess motivation. We found that chemogenetic activation of LH^{LEPR} neurons significantly increased PR performance, while inhibition of these neurons decreased PR behavioral responses. We then mapped LH^{LEPR} axonal projections and demonstrated that these neurons target the ventral tegmental area (VTA). Moreover, Channelrhodopsin (ChR2)-assisted circuit mapping (CRACM) revealed that LH^{LEPR} neurons form functional inhibitory synapses with non-dopaminergic neurons in the VTA. Furthermore, activation of these projections promotes motivation for food reward. Finally, we found that neurons expressing agouti-related peptide (AGRP) in the arcuate nucleus of the hypothalamus (ARC^{AGRP}) likely act as upstream inputs to the LH^{LEPR}-VTA pathway as activation of ARC^{AGRP}-LH projections also strengthens PR performance. Together, these results identify LH^{LEPR} neurons as a new integrator of the hypothalamic-ventral tegmental circuitry that gates motivation.

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Poster

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Title: Exploring the neural mechanism by which optogenetic stimulation of ventral tegmental area dopamine neurons prevents extinction of cued approach behavior

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Abstract: The nucleus accumbens (NAc) and its dopaminergic (DA) innervation from the ventral tegmental area (VTA) are involved in promoting reward-seeking behavior as well as strengthening cue-reward associations. Many NAc neurons exhibit cue-evoked excitations that are required for approach behavior elicited by a reward predictive cue. Additionally, a large body of literature suggests that DA neurons encode reward prediction errors (RPE), which serve to update the current state and alter the strength of cue-reward associations depending on the valence of the RPE. While RPEs presumably lead to changes in response probability, the downstream neural mechanisms from the VTA to NAc mediating this behavior remain unknown. We hypothesized that DA neuronal activity at the predicted time of reward delivery is sufficient to reinforce cued approach behavior by maintaining the magnitude of cue-evoked excitation of NAc neurons on subsequent trials, blocking the transference of a negative RPE. To test this hypothesis we recorded from neurons in the NAc of *Th::Cre* rats expressing channelrhodopsin (ChR2) or eYFP only in VTA DA neurons. Animals were trained on a conditioned stimulus (CS) task in which two distinct auditory tones were presented. One tone predicted availability of a liquid sucrose reward while the other was a non-rewarded. After training, rats were subjected to an *omission* session followed by an *omission + stimulation* session. During *omission* sessions there was a 30 min baseline in the CS task followed by omission of the reward. *Omission + stimulation* sessions introduced a 20 Hz, 1s photostimulation at the predicted time of reward. We found that the decline in behavioral responding during *omission* was prevented by photostimulation in ChR2 but not eYFP rats. Recording from NAc neurons during *omission +*

stimulation sessions revealed short latency firing of NAc neurons during stimulation. This was not seen in recordings of eYFP only animals. In addition, the reduction in cue-evoked excitations during omission was attenuated by stimulation at the time of predicted reward. These results suggest a mechanism by which VTA DA neuronal firing influences subsequent cue-evoked excitations and thus the probability of behavioral response to the cue. Stimulation of VTA DA neurons prevents the extinction of approach behavior and our results suggest that this effect is due to a reduction in the decline in cue-evoked excitations that drive the approach response. Further experiments are underway to investigate if short latency firing is required for the maintenance of approach behavior.

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Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

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Topic: G.02. Motivation

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Title: Effort-related choice behavior is regulated by cholinergic signaling in the ventral tegmental area

Authors: *J. L. HAIGHT, E. J. NUNES, D. J. RATHI, N. A. ADDY
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Abstract: Motivation to work for and obtain rewards in the environment is critically important for an individual's survival, and a lack of motivation (anhedonia) is considered a core component of the pathology of major depressive disorder. The choice to expend effort to pursue preferred rewards over other less preferred, but more easily available options is regulated by dopamine signaling in the nucleus accumbens (NAc). While the role of NAc dopamine signaling in motivational behaviors has been identified, our understanding of the regulation of the source of this signal, the dopamine cell bodies in the ventral tegmental area (VTA), is limited. It has been hypothesized that cholinergic tone in the VTA is a critical mediator of dopamine neuronal activity, and thus motivational activation. Recently, our lab and others have shown that cholinergic signaling in the VTA regulates downstream dopamine release in the NAc. In addition, our lab has demonstrated that cholinergic signaling in the VTA can regulate the ability of sucrose- and cocaine-paired cues to drive reward-seeking behaviors which are dependent on NAc dopamine signaling. Here, we examine the role of cholinergic signaling in the VTA in an effort-based decision making task. In this task, subjects have a choice between performing an operant response for a preferred food (lever presses for 45mg sucrose pellets) on a fixed-ratio 5

schedule, or consuming freely available but less-preferred rat chow. Previous work has demonstrated that the willingness to work for sucrose pellets in this task is dependent on dopamine transmission in the NAc, and blocking dopamine transmission in the NAc results in a reduced willingness to work. In this study, we assessed the effort-related choice effects of increasing cholinergic tone through systemic or VTA-specific administration of the acetylcholinesterase inhibitor, physostigmine. We found that systemic administration of physostigmine (0.125 mg/kg) resulted in a ≥25% reduction of lever responding in male and female rats, with no effect on chow consumption. Preliminary data also indicates that bilateral physostigmine infusion (2 ug per side) directly into the VTA had similar effects to systemic administration in male rats, reducing lever contacts while leaving chow consumption intact. These results show that cholinergic signaling, potentially in the VTA, regulates the motivation to work for a desirable reward. Further studies are currently underway to assess whether this motivational control is due to cholinergic signaling at specific nicotinic or muscarinic receptors in the VTA.

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Poster

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Topic: G.02. Motivation

Support: K99 MH116116
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Title: Role of reward expectation on dopaminergic signals and medium spiny neurons in the dorsal striatum

Authors: *C. H. DONAHUE[1], J. A. NADEL[2], A. C. KREITZER[3]
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Abstract: Dopamine is thought to play a central role in motivated behavior through its influence on striatal circuits, but how this occurs is still unclear. To investigate this, we trained mice to perform two complementary tasks where we systematically manipulated the amount of effort required to obtain reward. In a fixed-ratio task, animals were required to complete 1, 3, or 5 nose poke sequences to receive a reward. The number of required nose pokes was fixed in blocks of 40 trials to encourage the animals to build an expectation of the amount of effort required in each trial. The same mice were also trained on a variable ratio task, where an average of either 1, 3, or

5 nose poke sequences was required in each block, but the precise number could not be predicted on any given trial. The animals gradually became faster as they progressed through a nose poke sequence only in the fixed ratio task, suggesting that knowledge about their proximity to upcoming reward invigorated their movements.
We expressed a genetically-encoded calcium indicator (gCaMP6f) in the dorsal striatum and imaged single cell activity of direct- and indirect-pathway medium spiny neurons (dMSNs and iMSNs) with a head-mounted microscope as animals performed each task. In the fixed ratio task, both dMSNs and iMSNs were significantly more active when animals executed nose pokes early in the sequence when they were furthest from reward, and their responses progressively decreased as they got closer to reward**.** This effect was significantly more pronounced in the iMSN population. In the variable ratio task, where the number of required movements could not be predicted, activity did not modulate throughout the nose poke sequence, suggesting that knowledge about proximity to reward drove these responses. Next, we used fiber photometry to image dopaminergic projections to the dorsal striatum and found the opposite relationship: the magnitude of phasic responses associated with each movement progressively increased as the animals got closer to reward, and this occurred only in the fixed ratio task. Together, these results suggest that reward expectation modulates movement-related dopaminergic signals in the dorsal striatum, which could play a potential role in modulating ongoing striatal activity.

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Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

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Topic: G.02. Motivation

Title: Pramipexole enhances disadvantageous probabilisticdecision-making via D_3, but not D_2, dopamine receptors

Authors: *R. CADEDDU, M. ORRU´, H. STRATHMAN, M. BORTOLATO
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Abstract: Pramipexole (PPX) is a D_2 and D_3 dopamine receptor agonist, used in the treatment of Parkinson's disease (PD) and restless leg syndrome. PPX increases the risk of problem gambling and impulse-control disorders in vulnerable patients; the neurochemical bases of this effect, however, remain unclear. To study the effects of PPX on risky probabilistic decision-making, we recently designed a probability-discounting task to capture the effects of this drug in response to disadvantageous options. We found that PPX (0.3 mg/kg/day, SC) led to mild probability-discounting deficits, which were significantly exacerbated by a concurrent treatment with the monoamine-depleting agent reserpine (RES; 1 mg/kg/day, SC), at low doses that did not affect

baseline locomotor and operant responses. Building on this evidence, in this study we aimed at assessing the neurochemical mechanisms that facilitate and mediate the behavioral effects of PPX. First, we found that the same regimen of RES that facilitated the effects of PPX increased the binding of D_3, but not D_2 dopamine receptors, in the nucleus accumbens. Then, we verified that the effects of PPX were not affected by concurrent treatment with the highly selective D_2 dopamine receptor antagonist L,741-626 (0.1-1 mg/kg, IP); but they were partially reduced by the highly selective D_3 dopamine receptor antagonist SB277011-A (1-10 mg/kg, IP), at doses that did not significantly increase omissions (3 mg/kg, IP). Finally, we documented that the association of RES and PPX did not increase the proclivity to cross a suspended bridge, suggesting that the effects of RES and PPX on probability discounting do not reflect a generalized increase in risk taking.

Disclosures: R. Cadeddu: None. **M. Orru´:** None. **H. Strathman:** None. **M. Bortolato:** None.

Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 600.17/FFF7

Topic: G.02. Motivation

Title: MPA-induced drive-assisted steering (MIDAS) system for behavior experiment

Authors: *D.-G. KIM[1], Y.-C. JEONG[1], S.-G. PARK[1], P.-S. LEE[2], D. KIM[1]
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Abstract: We devised the MPA-induced drive-assisted steering (MIDAS) system, in which a head-mounted bait object swings (servo-motor) within the visual field and CaMKIIα+ MPA-vPAG photostimulation (LED) is remotely induced the hunting-like behavior. This closed-loop system can detect the mouse head positions and angles in real time through the CMOS camera and control the movement of mouse using head-mounted device by navigation algorithm. Using the system, we successfully guided mice to navigate specified routes in our 3D maze. Considering that the mice were able to pass through the various obstacles using appropriate behaviors under MIDAS control, we suggest that the MIDAS system could prove useful in behavioral experiments and other application.

Disclosures: D. Kim: None. **Y. Jeong:** None. **S. Park:** None. **P. Lee:** None. **D. Kim:** None.

Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 600.19/FFF9

Topic: G.02. Motivation

Support: Howard Hughes Medical Institute
NIH R01 MH100568
NIH U01 NS094191
HMS Department of Neurobiology Graduate Fellowship
HMS Stuart H.Q. & Victoria Quan Fellowship in Neurobiology

Title: Single cell transcriptomic profiling reveals distinct subtypes of serotonergic neurons in the mouse dorsal raphe nucleus

Authors: *K. HUANG[1], N. E. OCHANDARENA[1], A. C. PHILSON[1], M. HYUN[2], B. L. SABATINI[3]
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Abstract: The dorsal raphe nucleus (DRN) is an important source of neuromodulatory inputs that innervates a wide range of forebrain regions to regulate many physiological and behavioral processes. While most studied as a major source of serotonergic (5-HT) inputs, DRN neurons exhibit a large degree of heterogeneity at the anatomical and molecular level. Although this heterogeneity likely accounts for the DRN's diverse functions, efforts to establish clear relationships between the molecular identity and function of DRN cell types have been impeded by the lack of a well-annotated map between their molecular and anatomical features. In this study, we used high-throughput single cell RNA sequencing (scRNA-Seq) and profiled approximately 50,000 cells from the mouse DRN and surrounding ventrolateral periaqueductal gray. Through this unbiased survey of DRN cell types, we have identified several neuronal cell types and at least four distinct subtypes of 5-HT neurons. Using multiplexed fluorescence *in situ* hybridization, we have mapped these 5-HT neuron subtypes to different spatial domains within the DRN. Analysis of differentially expressed genes between DRN 5-HT neuron subtypes suggests that they co-release different neurotransmitters and peptides, potentially exerting distinct and competing effects both locally and on downstream circuits. Additionally, retrograde tracing studies have found that DRN neurons innervating functionally distinct target regions are also spatially segregated. By combining viral retrograde genetic tagging with both scRNA-Seq and fluorescence *in situ* hybridization, we are mapping 5-HT neuron subtypes defined by their spatial location and anatomical projection to their molecular profiles. Based on our findings, we

are also exploring intersectional and spatially resolved approaches towards investigating functional differences between 5-HT neuron subtypes.

Disclosures: K. Huang: None. **N.E. Ochandarena:** None. **A.C. Philson:** None. **M. Hyun:** None. **B.L. Sabatini:** None.

Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 600.20/FFF10

Topic: G.02. Motivation

Support: NIH-R01DA036612
Brain Research Foundation NARSAD 2014 YIG
Attias family foundation
T32MH018399
NIDA-INSERM postdoctoral fellowship program

Title: Glutamate and GABA neurons of the ventral pallidum: Opponent roles in motivated behavior

Authors: *L. FAGET, V. ZELL, E. SOUTER, A. MCPHERSON, R. RESSLER, D. DULCIS, T. S. HNASKO
UCSD, LA Jolla, CA

Abstract: The ventral pallidum (VP) is a structure central to reward pathways and the control of motivated behaviors. The VP is predominantly GABAergic but is neurochemically heterogeneous containing various distinct cell types including cells expressing the glutamatergic marker vesicular glutamate transporter (VGLUT2). Using reporter lines we find that VP glutamate neurons are concentrated in the rostro-central ventro-medial VP and are mostly distinct from other VP cell types - rarely coexpressing GABAergic and cholinergic markers. Using cell-type-specific tracing approaches we find that VP glutamate and GABA neurons share similar projection targets, distinct from those made by cholinergic neurons in VP. Using optogenetic manipulation, we observe that activation of VP GABA cell bodies elicit behaviors indicative of positive reinforcement and enhanced appetitive drive mainly through projections to VTA, while their inhibition produces avoidance behaviors. On the other hand, activation of VP glutamate neurons led to behavioral avoidance, particularly via their projections to the LHb. These findings highlight a potent role for bidirectional control of motivated behaviors by VP inhibitory and excitatory neurons, dysregulation of which could contribute to the emergence of deficits in reward functions associated with drug addiction and other neuropsychiatric disease.

Disclosures: L. Faget: None. **V. Zell:** None. **E. Souter:** None. **A. McPherson:** None. **R. Ressler:** None. **D. Dulcis:** None. **T.S. Hnasko:** None.

Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 600.21/FFF11

Topic: E.03. Basal Ganglia

Support: ANR-11-LABX-0042

Title: The anterior caudate nucleus supports impulsive choices triggered by pramipexole

Authors: *L. TREMBLAY[1], E. MARTINEZ[2], B. PASQUEREAU[3], Y. SAGA[4], V. SGAMBATO-FAURE[5]
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Abstract: Excessive impulsive behaviors are associated with various psychiatric and neurological disorders. In Parkinson's disease, Pramipexole (PPX, D2/D3 agonist) is known to reduce motor symptoms but often leads to impulse control disorders. Three well-categorized types of impulsive behaviors are described: action impulsivity, choice impulsivity and waiting impulsivity (Dalley and Robbins, 2017). Based primarily on the heterogeneity of cortico-striatal projections and the fact that the striatum is massively innervated by dopamine (DA) inputs, we hypothesize that those impulsive behaviors triggered by DA treatments may be supported by distinct striatal territories. The Caudate nucleus (CdN), dedicated to decision-making processes, could be related to the emergence of impulsive choices. The Putamen (Put) that is involved in motor processes could be related to action impulsivity. And, the Ventral Striatum (VS), well known to be involved in motivation and outcome expectation could be related to waiting impulsivity. Here, we compared systemic (0.1 mg/kg) and local (6µl) injections of PPX in monkeys trained to execute a delay discounting task. This behavioral paradigm allows detecting impulsive choices by measuring the tendency to choose small immediate rewards over large delayed ones. Local microinjections were alternatively performed inside the three striatal territories to determine the selective contribution of those subregions in DA-induced impulsive behaviors. First, we found that systemic injections of PPX induced impulsive choices in three monkeys by increasing their temporal discounting factors. Then, we reproduced those impulsive behaviors when PPX was directly injected into the CdN, while injections into the VS or the Put had no effect on monkeys' choices.
Together, our results confirm the involvement of the CdN in decisional processes and highlight the importance of this striatal sub-region in impulsive choices. These results are consistent with

clinical studies using PPX and allow us to emphasize the importance of dopamine modulation inside the Caudate nucleus in the neurobiological processes of impulsive behaviors.

Disclosures: L. Tremblay: None. **E. Martinez:** None. **B. Pasquereau:** None. **Y. Saga:** None. **V. Sgambato-faure:** None.

Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

Location: SDCC Halls B-H

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Program #/Poster #: 600.22/FFF12

Topic: G.02. Motivation

Support: MOE Tier 2 Grant (MOE2014-T2-2-016) (to R.Y.)
Chinese Postdoctoral International Exchange Program (to S.S.)

Title: Separate neural mechanisms underlie mean reward and reward variance in risky decision making

Authors: *S. SUN[1], R. YU[2]
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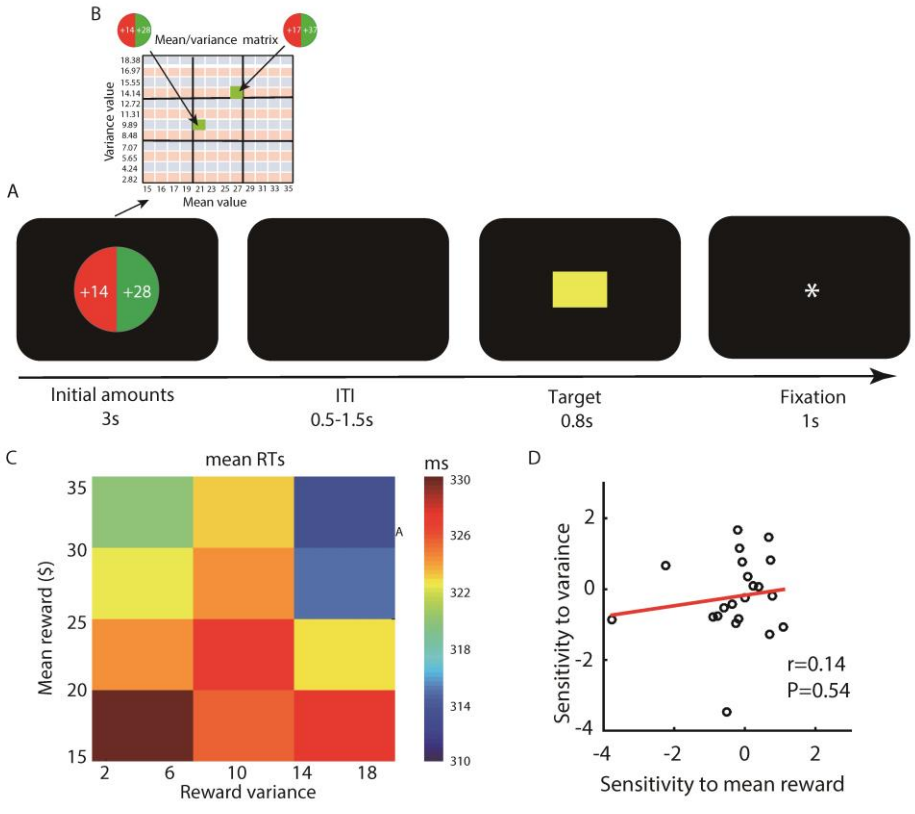
Abstract: Value-based choices are influenced both by potential gains and by the risk of potential gains or losses. How our brain represents expected value and potential risks, integrates such information, and leads to a decision is largely unexplored. Using an incentivized reaction time task in which mean reward and reward variance were parametrically manipulated and orthogonalized, we found that participants respond faster with increasing mean reward and reward variance. Besides, the faster reaction time is indicated by smaller pupil size and more gaze transitions between two rewards, suggesting more alertness, motivation, and efforts are involved in higher reward anticipation. Neuronally, the striatum encodes mean reward, whereas insula encodes reward variance with different neural circuits to integrate both motivational (e.g. amygdala and vmPFC, respectively) and cognitive (e.g. SFG and dACC, respectively) information. With a computational modeling, we further suggested that the putamen is involved in subjective (vs. objective) risk preference by integrating both expected rewards and risks. Taken together, our findings suggested that both rewards and risk work as positive agents which initiate actions with an engagement of cortical-striatal, cortical-limbic and salience networks, and thus modulate subsequent behaviors. Our findings provide new insights into the neural process of decisions under uncertainty.


B
Mean/variance matrix
Variance value
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A
+14
+28
Initial amounts
3s
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0.5-1.5s
Target
0.8s
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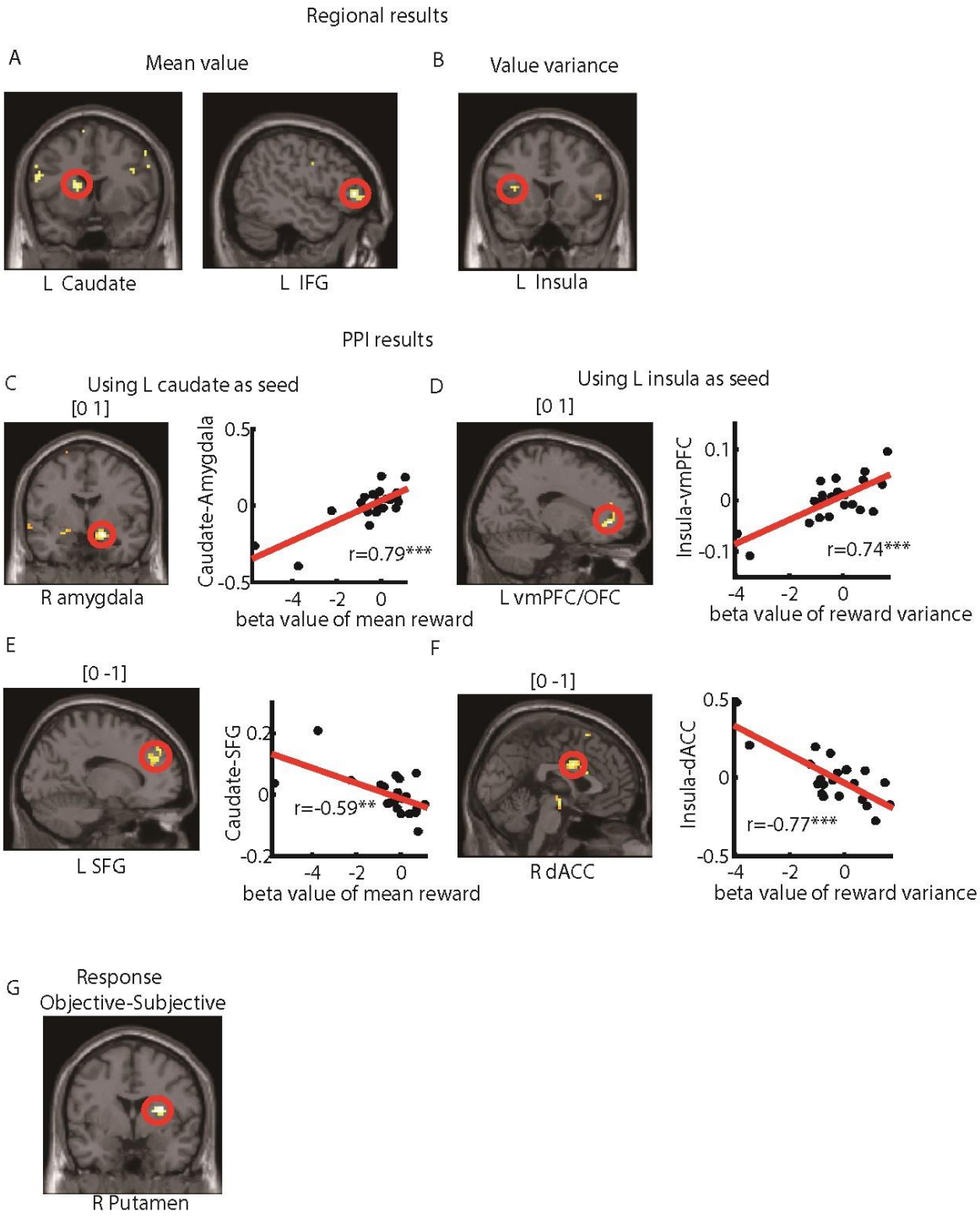

Disclosures: S. Sun: None. **R. Yu:** None.

Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

Location: SDCC Halls B-H

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Program #/Poster #: 600.23/FFF13

Topic: G.02. Motivation

Support: NIH Grant 5R00DA035251-05
NIH Grant R25GM055246

Title: GABAergic ventral pallidum neuron roles in risky decision making

Authors: *M. R. FARRELL, C. RUIZ, J. HEYER, S. MAHLER
Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA

Abstract: Drug addiction is fundamentally a disorder of decision making, in which addicts choose drugs despite mounting negative consequences of their use. However, the neural circuitry underpinning this maladaptive decision making remains poorly characterized. The ventral pallidum (VP), which lies at an anatomical nexus of reward and aversion neural circuits, is ideally positioned for influencing action selection during decisions made under motivational conflict. VP is highly heterogenous, with diverse neuronal subtypes that contribute differently to motivated behavior. For example, VP GABA neurons promote approach and reward seeking, while glutamate neurons instead mediate aversion and avoidance (Faget et al., 2018, *Nat. Commun.*; Tooley et al., 2018, *Biol. Psychiatry*). Interestingly, both populations of VP neurons innervate both reward- and aversion-related brain regions (ventral tegmental area, lateral habenula), opening the possibility for both playing roles in reward as well as aversion. Therefore, we here asked how manipulating activity of VP GABA neurons modulates behavior when both opportunity for reward and potential for punishment are present. Employing a modified version of the risky decision task in rats (Simon et al., 2009, *Neuropsychopharmacology*), we ask whether inhibitory (hM4Di) or excitatory (hM3Dq) DREADD stimulation or inhibition of VP GABA neurons alters risky decision-making behavior. Mildly food-deprived GAD1-Cre rats with hM3/4D DREADDs expressed exclusively in VP GABA neurons were trained to choose between two levers: pressing one delivers a “small” food reward (1 pellet) while the other delivers a “large” food reward (2 pellets). Over the course of a 1 hr session, the large reward lever also delivered an increasingly probable co-delivered footshock, such that the risk of footshock increased as the session progressed (0% for 20 trials, 25%, 50%, 75%, 100%). The “small” food reward lever never delivered footshock and always delivered 1 pellet. In addition to examining effects of stimulating or inhibiting VP GABA neurons on risky decision making, we will also explore effects of manipulating VP GABA projections to reward (VTA) and aversion (LHb)-related output regions. Our results will elucidate roles for VP GABA neurons in

controlling reward-seeking behavior under threat of harm, as often occurs in drug abuse and addiction.

Disclosures: M.R. Farrell: None. **C. Ruiz:** None. **J. Heyer:** None. **S. Mahler:** None.

Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 600.24/FFF14

Topic: E.03. Basal Ganglia

Title: Neural mechanism of valence control in the Brainstem

Authors: *W. SHIN, J. KIM
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Abstract: Balancing emotion and motivation is an important process to make appropriate actions for survival. This function exists in all animals from lower vertebrates to higher primates. The brainstem, called as primitive brain, is evolutionary conserved and essential region for survival. However, its causal role for valence control are largely unknown, due to their dense and complex nuclei compartments and various cell types. Our goal is to unravel the neural circuits and novel cell types in the brainstem that participate in valence control. Here we are going to introduce and discuss a novel circuit recently discovered using optogenetics, imaging and circuit specific cell characterization.

Disclosures: W. Shin: None. **J. Kim:** None.

Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 600.25/FFF15

Topic: G.02. Motivation

Support: CIHR Grant MOP89758
the National Key Research and Development Program of China 2017YFC1310405
the National Natural Science Foundation of China 31371035, U1736124

Title: Chemogenetic inhibition of neurons in the paraventricular thalamus that project to the nucleus accumbens has no effect on the expression of morphine conditional place preference

Authors: *X. DONG[1], S. LI[1], Y. LI[2,3], G. J. KIROUAC[1,4]
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Abstract: The paraventricular nucleus of the thalamus (PVT) is anatomically positioned to mediate addiction behaviors because it projects to multiple brain areas involved in appetitive motivation and drug-seeking. Indeed, experimental evidence shows that the PVT contributes to cocaine- and alcohol-seeking and that a projection from the PVT to the nucleus accumbens (NAc) may be involved in cocaine-seeking. In the present study, we examined the role of PVT-NAc projecting neurons in the expression of morphine conditioned place preference (CPP) in mice. We expressed Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) in the form of the inhibitory hM4Di in PVT neurons that project to the NAc using an intersectional dual-virus approach. This approach involved injections of AAVrg-Syn1-EBFP-Cre bilaterally in NAc and injections of the Cre-dependent AAV8-hSyn-DIO-hM4Di-mCherry or AAV8-hSyn-DIO-mCherry in the PVT. Following a recovery of 2-3 weeks, mice were trained using an unbiased CPP task in which mice received either morphine (10 mg/kg) or saline immediately before a 30-min training session. After four rounds of pairing, mice showed preference to the morphine paired side and clozapine (0.1 mg/kg, i.p.) had no effect on morphine CPP expression in mice expressing hM4Di. In a separate experiment, mice expressing hM4Di that were treated with clozapine showed a lower level of anxiety-like behavior in an open field compared to mice expressing hM4Di treated with saline or in mice expressing mCherry alone treated with clozapine. The number of PVT neurons with both mCherry and c-Fos was reduced specifically in hM4Di-expressing mice treated with clozapine validating that clozapine induced inhibition of neural activity specifically in hM4Di-expressing neurons. In summary, our results do not support a role of the PVT-NAc pathway in the expression of morphine CPP. This study also points to a potential role of the PVT-NAc projection in anxiety-like behavior.

Disclosures: X. Dong: None. **S. Li:** None. **Y. Li:** None. **G.J. Kirouac:** None.

Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

Location: SDCC Halls B-H

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Program #/Poster #: 600.26/FFF16

Topic: G.02. Motivation

Support: 1R15DA041694-01

Title: Dissociable effects of M4 and M3 muscarinic cholinergic receptor antagonism in the rostromedial tegmental nucleus on reward and locomotor activation

Authors: *S. STEIDL, R. HARB, L. RIEDY, S. SCHEINMAN
Psychology, Loyola Univ. Chicago, Chicago, IL

Abstract: GABAergic neurons of the rostromedial tegmental nucleus (RMTg), also known as the "tail" of the ventral tegmental area (VTA), project to and inhibit VTA dopamine neurons. The laterodorsal tegmental nucleus (LDTg) and the pedunculopontine tegmental nucleus (PPTg), two principle brainstem acetylcholine (ACh) cell groups, each provide cholinergic as well as non-cholinergic projections to the VTA that regulate VTA dopamine neuron activity. The RMTg also receives projections from each of the LDTg and the PPTg. We have recently shown that RMTg infusions of the M3 muscarinic ACh receptor antagonist 4-DAMP, but not of the M4 muscarinic ACh receptor antagonist Tropicamide, strongly increase open-field locomotion (Steidl et al., 2017). We now show that RMTg infusions of Tropicamide, but not of 4-DAMP, result in the acquisition of conditioned place preference (CPP). Taken together it appears that cholinergic inputs to the RMTg differentially contribute to rewarding and locomotor effects via M4 and M3 muscarinic ACh receptors, respectively. Current studies are focused on testing the rewarding effects of selective optogenetic inhibition of LDTg or PPTg cholinergic projections to the RMTg.

Disclosures: S. Steidl: None. **R. Harb:** None. **L. Riedy:** None. **S. Scheinman:** None.

Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 600.27/FFF17

Topic: G.02. Motivation

Support: Bourse de mobilité Idex Bordeaux
LIA OptiNutriBrain

Title: Exploring the effects of tetrahydrobiopterin on motivation, dopamine release and acute inflammation in mice

Authors: *H. FANET[1,3,4,5,6], A. OUMMADI[4], A. LO[7], F. DUCROCQ[4], M. TOURNISSAC[1,3], P. BOURASSA[1,3], F. MOUSSA[7], L. CAPURON[4], S. LAYÉ[4,5], S. CAILLE[8], P. TRIFILIEFF[4], F. CALON[2,5], S. VANCASSEL[4,5]
[1]Fac. of Pharm., [2]Fac Pharm. and CRCHUQ, Laval Univ., Quebec, QC, Canada; [3]Neurosciences axis, CHU de Québec - Res. Ctr., Quebec, QC, Canada; [4]NutriNeuro - UMR INRA 1286, Univ. of Bordeaux, Bordeaux, France; [5]OptiNutriBrain - Intl. associated laboratory, Quebec -

Bordeaux, QC, Canada; [6]Inst. of Nutr. and Functional Foods - Laval Univ., Quebec, QC, Canada; [7]LETIAM - IUT d'Orsay, Univ. Paris Sud, Paris, France; [8]CNRS UMR 5287, Bordeaux cedex, France

Abstract: Inflammation can affect mesodopaminergic system and mediates depressive symptoms related to motivation and locomotion. Precisely, pro-inflammatory cytokines can alter dopamine synthesis and thus availability. Tetrahydrobiopterin (BH4) is the mandatory co-factor for phenylalanine and tyrosine hydroxylase activities and therefore essential for dopamine synthesis. Interestingly, inflammation can decrease BH4 by acting on its synthesis and degradation. So, lower BH4 level could participate to the dopaminergic and motivational deficits that occur frequently in chronic inflammatory conditions. Despite its importance, the effects of BH4 administration on dopamine synthesis and related behaviors have been poorly characterized. We hypothesized that BH4 administration can improve dopaminergic function and motivational processes and could be used to counteract inflammation-induced alterations. We first demonstrated that peripheral administration of BH4 (50mg/kg;intraperitoneally) was sufficient to double BH4 brain content within 3h. Using in-situ brain perfusion, we found that the brain uptake clearance (Clup) of BH4 was approximately 0.08µl/g/sec, consistent with a modest transfer across the blood brain barrier. BH4 injection neither changed the expression of main enzymes involved in BH4 and DA synthesis nor total striatal dopamine content. However, using *in vivo* microdialysis in freely moving mice, we showed that BH4 administration induced a slight increase in dopamine release in the nucleus accumbens during food presentation and a higher amphetamine-induced DA release (3mg/kg). Furthermore, BH4 injection increased motivation in a progressive ratio task in operant conditioning without affecting sucrose consumption and anhedonia. Surprisingly, BH4 injection led to a moderate decrease in spontaneous locomotion and to a blunted locomotor sensitization after second exposure to amphetamine. Last, BH4 injection reduced brain pro-inflammatory cytokines expression in an acute inflammation model induced by lLipopolysaccharide injection (830µg/kg). Here, we showed that increased BH4 content leads to increased dopamine release and motivation, and reduces the proinflammatory response to an acute inflammatory challenge. This suggests that BH4 could be a promising treatment for behavioral deficits related to dopaminergic disturbances related to inflammatory condition.

Disclosures: H. Fanet: None. **A. Oummadi:** None. **A. Lo:** None. **F. Ducrocq:** None. **M. Tournissac:** None. **P. Bourassa:** None. **F. Moussa:** None. **L. Capuron:** None. **S. Layé:** None. **S. Caille:** None. **P. Trifilieff:** None. **F. Calon:** None. **S. Vancassel:** None.

Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 600.28/FFF18

Topic: G.02. Motivation

Support: MH063649
DA015188
DA007268

Title: Releasing motivation: Direct inhibition of nucleus accumbens shell neurons promotes motivated behaviors

Authors: *J. J. OLNEY[1], K. C. BERRIDGE[2]
[1]Dept. of Psychology, [2]Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: The nucleus accumbens shell (NAcSh) is a critical node in the mesolimbic reward circuit. A popular hypothesis proposes that it is primarily inhibition of NAcSh GABAergic neurons that promotes motivated behaviors. Such inhibition of GABAergic activity interrupts the inhibitory input onto downstream targets, which, in turn, generate intense motivation. In support of this theory, previous pharmacological studies from our lab have demonstrated that suppression of neuronal activity within the NAcSh via microinjections of the AMPA antagonist, DNQX, or the GABA agonist, muscimol, are capable of eliciting motivated behaviors such as feeding or defensive treading. What is more, depolarization of these neurons via the excitatory optogenetic construct, channelrhodopsin, blocks these DNQX-induced effects, indicating that hyperpolarization of the NAcSh is necessary to produce motivation. The purpose of the present study was to provide direct evidence regarding whether neuronal inhibition in NAcSh is sufficient to generate increases in motivated behaviors. Here, we used pharmacogenetics and optogenetic tools to directly inhibit NAcSh neurons. Preliminary findings suggest that food intake more than doubled following treatment with CNO to trigger DREADD inhibition of NAcSh neurons, indicating that neuronal inhibition produces appetitive motivation. Additionally, although the total amount of food eaten was not altered as a function of optogenetic inhibition, closer examination indicates that animals spent more than twice the amount of time eating or treading while the laser was on relative to when the laser was off, suggesting that laser inhibition of NAcSh neurons temporally organized motivated behaviors to mostly coincide with laser illumination rather than during the intervening laser-off periods. Taken together, preliminary data from these experiments suggest that neuronal inhibition in NAcSh is sufficient to generate increases in motivated behaviors. As a whole, these findings indicate that neuropsychiatric disorders characterized by pathologically high levels of motivation, such as addiction, may be a consequence of hypoactivity within the NAcSh. (Supported by NIH grants MH063649, DA015188, and DA007268).

Disclosures: K.C. Berridge: None.

Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 600.29/FFF19

Topic: G.02. Motivation

Support: NIH T32 Grant DA007281-22
NIH Grant DA015188
NIH Grant MH063649

Title: Optogenetic stimulation of the medial amygdala may focus pursuit for a cocaine reward

Authors: *E. E. NAFFZIGER, K. C. BERRIDGE
Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: *Optogenetic stimulation of the medial amygdala may focus pursuit for a cocaine reward* E. E. Naffziger and K. C. Berridge Previous research on the medial amygdala (MeA) has highlighted a crucial role of orchestrating sociosexual behaviors based-off incoming pheromone signals. Recent work from our lab suggests MeA may be contributing to the motivation for rewards outside of the realm of sociosexual behaviors, such that pairing MeA channelrhodopsin (ChR2) stimulation with an external sucrose reward was capable of increasing the motivation for the MeA ChR2-paired sucrose reward. Importantly, MeA belongs to the medial component of the extended amygdala system (EAS). The lateral counterpart of EAS (including the central amygdala) has been well-documented to play a role in motivation for drug rewards. However, the extent to which MeA may be involved in processing motivation for a drug reward has yet to be explored. In this study, rats received bilateral infusions of ChR2 into MeA before later receiving intra-jugular catheter implants. After recovery, animals underwent self-administration and had the choice to earn an infusion of cocaine or an infusion of cocaine paired with MeA ChR2 photostimulation. Preliminary data from self-administration suggests that pairing MeA ChR2 with one of two available cocaine infusions is capable of driving desire towards the MeA ChR2-paired infusion. This data could indicate that while historically the lateral EAS has been examined in addiction, there may be a role for the medial EAS as well. Importantly, this data helps inform our understanding of neuropsychiatric disorders that are characterized by abnormal motivation.

Disclosures: E.E. Naffziger: None. **K.C. Berridge:** None.

Poster

600. Subcortical Neurocircuitry in Motivated Behaviors

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 600.30/FFF20

Topic: G.02. Motivation

Title: Dissecting the addiction-like preference created by optogenetic stimulation of the central amygdala

Authors: C. L. POISSON[1], M. MIAN[2], D. VAAMONDE[2], J. M. CHABOT[2], H. XU[2], C. FREELAND[3], *M. J. ROBINSON[4]
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Abstract: Substance use disorders involve compulsive preference for drugs of abuse over better alternatives, and despite adverse consequences. We have recently shown that optogenetic stimulation of the Central Amygdala (CeA) creates an addiction-like preference for a stimulation-paired reward (Tom et al., 2018). However, little is known about the psychological mechanisms that help generate this persistent and compulsive preference. The present study evaluates the relative role of three theories of addiction in generating CeA-induced compulsive preference: 1) accelerated learning, 2) habit formation, and 3) increased incentive value of reward-associated stimuli. Rats were injected with Channelrhodopsin or a control virus into the CeA, which was optogenetically stimulated using laser light. The first experiment employed a novel decision-making task, where auditory cues signaled which of two levers would deliver a reward. The ability of CeA stimulation to enhance acquisition (1) of the task was examined by pairing laser stimulation only with correct responses, whereas habit formation (2) was tested by repeatedly pairing laser stimulation with responses on one of the two levers, whether responses were correct or incorrect. The ability of CeA stimulation to promote habit formation of a particular instrumental response was further examined in a task where laser stimulation accompanied an 8 sec timeout period following reward delivery, during which animals were either allowed or not to repetitively respond on the reward-paired lever. Finally, the ability of CeA stimulation to ascribe incentive value to reward-paired stimuli (3) and not just rewards, was examined by pairing laser stimulation with a particular response and its reward-paired cues, either only in the presence of reward delivery or when reward was omitted. Our results suggest that laser stimulation of the CeA does not create a compulsive preference by enhancing learning, promoting habit formation or increasing the incentive value or reward-associated choices and cues. Instead the CeA appears to create a narrow preference for one particular reward by enhancing the motivational value of reward outcomes. However this preference appears to display compulsive-like traits, and shows resistance to devaluation, only after the preference is initially acquired free of challenges. These results suggest that the CeA may play a role in the transition from casual use to the persistent and compulsive pursuit of a particular reward.

Disclosures: C.L. Poisson: None. **M. Mian:** None. **D. Vaamonde:** None. **J.M. Chabot:** None. **H. Xu:** None. **C. Freeland:** None. **M.J. Robinson:** None.

Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 601.01/FFF21

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH AA006420
NIH AA013498
NIH AA015566
NIH AA017447
NIH AA021491
FWF J-3942

Title: Alcohol dependence and withdrawal alter serotonergic modulation of GABA transmission in the CeA

Authors: S. KHOM, R. R. PATEL, D. HEDGES, F. P. VARODAYAN, *M. BAJO, M. Q. STEINMANN, R. VLKOLINSKY, D. KIRSON, M. ROBERTO
Dept. of Neurosci., The Scripps Res. Inst., La Jolla, CA

Abstract: Increased serotonergic neurotransmission plays a critical role in the etiology of alcoholism regulating both reward and stress circuits in the brain. Studies in humans and animal models have shown that modulating the serotonergic signaling can both increase and decrease ethanol consumption. Chronic ethanol intake enhances the activity of serotonergic neurons in the dorsal raphe nucleus, and in addition altered 5-HT_{2C} signaling in the extended amygdala contributes to anxiety-like behaviors during ethanol withdrawal.
The central nucleus of the amygdala (CeA) is the major output region of the amygdalar complex and a major player in the development of alcoholism. Our overall hypothesis is that ethanol dependence and withdrawal dysregulate 5-HT signaling in central amygdala. Our electrophysiological data show that 5-HT dose-dependently (0.5-50 μM) increases spontaneous GABA release in the CeA of naïve rats. Specifically, we found that 50 μM 5-HT significantly ($p<0.001$) increased (to 402±63% of control) the frequency but decreased (to 76±9% of control) the amplitude of spontaneous inhibitory postsynaptic currents (sIPSCs), indicating that 5-HT increases action-potential dependent GABA release and decreases $GABA_A$ receptor functions in the CeA of naïve rats. Interestingly, 50 μM 5-HT significantly increased frequency of sIPSCs in both CeA neurons of ethanol-dependent (153±24% of control) and 14 days withdrawn rats (219±49% of control), however this increase was significantly less pronounced compared to naive rats. In addition, the 5-HT-induced decrease in the amplitude of sIPSCs observed in naive

CeA neurons was lost in ethanol-dependent and withdrawn rats. Moreover, 50 μM 5-HTabolished spontaneous firing of CeA neurons in both naïve and ethanol-dependent rats. The selective 5-HT_{2C} agonist WAY161503 significantly increased sIPSC frequency in ethanol-naïve CeA neurons (217±44%, $p<0.05$), but had mixed effects on sIPSC frequency on CeA neurons from ethanol-dependent and ethanol-withdrawn rats. Overall, we find that 5-HT signaling profoundly modulates GABA transmission in the CeA of naïve rats and that ethanol dependence and withdrawal produces adaptive changes in the 5-HT system.

Disclosures: S. Khom: None. **R.R. Patel:** None. **D. Hedges:** None. **F.P. Varodayan:** None. **M. Bajo:** None. **M.Q. Steinmann:** None. **R. Vlkolinsky:** None. **D. Kirson:** None. **M. Roberto:** None.

Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

Location: SDCC Halls B-H

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Program #/Poster #: 601.02/FFF22

Topic: G.08. Drugs of Abuse and Addiction

Support: PHS NIH Grant AA020919
PHS NIH Grant DA035958

Title: Ethanol enhancement of dopamine release in the nucleus accumbens and ethanol reward are mediated by peripheral neuroimmune interactions

Authors: *J. D. OBRAY[1], E. Y. JANG[3], T. J. CLARKE[2], A. KLOMP[1], A. P. RICHARDSON[2], M. PARSONS[2], C. H. YANG[3], J. T. YORGASON[2], S. C. STEFFENSEN[1]
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Abstract: The prevailing view is that enhancement of dopamine (DA) transmission in the mesolimbic DA system originating in the midbrain ventral tegmental area (VTA) and projecting to the nucleus accumbens (NAc) underlies the rewarding properties of alcohol. Despite the fact that many labs have shown that DA release is enhanced by acute ethanol in vivo, the story is mixed ex vivo, with some labs showing enhancement, while others inhibition, of DA release in the NAc. Further complicating this story is that ethanol injected directly into the VTA has no effects on DA release in the NAc. The aim of this study was to determine the role of peripheral neuroimmune responses in mediating ethanol enhancement of DA release in the NAc and ethanol reward. Using microdialysis and HPLC, systemic administration of ethanol (0.5-4.0 g/kg) markedly enhanced DA release in the NAc in male subjects. Ethanol (IP) or IV DA enhancement of DA release in the NAc was abolished by administration of the peripheral-only acting D2 receptor (D2R) antagonist domperidone. A place conditioning paradigm was used to

test rats for ethanol preference. Domperidone (1 mg/kg, IP) administered before ethanol conditioning trials was found to prevent acquisition of ethanol conditioned place preference. Locomotor activity and motor coordination were tested using open field and rotarod paradigms, respectively. Domperidone (1 mg/kg, IP) was found to attenuate ethanol suppression of locomotor activity at large doses of ethanol (2.0 - 4.0 g/kg, IP) while not affecting ethanol impairment of motor coordination. These findings suggest that ethanol enhancement of DA release and ethanol reward is in part mediated by a peripheral mechanism involving D2Rs. These results challenge the dogma regarding direct ethanol actions on mesolimbic DA transmission. Experiments are ongoing to evaluate ethanol mediated changes in plasma catecholamine concentrations, ethanol effects on DA release in animal models of monocyte/macrophage/microglia depletion, and domperidone effects on ethanol mediated enhancement of DA neuron firing rate in vivo.

Disclosures: J.D. Obray: None. **E.Y. Jang:** None. **T.J. Clarke:** None. **A. Klomp:** None. **A.P. Richardson:** None. **M. Parsons:** None. **C.H. Yang:** None. **J.T. Yorgason:** None. **S.C. Steffensen:** None.

Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

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Program #/Poster #: 601.03/FFF23

Topic: G.08. Drugs of Abuse and Addiction

Support: 1R01AA024798

Title: Effects of low-dose embryonic ethanol on the early development of hypocretin/orexin neurons and behavior in larval zebrafish

Authors: *A. COLLIER, V. HALKINA, S. MIN, O. KARATAYEV, G. Q. CHANG, S. F. LEIBOWITZ
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Abstract: Embryonic exposure to ethanol is known to increase alcohol drinking and have long-term effects on neurochemical systems in the brain. With zebrafish (ZF) emerging as an advantageous model for elucidating neural mechanisms of numerous brain disorders, we recently established (Sterling et al., 2015) a model of voluntary ethanol-gelatin consumption in adult ZF and demonstrated that consumption of ethanol significantly stimulates the neuropeptide, hypocretin/orexin (hcrt/ox), in the anterior hypothalamus. We also showed (Sterling et al., 2016) that embryonic ethanol exposure stimulates the proliferation and expression of hcrt/ox and increases voluntary consumption of ethanol-gelatin in adult ZF as well as locomotor activity, anxiety, and aggression. In the present study, we utilized live imaging confocal microscopy and

Imaris software to investigate how low-dose embryonic ethanol (0.5%) affects the early development of hcrt/ox neurons and how this effect relates to behavior in larval ZF. In control animals examined from 24-28 hpf, we found hcrt/ox neurons to exist primarily in tight clusters, which from their original position in the medial/anterior/ventral region of developing hypothalamus migrated in a lateral/posterior/dorsal direction. Embryonic ethanol exposure increased the proliferation of hcrt/ox neurons and significantly disrupted their migratory path, reducing the straightness of their movement and disrupting their migration in medial-lateral and anterior-posterior directions. Interestingly, these effects occurred mostly on the right side of the brain. The ethanol-induced changes in migration ultimately altered the anatomical distribution of hcrt/ox neurons in lateral hypothalamus from 6-12 dpf, causing the hcrt/ox neurons to be more dispersed and located more medial and posterior again on the right side. Ethanol also affected hcrt/ox neuronal projections, increasing their number and arborization on the right. These neuronal changes induced by embryonic ethanol were closely associated with changes in behavior at 6-12 dpf. Using a new model for measuring voluntary ethanol-gelatin consumption in larval ZF, we found that, similar to our results with adult ZF, embryonic ethanol stimulated voluntary ethanol consumption at 12 dpf and increased locomotor activity and anxiety-like behavior. These results demonstrate that low-dose ethanol markedly affects migration, morphology and anatomical distribution of hcrt/ox neurons and behavior in the same fish, suggesting a causal relationship and demonstrating the importance of hcrt/ox developmental asymmetry in normal neuronal and behavioral functioning.

Disclosures: A. Collier: None. **V. Halkina:** None. **S. Min:** None. **O. Karatayev:** None. **G.Q. Chang:** None. **S.F. Leibowitz:** None.

Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

Location: SDCC Halls B-H

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Program #/Poster #: 601.04/FFF24

Topic: G.08. Drugs of Abuse and Addiction

Support: PAPIIT IN301717

Title: Effects of systemic and intra-ventral tegmental area administration of 5-HT1B receptor agonist CP94253 on oral self-administration of ethanol in rats

Authors: *J. C. JIMÉNEZ, F. CORTES-SALAZAR, L. N. CEDILLO, R. I. RUÍZ-GARCÍA, C. E. MÉNDEZ-CORONEL, A. I. BARRIENTOS-NORIEGA, F. MIRANDA-HERRERA*
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Abstract: GABAA receptors are expressed in cell body of ventral tegmental area (VTA) GABA interneurons, and other sites, in the reward system and play a key role in the addictive actions of

ethanol (EtOH). Activity of VTA GABA interneurons is regulated by postsynaptic 5-HT1B (hetero) and presynaptic 5-HT1B (auto) receptors. Activation of 5-HT1B heteroreceptors in the VTA reduces GABA release onto VTA dopamine (DA) neurons resulting in their desinhibition and consequently increasing DA release in nucleus accumbens. On the other hand, activation of 5-HT1B autoreceptors has opposite effects on GABA and DA release. One strategy to find out if the 5-HT1B receptor agonist administration produces its behavioral effects activating 5-HT1B auto or heteroreceptors is to observe the behavioral effects after systemic or intra-VTA injection of 5-HT1B receptor agonist. Here we evaluate the effects of the systemic and intra-VTA injection of the 5-HT1B receptor agonist CP94253 on oral self-administration of EtOH in rats. Male Wistar rats (250-300 g) were used. Rats were water deprived for 24 h, and then trained to lever-press for water reinforcement on a FR1 schedule (30-min session) by 3 days. Then, rats were trained to lever-press for EtOH (0.01 ml of EtOH in water at 12%) on a FR1 schedule (30-min session) by 3 days. After this training, the reinforcement contingency was changed to FR3 for EtOH access (30-min session) until response rate remained stable at 80% by 3 consecutive days. Then rats were randomly assigned to one of the 6 groups (n=10). Three groups of rats received a systemic injection of 5-HT1B receptor agonist CP94253 (2.0, 4.0 and 8.0 mg/kg, one dose per group) before rats were under FR3 schedule of reinforcement for EtOH access by one session. Other three groups of rats received intra-VTA injection of CP94253 (0.625, 1.25 and 2.5 µg, one dose per group, cannulae were implanted 2 mm dorsal to the VTA at AP -5.1 mm of Bregma, ML ± 0.9 mm, DV -7.8 mm). The data showed that both systemic and intra-VTA injections reduces oral self-administration of EtOH. These findings suggest that 5-HT1B autoreceptors may modulate the reduction of oral self-administration of EtOH in rats.This study was supported by PAPIIT IN301717 (UNAM, Mexico)

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Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 601.05/GGG1

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R01-009411-19
NIH Grant T32-DA028874-07

Title: Acute stress and alcohol exposure produce a common alteration in ventral tegmental area inhibitory signaling

Authors: *B. A. KIMMEY, A. OSTROUMOV, R. WITTENBERG, J. A. DANI
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Drugs of abuse, including alcohol, produce robust synaptic plasticity adaptations in the mesolimbic reward pathway that are paralleled by stress exposure. We recently found that acute stress in rats leads to long-lasting alterations in inhibitory signaling in the ventral tegmental area (VTA), which is mediated by a deficit in the function of the potassium-chloride cotransporter 2 (KCC2) in VTA GABA neurons. Impairment of KCC2-mediated chloride extrusion shifts ethanol-induced VTA GABAergic signaling from inhibitory toward excitatory via a depolarized GABA reversal potential (E_{GABA}) in GABA neurons. Here, we replicate our stress findings in mice and show that after four weeks of ethanol drinking experience under the intermittent two-bottle choice paradigm in unstressed mice, E_{GABA} in VTA GABA neurons is depolarized relative to mice which drank saccharin for the same duration. This depolarizing shift in E_{GABA} following ethanol consumption mirrors the effect of acute stress. Moreover, western blot analysis revealed that functional KCC2 phosphorylation was decreased in ethanol-drinking mice when compared to saccharin drinking mice, as we have found following acute stress. These data suggest that VTA KCC2 is a common molecular adaptation arising from acute stress and alcohol exposure, which may contribute to subsequent alcohol abuse. Targeting KCC2, therefore, may provide a novel therapeutic avenue for limiting the progression to alcohol use disorder.

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Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

Location: SDCC Halls B-H

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Program #/Poster #: 601.06/GGG2

Topic: G.08. Drugs of Abuse and Addiction

Support: AA020930
AA023288

Title: Plasticity of cingulate cortex intrinsic excitability following voluntary ethanol consumption

Authors: *R. D. CANNADY[1], P. J. MULHOLLAND[2]
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Abstract: Exposure to ethanol promotes plasticity of intrinsic excitability in several brain regions and is implicated in the dysregulation of learning processes and effective integration of

synaptic signaling. Several studies have examined how passive ethanol exposure and ethanol withdrawal alter intrinsic excitability. However, few studies have examined how voluntary ethanol consumption alters neuronal firing. The anterior cingulate cortex (ACC) is a key region that integrates input from several reward-related brain regions, but the mechanisms that promote sensitivity to ethanol exposure and withdrawal have not been fully investigated. Moreover, it is not known if voluntary ethanol consumption alters intrinsic excitability of ACC neurons. To address this gap in understanding, male C57BL/6J mice were given access to 20% ethanol and water using a chronic intermittent two-bottle choice drinking procedure. The brains of these mice were extracted for patch-clamp electrophysiology recordings after 1 day, 1 week, 4 weeks, or 7 weeks of voluntary intermittent access to ethanol. Current pulses were injected into deep layer ACC pyramidal cells to evoke action potentials and to examine excitability following voluntary consumption. A single day of ethanol consumption significantly increased evoked action potentials relative to water-drinking control mice. Mice that consumed ethanol for 1 week exhibited reduced spiking in the ACC relative to controls. The changes in spiking were transient and dependent on drinking history as mice that consumed ethanol for 4 or 7 weeks showed no significant alterations in action potential firing between ethanol and water consuming mice. These data indicate that voluntary ethanol consumption produces unique and transient alterations in ACC intrinsic excitability. Thus, the ACC may be involved in encoding ethanol-specific information after early consumption with implications as a predictive indicator of responsiveness to consumed ethanol.

Disclosures: R.D. Cannady: None. **P.J. Mulholland:** None.

Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

Location: SDCC Halls B-H

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Program #/Poster #: 601.07/GGG3

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R01AA024774
NSF HRD 0450339
NIH-NIGMS 5R25GM099649-03

Title: Effect of voluntary binge drinking on microglial cells in the medial prefrontal cortex and hippocampus of male and female adolescent rats

Authors: *A. SILVA-GOTAY[1], W. VARGAS RIAD[2], E. TAVARES[1], A. LIN[1], M. K. HOLDER[3], H. N. RICHARDSON[1]
[1]Psychological and Brain Sci., Univ. of Massachusetts Amherst, Amherst, MA; [2]BGB Group, New York, NY; [3]Georgia State Univ., Atlanta, GA

Abstract: Adolescence is a period of development when teenagers are engaging in risky behaviors including binge drinking. Heavy alcohol use may be particularly hazardous at this time because brain circuits in the frontal lobes are undergoing maturational processes important for higher cognitive function and behavioral control in adulthood. Alcohol has been shown to induce inflammation in the brain. Microglia, the brain immune cells, not only mediate inflammation but also play a role in neural development. The goal of the present study was to test the hypothesis that voluntary alcohol activates microglia in the medial prefrontal cortex (mPFC) and dorsal hippocampus early in adolescence, as this could alter the trajectory of neural circuit development and function. Adolescent male and female Wistar rats underwent two weeks of operant binge alcohol self-administration of sweetened alcohol or sweetened water (PD 28-42). Brains were collected one day after the last drinking session and microglia were labeled using an ionized calcium-binding adapter molecule 1 (Iba1) antibody. We found that higher levels of binge drinking were associated with increased Iba1 immunoreactivity in the mPFC of males and females. There was also a trend of increased Iba1 immunoreactivity in the dentate gyrus and CA1 field of the hippocampus in alcohol males. These findings suggest voluntary binge drinking may elicit an inflammatory state in the brain. Future studies will determine whether higher immunoreactivity is due to increased cell number, cell size, and/or thicker proximal processes, as well as elevated neuroinflammatory cytokines. Overall, these findings highlight the potential risk moderate to high voluntary intake could have on the developing brain.

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Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

Location: SDCC Halls B-H

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Program #/Poster #: 601.08/GGG4

Topic: G.08. Drugs of Abuse and Addiction

Support: This study was supported by Mahajan Imaging Centre Pvt. Ltd., Delhi

Title: Better insights with ISC when using a multi-sensory fMRI paradigm

Authors: *D. SINGLA[1], J. KAUR[2], A. DHAWAN[3], V. MAHAJAN[4], R. GARG[2]
[1]Electrical Engin., [2]Computer Sci. & Engin., Indian Inst. of Technology, Delhi, New Delhi, India; [3]All India Inst. of Med. Sci., Delhi, India; [4]Mahajan Imaging Pvt. Ltd., Delhi, India

Abstract: Cue reactivity tasks have been widely employed in fMRI studies. Due to ease of use and compatibility with General Linear Model (GLM), visual cues are predominantly adopted despite their limitation in terms of replicating real life scenario. We propose using Intersubject Correlation Analysis **(ISC)** to analyse multi sensory paradigms over GLM based analysis and

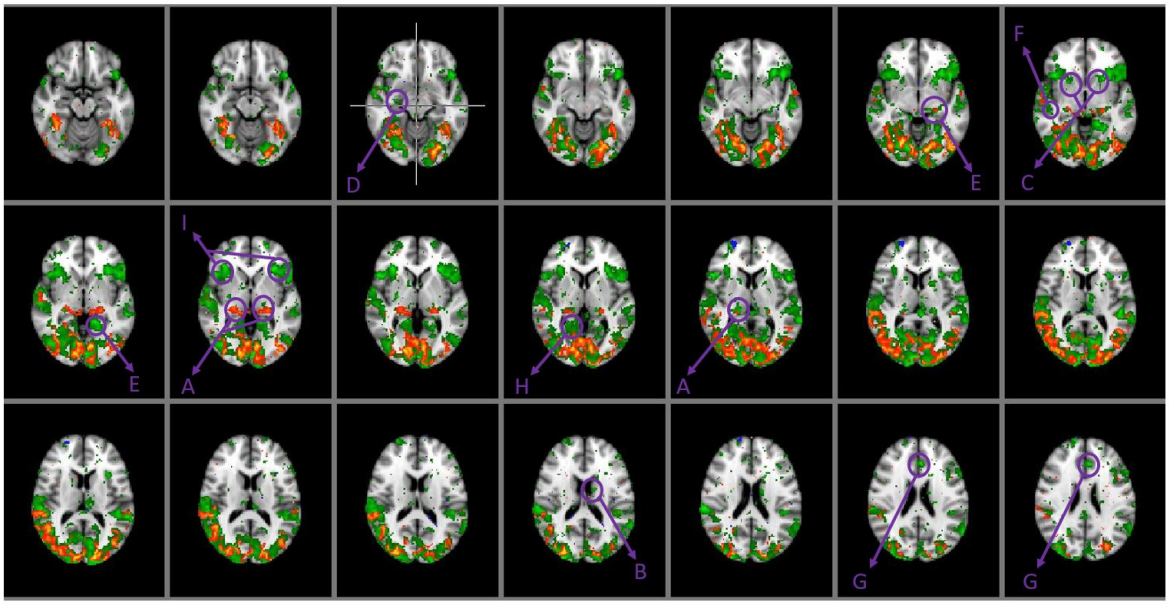
demonstrate advantages of ISC in a multi sensory paradigm using a case study of craving for alcohol in subjects with heavy alcohol use.

Four male young adults (mean age of 24) with heavy alcohol use whose score on Alcohol Use Disorder Identification Test (AUDIT) was greater than 8, were scanned using a 3T GE MRI Scanner while undergoing a multi sensory craving paradigm. The paradigm included 20 blocks with short videos with fixation cross after every block. Ten videos contained alcohol which were matched with neutral videos based on colour, background, presence of faces, emotions, etc. The order of blocks was randomized once and then kept same across all subjects.

Preprocessing of fMRI data included BET extraction, slice timing correction (ascending interleaved), spatial smoothing (FWHM of 5mm) and temporal filtering of 0.01Hz using FSL. Contrast between alcohol cues and fixation was computed using GLM analysis and compared with statistical maps obtained using ISC analysis. Both the statistical maps were corrected for multiple comparisons using False Discovery Rate (FDR) of 0.05.

With GLM analysis, both visual and auditory regions were observed to be activated along with thalamus. With ISC analysis, regions previously known to be involved in craving such as insula, amygdala, hippocampus, caudate, putamen, anterior cingulate cortex (ACC), posterior cingulate cortex (PCC), orbitofrontal cortex (OFC) were also activated. Refer to the attached figure for the two statistical maps and the activated areas.

We hypothesize that craving is nonlinear in nature. Linear Time Invariant (LTI) assumption of GLM makes it harder to capture craving regions when applied to multi-sensory cues. ISC analysis is a better option in this case.



Red-Yellow represent regions activated by General Linear Model (GLM) analysis while Blue represent regions deactivated by GLM analysis. The Green color represent regions given by Inter-Subject Correlation (ISC)
A: Left & Right Thalamus, B: Left Caudate, C: Left & Right Putamen, D: Right Amygdala, E: Left Hippocampal divisions, F: Parts of Insula, G: Parts of ACC, H: Parts of PCC, I: Parts of OFC

Disclosures: D. Singla: None. **J. Kaur:** None. **A. Dhawan:** None. **V. Mahajan:** None. **R. Garg:** None.

Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 601.09/GGG5

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH P01AG031862
NIH GM110174

Title: Liver alcohol metabolism directly fuels histone acetylation in the brain

Authors: *P. MEWS[1], G. EGERVARI[1], S. SIDOLI[1], R. NATIVIO[1], G. DONAHUE[1], D. C. ALEXANDER[1], E. J. NESTLER[2], B. A. GARCIA[1], S. L. BERGER[1]
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Abstract: In the adult brain, epigenetic control of gene expression has important roles in the processing of neural activity. Emerging evidence suggests that epigenetic regulation is dependent on metabolic state, implicating specific metabolic factors in neural functions that drive behavior. In neurons, histone acetylation is dependent on the metabolite acetyl-CoA that is produced from acetate by chromatin-bound ACSS2. Here, using in vivo stable isotope labeling, we show that liver alcohol metabolism rapidly fuels histone acetylation in the brain by direct deposition of alcohol-derived acetyl groups onto histones in an ACSS2-dependent manner. A similar induction was also observed with heavy labeled acetate injection in vivo. In addition, injection of labeled alcohol into a pregnant mouse results in incorporation of labeled acetyl groups into the brains of the gestating fetuses. In isolated primary hippocampal neurons in vitro, extracellular acetate induced learning and memory-related transcriptional programs that were sensitive to ACSS2 inhibition. These findings establish a novel and direct link between hepatic alcohol metabolism and neuronal histone acetylation, providing the first evidence for dynamic signaling from liver metabolism directly to epigenetic regulation in neurons.

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Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

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Program #/Poster #: 601.10/GGG6

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIMHD 2G12MD007592
NIH/NIAAA R15AA020996
NIH/NIMHD-RCMI 5G12MD007592

Title: D2 dopamine receptor in ethanol-induced behaviors

Authors: *N. M. DELGADO, A. CEBALLOS, P. R. SABANDAL, K.-A. HAN
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Abstract: Alcohol is one of the most widely used drugs worldwide. Alcohol consumption has many effects including euphoria and sedation. Excessive alcohol intake causes motor impairment and possibly risky sexual behavior and substance use disorder. Numerous studies have identified dopamine as a critical neuromodulator mediating several effects of ethanol. In the fruit fly *Drosophila melanogaster*, exposure to ethanol causes changes in behavior such as alterations in locomotor activity, tolerance and behavioral disinhibition similar to other animals and humans. The goal of this study is to uncover the role of the D2 dopamine receptor in alcohol-induced behaviors. We exposed wild-type *Canton-S* and D2 receptor mutant *d2r* flies to ethanol and monitored behavioral changes. *d2r* mutant flies exhibited initial sensitivity to the sedative effect of ethanol comparable with that of *Canton-S* but showed abnormal locomotor activities, sedation, tolerance and behavioral sensitization to disinhibited courtship. Our findings suggest that D2 receptor plays multiple roles in alcohol-induced behaviors. We are currently investigating the underlying mechanism. Our research may provide novel insights into the neurobiological mechanisms underlying alcohol use and addiction. This work was supported by the NIH grants NIMHD 2G12MD007592 and NIAAA R15AA020996.

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Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

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Program #/Poster #: 601.11/GGG7

Topic: G.08. Drugs of Abuse and Addiction

Support: University Foundation Research Grant, Univ. of Pennsylvania

Title: Optogenetic modulation of dopamine release in the nucleus accumbens and ethanol self-administration

Authors: *W. M. DOYON, JR[1], S. VILLATORO[2], D. A. CONNOR[1], J. A. DANI[1]
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Abstract: Our previous studies have correlated increased ethanol self-administration, following nicotine or stress exposure, with blunted dopamine signaling in the medial nucleus accumbens (NAc). However, a causal role for dopamine signaling and increased ethanol self-administration has not been confirmed. To examine the functional consequences of dopamine release in the medial NAc, we are using optogenetics in male and female Long-Evans rats that express Cre-recombinase to modulate release at specific target areas. Viral vectors were injected into the midbrain to express channelrhodopsin (ChR2-YFP), halorhodopsin (NpHR3.0-YFP), and YFP alone in a Cre-dependent manner. In ChR2-expressing rats, blue light stimulation in the medial NAc shell (20 Hz/5 sec for 5 min) transiently increased extracellular dopamine levels as measured by in vivo microdialysis (n = 3 rats). By contrast, in NpHR-expressing rats, green light stimulation attenuated dopamine levels (n = 2 rats). To determine the impact of light stimulation on ethanol intake, rats were trained for operant self-administration of 4% ethanol (+0.1 % saccharin). In ChR2-expressing rats, light stimulation during self-administration caused a decrease in daily ethanol/saccharin consumption (n = 4 rats), but did not alter intake of saccharin alone. Ethanol/saccharin intake was unaffected by light stimulation in one YFP-expressing control rat. These preliminary results and ongoing experiments could reveal a causal role of specific dopamine neural projections in ethanol reinforcement and motivated behavior.

Disclosures: W.M. Doyon: None. **S. Villatoro:** None. **D.A. Connor:** None. **J.A. Dani:** None.

Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 601.12/GGG8

Topic: G.08. Drugs of Abuse and Addiction

Support: NIAAA R01AA021505
NIAAA U01AA025932

Title: Excessive ethanol consumption elevates GABAergic inputs onto cholinergic interneuron in the dorsomedial striatum

Authors: *H. GANGAL, J. LU, X. WANG, J. WANG
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Abstract: The dorsomedial striatum (DMS) and the cholinergic system are known to play a critical role in behavior flexibility, the ability to adjust to salient stimuli in the environment. This flexibility is impaired under the influence of excessive ethanol consumption. This study is aimed to investigate the neural mechanism underlying this impairment. Chat-eGFP transgenic mice were used to allow the fluorescent visualization of cholinergic interneurons (CINs) in the DMS. Excessive ethanol consumption was established using the intermittent-access to 20% ethanol two-bottle choice drinking procedure, and whole-cell patch-clamp electrophysiology was used to record in the DMS CINs. We discovered that the average spontaneous firing frequency of the DMS CINs was reduced in the alcohol mice as compared to their water controls. We also found that there was a significant increase in the frequency of spontaneous inhibitory post synaptic currents (sIPSCs) to the DMS CIN. Given our previous finding that activity of dopamine D1 receptor-expressing medium spiny neurons (MSNs) in the DMS were elevated following excessive alcohol intake (Cheng et al., Biological Psychiatry, 2017) and that these neurons are GABAergic, we hypothesize that the DMS CINs receives inhibitory inputs from these D1R-MSNs; these inputs are strengthened by excessive ethanol intake. Using triple transgenic mice, D1Cre;Ai32;Chat-GFP, which allows optical stimulation of D1R-MSNs and recording from DMS CINs at the same time, we discovered that a series of light stimulation of D1R-MSNs with increasing intensities caused increased amplitudes of IPSCs. These IPSCs were blocked by a $GABA_A$ receptor antagonist, suggesting that DMS D1-MSNs form GABAergic connections with striatal CINs. We are currently examining whether the IPSCs are enhanced by excessive alcohol consumption. These results indicate that ethanol-mediated potentiation of D1R-MSN activity may down-regulate DMS CIN activity, which may contribute to impaired flexibility in alcohol use disorder.

Disclosures: H. Gangal: None. **J. Lu:** None. **X. Wang:** None. **J. Wang:** None.

Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA022707
NIH Grant AA024527

Title: Effects of different modes of alcohol administration on hypothalamic synaptic plasticity and HPA axis hormonal and behavioral responses to stress

Authors: *V. N. MARTY, Y. MULPURI, J. MUNIER, S. LELE, R. H. VO, I. YENOKIAN, I. SPIGELMAN
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Abstract: Alcohol use disorder is associated with a persistently dysregulated hypothalamic-pituitary-adrenal (HPA) axis and corticotropin-releasing factor (CRF) signaling that leads to inappropriate stress responses, thereby increasing relapse susceptibility in abstinent alcoholics. Here we investigated the effects of two different modes of ethanol (EtOH) administration inducing neuroadaptive changes responsible for the dysregulation of the HPA axis. Using whole-cell patch-clamp recordings, we showed that stress induces a CRF-dependent depression of NMDAR function in parvocellular neurosecretory cells (PNCs) in the paraventricular nucleus of the hypothalamus (PVN), which allows for short-term potentiation (STP) of AMPAR-mediated currents following high-frequency stimulation (HFS, 100Hz for 1sec, x4). This stress-induced STP can be evoked for several days and provides a mechanism by which the HPA axis responds adaptively to subsequent stressors. Chronic intermittent EtOH (CIE) was administered either by oral gavage (30 doses, 5g/kg of EtOH once every other day for the first five doses, and 6g/kg of EtOH once every day for the following 25 doses), or by EtOH vapor (12h daily for 6 weeks). All experiments were performed after at least 40 days of withdrawal. We found that HFS-induced STP was impaired in PNCs of stressed CIE-gavage and -vapor rats. NMDAR inhibition by intracellular MK-801 restored stress-induced STP suggesting that the loss of CRFR1-mediated NMDAR blockade in CIE rats may prevent stress-induced STP. To relate the expression of STP to the HPA axis hormonal response, we examined ACTH and CORT plasma levels in response to repeated (at 72hr-intervals) restraint stress. In both CIE-gavage and -vapor rats, the ACTH response to the 3rd stress was blunted independent of plasma CORT levels, indicative of enhanced negative feedback. Stress-induced increases in self-grooming behavior, an adaptive response to stress involving CRF-expressing PNCs, were impaired in CIE-gavage, but not in CIE-vapor rats. These data indicate that CIE-induced alteration of stress-induced PNC synaptic plasticity could be responsible for the HPA axis maladaptive hormonal responses to stress, and that the mode of EtOH administration remains a key variable in studying the effects of chronic alcohol on brain function. NIH grants AA022707 & AA024527

Disclosures: V.N. Marty: None. **Y. Mulpuri:** None. **J. Munier:** None. **S. Lele:** None. **R.H. Vo:** None. **I. Yenokian:** None. **I. Spigelman:** None.

Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

Location: SDCC Halls B-H

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant P20 GM113109-01A1

Title: Individual differences in alcohol consumption in relation to instrumental extinction learning and dorsomedial striatal parvalbumin expressing neurons

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Abstract: In humans, there is a relationship between levels of alcohol use and response inhibition abilities. Relationships between alcohol consumption and response inhibition have also been found in rodent models. We have previously found that, in outbred Long-Evans rats given chronic intermittent access to alcohol (CIA) during adolescence and early adulthood, rats that consume high levels of alcohol also exhibit lower conditioned fear, faster instrumental extinction and lower errors in a reversal learning task. These findings suggest a generalized behavioral phenotype, which we have termed the HALF-FIELDER rat (Pajser et al., 2018). However, the neurobiological substrates of the HALF-FIELDER rat are unknown. Here, we determined whether alcohol consumption would correlate with instrumental extinction in a task specifically designed to assess operant extinction. We also determined whether striatal parvalbumin-positive (PV+) neurons would correlate with alcohol consumption and instrumental extinction, as preliminary data from our lab suggested a possible association.
Rats received CIA (n = 21) or water-only (n = 15) access for 6 weeks (PND 26-66). Ten days after completion of the CIA paradigm, rats began behavioral training. Once free-operant lever pressing had been established, the rats received 2 once-daily sessions of cued instrumental training, in which a lever-light compound was presented during 40-sec cues and lever-presses could earn 2 food pellets/trial on an intermittent reinforcement schedule. Then, rats underwent extinction training in which no food was earned. The rats were then euthanized and their brains were processed to stain for PV+ neurons in the dorsomedial striatum (DMS) and dorsolateral striatum (DLS)using immunohistochemistry.
Contrary to prior results, we found that rats with prior alcohol access exhibited faster instrumental extinction than rats only given water. We also found that alcohol consumption and extinction correlated, such that higher drinking rats showed a faster rate of extinction. Preliminary data suggest that PV+ neurons in the DMS, but not DLS, are correlated both with alcohol consumption and the rate of instrumental extinction. These results suggest that rats that consume more alcohol show a faster rate of extinction, and both of these behavioral traits correlate with the number of PV+ neurons in DMS. Our findings support the reliability of associations between behavioral traits in the HALF-FIELDER phenotype, and suggest a possible neuronal substrate for these traits.

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Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

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Topic: G.08. Drugs of Abuse and Addiction

Support: NSERC Grant RGPIN 06615

Title: Amino acid neurotransmitter release in the nucleus accumbens differs between mice exhibiting low and high sensitization to ethanol

Authors: *M. G. NASHED, D. CHATTERJEE, D. NGUYEN, M. DIWAN, J. N. NOBREGA
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Abstract: Ethanol-induced behavioural sensitization (EBS) is a neurobehavioral model of the adaptive neurochemical changes that occur following repeated exposure to the same dose of ethanol (EtOH). Interestingly, EBS does not occur uniformly in all mice exposed to the sensitization paradigm, even among animals from the same strain and cohort. Indeed, low-sensitized (LS) mice can readily be differentiated from high-sensitized (HS) mice, suggesting innate differential responses to EtOH in the reward circuitry of individual animals. Although this phenomenon remains poorly understood, we have recently reported that glutamate is variably regulated in the nucleus accumbens (NAc) of LS and HS mice during the expression phase of EBS. Here, we expand on these findings by examining both excitatory amino acid (EAA) and inhibitory amino acid (IAA) neurotransmitter release in the NAc during the expression phase of EBS. Male DBA mice (N = 32) received 5 EtOH (2.2 g/kg; n = 24) or saline (n = 8) injections twice per week, and 15-minute locomotor activity (LMA) was assessed immediately following injections 1, 3, and 5. Of the 24 EtOH mice, eight were classified as LS and eight were classified as HS on the basis of injection 5 LMA. Two weeks following injection 5, mice were challenged with EtOH (1.8 g/kg), and either their LMA was evaluated (n = 12) or in vivo microdialysis samples were periodically collected via implanted cannulae targeting the NAc core (n = 12). In response to EtOH, LS mice did not exhibit increased LMA, while HS mice exhibited a 110% increase in LMA compared to saline mice. Analysis of the microdialysis samples revealed that EAAs and IAAs were differentially elevated in the NAc of mice predominantly in the first 20 minutes following EtOH challenge. In LS mice, post-EtOH glutamate and aspartate (EAAs) peaked at 140% and 141% of baseline, respectively. The IAAs GABA, glycine, and taurine peaked at 423%, 553%, and 676% of baseline, respectively. In HS mice, post-EtOH glutamate and aspartate peaked at 184% and 170% of baseline, respectively. GABA, glycine, and Taurine peaked at 277%, 168%, and 212% of baseline, respectively. Interestingly, while LS mice exhibited similar levels EAAs compared to saline mice, they exhibited higher levels of IAAs, particularly taurine. By contrast, HS mice exhibited higher levels of EAAs and lower levels of

IAAs compared to both saline and LS mice. These results suggest that differential amino acid neurotransmitter regulation in the NAc may underline the innate neurobehavioral differences observed in LS and HS animals. In particular, the role of glycine receptors in mediating resistance to EBS should be further investigated.

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Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

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Topic: G.08. Drugs of Abuse and Addiction

Support: R00AA021782

Title: Pituitary adenylate cyclase-activating polypeptide in the nucleus accumbens shell reduces ethanol drinking

Authors: *A. T. GARGIULO[1], L. SANZALONE[1], P. S. SHAH[2], J. R. BARSON[1]
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Abstract: Alcohol use disorder is pervasive and multifaceted, but currently available pharmacotherapies are not widely effective. Thus, a better understanding of the neurobiological mechanisms underlying alcohol use disorder may help in identifying novel targets for the development of efficacious treatments. Very limited evidence suggests that one neuropeptide that may play a role in ethanol drinking is pituitary adenylate cyclase-activating polypeptide (PACAP), and recent results from our laboratory suggest that the PACAP protein isoform, PACAP-27, could be involved. Notably, while PACAP-38 is more ubiquitously expressed in the brain and has been associated with a number of stress-related behaviors, PACAP-27 is more selectively expressed and has not been associated with such behaviors. In the present study, we examined the effects of the PACAP isoforms on ethanol drinking, testing both the nucleus accumbens shell (NAcSh) and core (NAcC), which have both been implicated in motivated behavior. Notably, the NAcSh shows some of the strongest binding of PACAP-27 in the brain. Therefore, we trained male, Long-Evans rats to drink 20% ethanol using the intermittent-access two-bottle choice paradigm. Once they established pharmacologically-relevant drinking, we implanted the rats with bilateral cannulae into either NAcSh (n = 10) or NAcC (n = 7) and bilaterally injected them prior to their daily ethanol access with PACAP-27 or PACAP-38 (25 pmol, 50 pmol), compared to saline vehicle (0.3 ul). For the NAcSh, we found that PACAP-27 significantly reduced ethanol drinking without affecting intake of simultaneously-available

chow. In contrast, drinking was not significantly affected by PACAP-38 when injected into the NAcSh or by either PACAP isoform when injected into the NAcC. Ongoing experiments are examining the effects of these isoforms on ethanol drinking in female rats and on sucrose drinking. Thus far, the results demonstrate that PACAP-27, acting in the NAcSh but not NAcC, can significantly inhibit pharmacologically-relevant ethanol drinking. We propose that this peptide should be further investigated for its utility as a novel pharmacological target for the treatment of alcohol use disorder.

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Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

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Program #/Poster #: 601.17/GGG13

Topic: G.08. Drugs of Abuse and Addiction

Support: AA023648
022082

Title: Identifying neural ensembles that mediate EtOH seeking by stimuli conditioned to withdrawal alleviation by EtOH in dependent subjects using pharmacogenetic inactivation in transgenic rats

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Abstract: Alcoholism is a chronically relapsing disorder characterized by compulsive ethanol (EtOH) seeking and use. One major factor implicated in vulnerability to relapse includes learned responses induced by contextual stimuli that have become associated with the subjective actions of EtOH. In alcoholics, the severity of EtOH craving evoked by environmental cues is highly correlated with the degree and history of EtOH dependence. This is hypothesized to result from repeated experiences of EtOH consumption during withdrawal, which modifies the individual's reinforcement history to include the subjective effects of EtOH during this state, thereby enhancing EtOH's reinforcing actions. We have previously shown that environmental stimuli conditioned to EtOH availability during withdrawal produces significant reinstatement and that stimuli conditioned to EtOH availability in the same rats during the nondependent state lose their efficacy to elicit EtOH seeking. We have also shown that stimuli conditioned to EtOH availably during withdrawal, unlike in nondependent rats without this history, elicit compulsive-like EtOH seeking as revealed by resistance to suppression of cue-induced responding despite adverse consequences and rats' willingness to expend greater effort to obtain EtOH. Here, we extend our

previous findings by examining the neural regulation of withdrawal-related conditioning on EtOH seeking and vulnerability to relapse using Daun02 pharmacogenetic inactivation of key sites within the incentive motive circuit in cfos-LacZ transgenic rats. At the present time, the data suggest that inactivation of EtOH stimuli-responsive neural ensembles in the mPFC eliminates EtOH seeking associated with relief from withdrawal in dependent rats, but not EtOH seeking induced by stimuli conditioned to EtOH availability in the nondependent state in the same rats. (Support: NIH-NIAAA AA023648; AA022082).

Disclosures: O.O. Kozanian: None. **F. Weiss:** None.

Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

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Topic: G.08. Drugs of Abuse and Addiction

Support: This work was supported by PHS NIH grants AA020919 and DA035958 to SCS

Title: Interleukin 10 increases dopamine neuron activity in the ventral tegmental area and increases dopamine release in the nucleus accumbens via reduction of GABA inhibition

Authors: *A. J. PAYNE, S. D. WILLIAMS, T. J. CLARKE, J. D. OBRAY, E. EISINGER, N. LEWIS, M. C. WOODBURY, S. C. STEFFENSEN
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Abstract: Dopamine (DA) transmission is a key player in the rewarding aspects of ethanol as well as ethanol dependence. The current dogma is that DA transmission is increased during ethanol exposure via the inhibition of ventral tegmental area (VTA) GABA neurons and that excitation of VTA GABA neurons during withdrawal results in decreased DA transmission. Microglia, the major neuroimmune effector in the brain, may be a key mediator in this process by releasing cytokines following activation. It is also thought that BDNF may mediate this effect. We evaluated the effect of ethanol on cytokine concentrations in the VTA and nucleus accumbens (NAc), and found that low dose ethanol (1.0 g/kg) decreased interleukin (IL)-10 levels, but high dose ethanol (4.0 g/kg) increased IL-10 levels. We also used standard cell-attached mode electrophysiological techniques to evaluate the effects of select cytokines and BDNF on VTA neuron firing rate *in vitro*. We found no change in firing rate in response to IL-6 and BDNF, but an increase in firing rate in VTA DA neurons in response to IL-10. Consistent with the changes in firing rate, optically-evoked IPSCs were also found to be decreased in response to IL-10. *Ex vivo* voltammetry and *in vivo* microdialysis were done to determine whether IL-10 can directly result in an increase in DA release. Although *ex vivo* voltammetry showed no change in DA release, IL-10 increased DA release *in vivo*. These findings suggest

that the rewarding and/or addictive effects of ethanol may be mediated by cytokines, specifically the anti-inflammatory cytokine IL-10.

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Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA020919
NIH Grant DA035958

Title: Acute ethanol increases monocyte infiltration of the cns and influences microglia activation

Authors: *S. C. STEFFENSEN[1], T. J. CLARKE[2], J. D. OBRAY[3], J. BRUNDAGE[1], D. RUTTER[1], S. B. WILLIAMS[1], J. T. YORGASON[1], S. HOPE[1]
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Abstract: Microglia are the primary immune cell in the central nervous system (CNS) and are known as “resident” macrophages, although their role has more recently been shown to extend far beyond immunity. The effects of ethanol on the brain are closely linked to neuroimmune responses mediated by microglia that are present in the healthy brain from the time of development. For example, post-mortem studies of alcoholic brains show increases in microglial markers, and high dose ethanol has been shown to cause the activation of CNS microglia. Normally, the blood-brain barrier (BBB) prevents the infiltration of cells and foreign pathogens from crossing from the periphery into the CNS. However, peripheral monocytes are known to infiltrate the CNS in response to seizures, traumatic brain injury, infection, and multiple sclerosis. Whether or not these cells engraft and become microglia is still a topic of debate. The aim of this study was to determine the effect of acute ethanol on microglia activation and monocyte infiltration into the CNS. Using the MaFIA mouse model (GFP+ on Csf1r promoter), fluorescent microscopy, and flow cytometry, we assessed the presence and phenotype of microglia and infiltrating monocytes following 1, 2, and 4 g/kg ethanol at 0.5, 1, and 2 hours post injection. We found that acute ethanol significantly increased ventral tegmental area and nucleus accumbens microglia volume/surface area by up to 30%, suggesting activation. We also found that GFP+ MaFIA macrophages injected into C57BL6/J mice will cross the BBB in response to acute doses of ethanol. These findings suggest a neuroimmune interaction with acute, low doses of ethanol, and challenge the dogma that ethanol has exclusively central effects, and

neuroimmune effects only at high doses. Further research is being performed to examine the implications of this effect, and what effects a conditional knockdown of monocytes in MaFia mice has on ethanol intoxication and reward.

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Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIAAA AA025481

Title: Effects of chronic ethanol and stress on spontaneous and sensory-evoked responses of locus coeruleus noradrenergic neurons

Authors: *C. R. DEN HARTOG[1], D. E. MOORMAN[2], E. M. VAZEY[3]
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Abstract: The locus coeruleus NE (LC-NE) system regulates various brain processes including cognitive control and stress responses through ascending projections to regions including mPFC and BLA. Dysregulation of LC-NE transmission by chronic ethanol is thought to play a critical component in the addiction cycle by driving reward-seeking behavior and withdrawal-induced stress responses. Exposure to repeated chronic stress also can alter CRF-mediated LC tone and dysregulate LC-NE function. LC noradrenergic function is critical for regulating alcohol consumption and stress responses, yet the effects of chronic ethanol and stress interactions on LC-NE circuits remain to be elucidated.In this study, we looked at the effects of chronic intermittent ethanol (CIE) and repeated forced swim stress (FSS) exposure on spontaneous and footshock-evoked (FS-evoked) sensory responses of LC neurons before and after acute ethanol. We recorded LC neurons from both male and female mice 3-7 days following the completion of 4-5 cycles of intermittent CIE vapor and stress exposure across four exposure groups - Air/No stress (NS), Air/FSS, CIE/NS, and CIE/FSS. As we have seen previously, after repeated CIE and/or FSS animals robustly escalated volitional drinking. LC neurons were identified by their firing rate and waveform characteristics. Baseline LC activity was collected before and after FS-evoked sensory responses to increasing stimulation intensities (1, 3, and 10 mA). Mice were then injected with a dose of ethanol equal to their last weekly average intake during 1hr access to 15% ethanol before repeating baseline LC activity and sensory-evoked activity collection. A main

effect of vapor and stress exposure was observed on sensory evoked LC activity in which CIE mice had enhanced FS-response magnitudes compared to Air/NS controls. Acute ethanol blunted sensory evoked magnitudes across all stimulus intensities and normalized CIE exposed response magnitudes to levels seen in air controls. We also saw changes in evoked response latencies and durations between air and CIE or stress exposed animals. These data demonstrate that chronic ethanol and stress can lead to persistent changes in LC function that are sensitive to acute ethanol. These findings provide important insight into the mechanism by which ethanol and stress can alter LC circuitry.

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Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

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Topic: G.08. Drugs of Abuse and Addiction

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Title: Voluntary binge drinking disrupts myelin-associated proteins in the medial prefrontal cortex and hippocampus of adolescent rats

Authors: *S. AKLI[1], E. R. TAVARES[1], A. SILVA-GOTAY[1], R. WYROFKSKY[2], W. M. VARGAS RIAD[3], E. VAN BOCKSTAELE[2], H. N. RICHARDSON[1]
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Abstract: Myelination of axons during adolescence is thought to lead to efficient neural communication between prefrontal and subcortical brain regions, thus improving cognitive abilities and emotional regulation in adulthood. We have previously found in male rats that adolescent binge drinking decreased myelin fiber density in the medial prefrontal cortex (mPFC). High alcohol intake also predicted poor performance on a working memory task in adulthood. The goal of the current study was to determine the effect of alcohol on proteins important for the structure and maintenance of myelin sheaths. Adolescent male and female Wistar rats underwent two weeks of operant self-administration of alcohol (postnatal days 28-42). Brains were then processed for Western blot analysis of myelin-related protein levels or for immunofluorescent labeling and confocal analysis of proteins in specialized axonal domains at the node of Ranvier. Our findings suggest that myelin basic protein is reduced by alcohol in regions undergoing plasticity during adolescence (mPFC and hippocampus), but not in the striatum. This effect was

greater in males compared to females. Alcohol also impacted the nodal domains of myelinated axons in the mPFC in both males and females. These molecular and microstructural changes in myelinated axons could have lasting effects on neural transmission, which may explain some of the cognitive and emotional deficits linked to binge drinking.

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Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R01 AA-023410
R21 AA-024036

Title: Ethanol and migration of immature gabaergic interneurons: From chloride to calcium

Authors: *S. M. LEE, P. W. L. YEH, H. H. YEH
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Abstract: Prenatal exposure to ethanol disrupts the normal pattern of tangential migration of cortex-bound primordial GABAergic interneurons, and this has been postulated to contribute to the etiology of fetal alcohol spectrum disorder (FASD). There is evidence that ethanol interacts with $GABA_A$ receptors that are tonically activated by ambient GABA to exert this effect but, beyond this, the cellular and subcellular mechanisms are largely unexplored. Here we initiated a project to establish the experimental premise for investigating the mechanisms linking ethanol, $GABA_A$ receptor activation and aberrant migration.
First, we asked whether ethanol exposure enhances $GABA_A$ receptor-induced depolarization. Perforated patch clamp recordings were performed on tdTomato-positive GABAergic interneurons from acute E14.5 Nkx2.1Cre/tdTomato slices to measure $GABA_A$ receptor reversal potential (E_{GABA}) before and during 6.5mM ethanol (30mg/dl or 0.03% equivalent) exposure. Ethanol exposure shifted E_{GABA} to being more depolarized compared to control. Ethanol also potentiated the amplitude of current responses to 50μM GABA. Pre-treatment of slices to 20μM bumetanide blocked the degree of ethanol-enhanced GABA depolarization.
Second, we asked whether the ethanol-enhanced GABA chloride flux is linked to a rise in intracellular calcium via voltage-gated calcium channels, since calcium is implicated in regulating neuronal migration and cytoskeletal dynamics. To this end, organotypic slice cultures were prepared from E14.5 Nkx2.1Cre/tdTomato embryos and assigned to four groups: Control, 20μM Nifedipine, 20μM Nifedipine+6.5mM Ethanol and 6.5mM Ethanol. The slice cultures

were maintained for 27 hours, fixed and tdTomato-fluorescent cells were counted to provide an index for the extent of tangential migration. The addition of the calcium channel blocker nifedipine prevented the ethanol-induced aberrant tangential migration. Ongoing experiments are employing the ratiometric calcium indicator Fura-2 to monitor ethanol-induced changes in intracellular calcium and correlate this with changes in growth cone dynamics.
The present study sets the stage for filling mechanistic gaps that link chloride homeostasis to calcium dynamics in regulating the migration of immature GABAergic cortical interneurons.

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Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 601.23/GGG19

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA020022

Title: Adolescent intermittent binge ethanol alters the expression of GABAergic interneurons in the prelimbic cortex of adult rat brain

Authors: *W. LIU, F. T. CREWS
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Abstract: The maturation of adolescent brain is a vulnerable to repeated exposure to alcohol, particularly for prefrontal cortex (PFC) that play critical roles in inhibitory control and executive functions. Studies have shown that alcohol has significant effects on the structure and function of the PFC, but it is not clear about the role of GABAergic interneurons in the PFC. The current study examined the effect of adolescent intermittent binge ethanol on GABAergic interneurons in the adult PFC. Male Wistar rats were bred and reared in our vivarium, on postnatal day 1 (P1) litters were culled to 10 pups. At weaning on P21, male offspring were weight matched, pair-housed and assigned into control water or adolescent intermittent ethanol (AIE, 5g/kg/day, i.g., P25-P55; 2 days alcohol, 2 days off) groups. Animals were sacrificed at P80 in young adulthood. The impacts of adolescent intermittent binge ethanol (AIE) on the interneurons was determined using immunohistochemistry in the PFC (including the prelimbic, PrL and infralimbic cortex, IL). The interneuron markers, parvalbumin (PV), somatostain (SST), 5-hydroxytryptamine 3a receptor (5HT3aR), vasoactive intestinal peptide (VIP), calretinin (CR), Calbindin (CB), neuropeptide cholecystokinin (CCK), neuropeptide Y (NPY), reelin (Rln), Nitric oxide synthase (NOS), and Glutamate decarboxylase 67 (GAD67) have been used. Results showed that the PrL have a greater number than the IL in controls compared with AIE group. AIE exposure significantly affected PrL, reducing SST+ (28%, $p<0.05$), PV+ (21%, $p=0.05$), CCK+ (49%

$p<0.001$), Rln+ (37%, $p<0.01$) and NOS+IR expression (35%, $p<0.05$), but not 5HT3aR+, VIP+, CB+ and CR+IR expression at P80. Interestingly, AIE exposure increased NPY+ (51%, $p<0.05$), GAD67+, (69%, $p<0.05$) and pCaMKII+IR expression (293%, $p<0.001$). There is a correlation between the increase of NPY+ and GAD67+IR expression in AIE group. Only the decrease in Rln+IR (42%, $p<0.01$) and the increase in pCAMKII+IR expression (461%, $p<0.01$) by AIE exposure were found in IL. These findings suggest that adolescent intermittent binge ethanol exposure alters the neuronal phenotypes of the adult PFC, particularly the PrL (Funded by the NADIA from NIAAA).

Disclosures: W. Liu: None. **F.T. Crews:** None.

Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 601.24/GGG20

Topic: G.08. Drugs of Abuse and Addiction

Support: PHS NIH R01 AA-023410
PHS NIH R21 AA-024036
PHS NIH F30 AA025534

Title: Altered pyramidal neuron function persists in the somatosensory cortex following prenatal ethanol exposure

Authors: *L. C. DELATOUR, H. H. YEH
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Abstract: Exposure to ethanol during gestation can lead to a broad range of brain and behavioral abnormalities which constitute Fetal Alcohol Spectrum Disorder (FASD), a leading cause of preventable intellectual disability. We previously reported that binge-type prenatal ethanol exposure disrupts the radial migration of primordial pyramidal neurons during embryonic corticogenesis. This exposure also leads to aberrances in pyramidal neuron form and function. Specifically, there is an imbalance of GABA and glutamate-mediated neurotransmission, favoring glutamate. This was found in the somatosensory cortex during the early postnatal period, which is a critical time in synapse development. However, the underlying mechanisms for these changes and whether neurotransmission remains altered later in development, are unknown. We therefore asked whether prenatal ethanol exposure affects the excitatory/inhibitory balance, apical dendritic spines, synaptic efficacy, and synaptic strength in somatosensory cortex pyramidal neurons in early adolescent mice (postnatal day 28-32).
We employed a binge-drinking paradigm in which pregnant mice were exposed to ethanol (5% in liquid food) from embryonic day (E) 13.5 through E16.5, spanning the height of cortical

neurogenesis and migration. Using whole cell patch clamp electrophysiology, pharmacologically isolated spontaneous inhibitory and excitatory postsynaptic currents were recorded in layer V/VI somatosensory cortex pyramidal neurons in male and female mice. Our results indicate a shift in the excitatory/inhibitory balance, favoring excitation, in ethanol-exposed mice. Ongoing studies are also examining changes in action potential-independent miniature inhibitory and excitatory postsynaptic currents.
To investigate synaptic strength and efficacy, optogenetically-evoked excitatory and inhibitory synaptic currents are recorded in transgenic mice which express channelrhodopsin in either pyramidal neurons or GABAergic interneurons, respectively. Thus far, our studies reveal a change in the paired-pulse ratio of excitatory postsynaptic currents in ethanol-exposed mice compared to controls, indicative of diminished presynaptic release probability and a weaker synapse. Given this change in excitatory synaptic strength, ongoing experiments are investigating the AMPA/NMDA ratio as well as spine density and morphology.
Our findings to date indicate that prenatal ethanol exposure has persistent effects on neurotransmission and alters synaptic strength. These data strongly implicate the somatosensory cortex as an important area of study to understand the sensory deficits that hallmark FASD.

Disclosures: L.C. Delatour: None. **H.H. Yeh:** None.

Poster

601. Drugs of Abuse and Addiction: Alcohol: Neural Mechanisms II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 601.25/GGG21

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R01AA024774 (HNR)
NIH-NIGMS 5R25GM099649-03 (AS)

Title: Adolescent exposure to cuprizone leads to demyelination and increased microglia cell number in the corpus callosum of male rats

Authors: *E. TAVARES[1], A. SILVA-GOTAY[2], A. LIN[1], G. MOLICA[1], S. HURWITZ[3], H. N. RICHARDSON[3]
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Abstract: Adolescence is an active period of brain plasticity where axonal pathways in the frontal lobes are undergoing myelination. We have found that exposure to toxic substances such as alcohol during early adolescence can lead to reduced myelin in the prefrontal cortex in male rats. To gain a better understanding of the mechanisms by which myelin can be altered during this developmental period, we tested how these axons are impacted by exposure to a

demyelinating agent. From postnatal day 28-42, male adolescent rats were fed a diet containing cuprizone, a copper chelator, or a control diet (n=6/group). Brains were then processed for analysis of demyelination in white and grey matter regions of the frontal lobes. Cuprizone exposure elicited a substantial decrease in myelin in frontal white matter fiber tracts such as the anterior branches of the corpus callosum (visualized by Luxol Fast Blue). We then used immunofluorescence and confocal microscopy to test for evidence of neuroinflammation. Microglia, the resident immune cells of the brain, activate in response to toxic substances and signal the release of pro-inflammatory cytokines. Microglia were visualized using an antibody against ionized calcium binding adaptor molecule 1 (iba1). There was almost a four-fold increase in the number of microglia within the corpus callosum of cuprizone-treated animals compared to controls. Although microglia cell number was also increased in the cortex, this change was modest compared to white matter structures. We are currently exploring whether the degree of myelin loss and microglial infiltration relates to expression of estrogen receptor alpha (ERα). Estradiol has been shown to decrease expression of pro-inflammatory factors and protect against cuprizone-induced demyelination. In addition, we have recently found ERα is expressed in microglia in the prefrontal cortex; thus, estradiol could act directly on microglia to mediate sensitivity to cuprizone.

Disclosures: E. Tavares: None. **A. Silva-Gotay:** None. **A. Lin:** None. **G. Molica:** None. **S. Hurwitz:** None. **H.N. Richardson:** None.

Poster

602. Cannabinoids: Neural Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 602.01/GGG22

Topic: G.08. Drugs of Abuse and Addiction

Support: DGAPA-UNAM to AERC Grant IN217918
DGAPA-UNAM to OPG Grant IN215218
DGAPA-UNAM to MMD Grant IA205218

Title: Cnr1 polymorphism, adverse childhood experience and their interaction on cannabis use and resilience abilities

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Abstract: Adolescents who had experienced childhood trauma are almost five times more likely to use cannabis than those who had not and can adversely affect resilience abilities (i.e., the ability to cope with stressful or disturbing events). A significant prevalence of childhood trauma and lower resilience levels have been observed in cannabis users, compared to healthy controls. Previous studies indicate that *CNR1*, the gene that codes for the cannabinoid receptor 1, is associated with drug dependence, but it is unknown if this gene is involved in expressing resilience abilities. The aim of the present study was to evaluate if the genotypes of the rs2180619 of CNR1 gene, childhood trauma and their interaction would influence cannabis consumption vulnerability and resilience abilities among Mexican young adults. Cannabis users and healthy controls were interviewed and were asked to answer a computerized Childhood Trauma Questionnaire (CTQ) and a Resilience Scale; finally, participants provided a saliva sample for genotyping. The exposure to childhood trauma was associated with cannabis consumption; there were not differences associated with the rs2180619 (AA, AG, GG). However, a subsequent analysis only with cannabis users showed that GG who presented higher frequency of episodes of cannabis consumption also presented higher cannabis abuse and lower resilience scores. Our results suggest that the rs2180619 of CNR1 gene as well as childhood trauma play a role in resilience and cannabis consumption. The exposure to at least one type of trauma during childhood confers a differential vulnerability for cannabis consumption and reduce the ability to cope with stressful or disturbing events, depending on the rs2180619 genotype of the CNR1 gene.

Disclosures: E.I. Ortega mora: None. **U. Caballero-Sánchez:** None. **T.V. Román-López:** None. **J.A. Gonzalez-Barrios:** None. **M. Méndez-Díaz:** None. **O. Prospéro-García:** None. **A.E. Ruiz-Contreras:** None.

Poster

602. Cannabinoids: Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 602.02/GGG23

Topic: G.08. Drugs of Abuse and Addiction

Support: Bert Moore Endowed Chair in BrainHealth at the University of Texas at Dallas

Title: Testing the role of the posterior cingulate cortex in processing salient stimuli in cannabis users: An rTMS study

Authors: *S. PRASHAD, E. S. DEDRICK, W. TO, S. VANNESTE, F. FILBEY
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Abstract: The posterior cingulate cortex (PCC) and precuneus are hubs in the default mode network and play a role in processing external salient stimuli. Accordingly, activation in these

regions has been associated with response to salient stimuli using drug cue-reactivity paradigms in substance using populations. These studies suggest that the PCC and precuneus may underlie deficits in processing salient stimuli that contribute towards the development of substance use disorders. The goal of this study was to directly test this hypothesis using repetitive transcranial magnetic stimulation (rTMS). Using a double-blind, placebo-controlled design, we used rTMS to target the PCC and precuneus with a double-cone coil at 10 Hz (high frequency; HF) and 1 Hz (low frequency; LF) in 10 adult cannabis users and 10 age- and sex-matched non-using controls. EEG data were collected before and after rTMS during a modified oddball paradigm with neutral, oddball, self-relevant, and cannabis-related stimuli. We hypothesized greater ERP response (P2, N2, and P3 components) to self-relevant stimuli in both groups as well as greater response to cannabis-related stimuli in users only during baseline compared to after HF rTMS. Cannabis users exhibited increased amplitude in P3 (p = 0.04) and faster latencies in the P3 (p = 0.02), N2 (p = 0.02), and P2 (p = 0.04) components in response to self-relevant stimuli compared to controls during baseline that normalized after rTMS. Cannabis users also exhibited greater N2 amplitude (p = 0.02) after LF rTMS and faster N2 (p = 0.04) and P2 (p = 0.003) latencies during baseline to cannabis-related stimuli. These results suggest that cannabis users exhibited heightened salience to external stimuli that were modulated after rTMS. PCC dysfunction in cannabis users may be related to abnormalities in processing salient stimuli, such those during cue-reactivity, and provides a potential target for cannabis use disorder intervention.

Disclosures: E.S. Dedrick: None. **W. To:** None. **S. Vanneste:** None. **F. Filbey:** None.

Poster

602. Cannabinoids: Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 602.03/GGG24

Topic: G.08. Drugs of Abuse and Addiction

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FEDER, POR Lisboa 2020 and Fundação para a Ciência e a Tecnologia - LISBOA-01-0145-FEDER-007391

Title: (Un)like two peas in a pod? Unexpected results from a preliminary study on the persistent depressive-like phenotype induced by chronic exposure to HU-210 during adolescence in female rats

Authors: *M. FERREIRA[1,2], F. M. MOURO[1,2], A. M. SEBASTIÃO[1,2]
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Abstract: Cannabis is the most widely consumed illegal drug in the world, especially amongst adolescents. Because adolescence is a period of heightened vulnerability to the impact of external influences on brain development, the persistent consequences of chronic cannabinoid use during this period are of high relevance. Animal studies have shown that chronic adolescent exposure to cannabinoid receptor (CBR) agonists induces impairments in both the cognitive (e.g. recognition memory) and affective (e.g. depressive-like phenotypes) domains, that persist into adulthood, even after drug exposure has ceased, suggesting a long lasting impact. Moreover, the affective deficits have been predominantly reported in female animals, highlighting a possible sex-specific vulnerability to effects of adolescent CBR exposure.
This work reports the results of an attempt to replicate the findings relating to the affective impact of chronic cannabinoid exposure, using a CBR agonist (HU-210) yet untested for this purpose. To that end, adolescent female Wistar rats were administered daily intraperitoneal injections for 15 days, in an escalating dosing schedule, of either HU-210 (N=10; PND35-39: 25μg/kg; PND42-46: 50μg/kg; PND49-53: 100μg/kg) or vehicle (N=10). Behavioral testing occurred after a 26-day washout (PND80) and consisted of the Elevated Plus Maze (EPM), Open Field (OFT), Social Interaction (SIT), Forced Swimming (FST), Sucrose Preference (SPT) and Marble Burying (MBT) tests.
Results showed that HU-210 decreased weight gain during the administration period, but this effect did not persist into adulthood. There were no differences between groups in either EPM, OFT or MBT performance, suggesting no changes in anxiety. Similarly, social anxiety, as indexed by the SIT, was not altered by HU-210. In the FST, HU-210 exposed animals showed diminished climbing time, but no differences in either swimming or immobility times – suggesting some alterations at the level of stress coping. During the SPT, HU-210 treated animals consumed less food than controls, but no differences were found for either sucrose preference or consumption – indicating absence of impact on the reward system.
In summary, we could observe signs of impaired stress-coping but no marked signs of a depressive-like state. While the discrepancies with previous reports might result from differences in the experimental protocol, it is also possible that HU-210 might be qualitatively different from other CBR agonists. If so, our data may raise questions as to the comparability of studies using different CBR agonists, and as to why this compound differs from others in its effects.

Disclosures: M. Ferreira: None. **F.M. Mouro:** None. **A.M. Sebastião:** None.

Poster

602. Cannabinoids: Neural Mechanisms

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Program #/Poster #: 602.04/GGG25

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant 5R21DA032821-02

Title: Effects of parasympathetic activation on large-scale brain networks in adolescents with cannabis use disorder in withdrawal

Authors: *D. G. GHAHREMANI[1], L. KESSLER[2], N. AZZIZI[2], D. SARRAF[3], A. C. DEAN[2], E. D. LONDON[1]
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Abstract: Heavy adolescent cannabis use is often associated with withdrawal symptoms during periods of cessation. These symptoms arise from activation of neural mechanisms of stress during this allostatic state (Koob & Le Moal, 2001) and are linked with resumption of drug use. We sought to determine whether parasympathetic activation during this period would relieve withdrawal symptoms and alter connectivity of large-scale resting state brain networks. Adolescents (age 17-19, Mean=18) with severe cannabis-use disorder (CUD, N=18, 5 female) completed fMRI scans on two separate days after 48-hours of abstinence from cannabis use. Withdrawal symptoms were measured via self-report questionnaires; parasympathetic activation was induced through controlled breathing techniques and was measured via heart rate variability (HRV). On each of the two testing days, withdrawal symptoms and HRV were measured before and after either undergoing controlled breathing or a guided relaxation exercise (control), directly prior to fMRI scanning. Participants showed changes in HRV after controlled breathing and reduced withdrawal symptoms. Analysis of RSFC showed decreased thalamocortical connectivity after controlled breathing vs. guided relaxation. These preliminary results suggest that strategies for parasympathetic activation during withdrawal states reduce withdrawal symptoms and connectivity of large-scale networks. They hold promise for helping to maintain abstinence during initial periods of cessation from drug use when allostatic load and potential for relapse is high.

Disclosures: D.G. Ghahremani: None. **L. Kessler:** None. **N. Azzizi:** None. **D. Sarraf:** None. **A.C. Dean:** None. **E.D. London:** None.

Poster

602. Cannabinoids: Neural Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 602.05/GGG26

Topic: G.08. Drugs of Abuse and Addiction

Title: Impaired segregation between cognitive and emotional processes in cannabis dependence

Authors: *P. MANZA[1], E. SHOKRI-KOJORI[2], N. D. VOLKOW[3]
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Abstract: Addiction is characterized by an erosion of cognitive control towards drug taking that is accentuated by negative emotional states. Here we tested the hypothesis that the enhanced interference on cognitive control reflects a loss of segregation between cognition and emotion in addiction. We analyzed Human Connectome Project data from 1204 adults aged 22-35, of whom 89 had cannabis dependence (CD). Two composite factors, one for cognition and one for emotion, that accounted for close to 50% of the variance on a large battery of neuropsychological tests, were derived using principal component (PC) analyses. Component scores for these two PCs in the CD group were significantly correlated, such that negative emotionality was associated with poor cognition. However, the corresponding component scores were uncorrelated in matched controls and in non-dependent recreational cannabis users. In CD, but not in controls or recreational users, fMRI brain activations to emotional stimuli (angry/fearful faces > shapes) correlated with the activations to cognitive demand (working memory; 2-back > 0-back). Canonical correlation analyses linked the individual differences in the cognitive and emotional component scores with the brain activations. In CD there was a substantial overlap between cognitive and emotional brain-behavior associations, but in the controls the associations were more restricted to the cognitive domain. These findings support our hypothesis of an impaired segregation between cognitive and emotional processes in CD that might contribute to impaired cognitive control under conditions of increased emotional demand. Interventions aimed at buffering negative emotionality and/or strengthening cognitive control might help restitute the loss of segregation between emotional and cognitive networks in addiction.

Disclosures: P. Manza: None. **E. Shokri-Kojori:** None. **N.D. Volkow:** None.

Poster

602. Cannabinoids: Neural Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 602.06/GGG27

Topic: G.08. Drugs of Abuse and Addiction

Title: White matter alterations in heavy cannabis users

Authors: *G. P. MONTEVERDE[1,2], A. ANGULO[2], L. NAVA[2], S. ALCAUTER[2]
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Abstract: Contradictory evidence regarding which brain areas may be compromised with high levels of marijuana consumption has been reported in previous studies. We have explored tissue integrity of the main white matter (WM) tracts using the MRI Diffusion Tensor Imaging (DTI) Technique, and evaluated cognitive function using neuropsychological tests to study the effects of chronic exposure to exogenous cannabinoids by comparing brain structure of regular users to a healthy non-consuming control group. Participants comprised 31 heavy marijuana users (main

illegal substance for recreational use, at least 16 joints per month in the previous year) and 32 controls. High resolution (voxel size of 1x1x1 mm^3) T1 weighted images were acquired in addition to 64 diffusion weighted images in a 3T MR scanner. Images were anonymized and processed using FSL´s Diffusion Toolbox; the main WM fiber segments for analysis were selected using John Hopkins University Atlas, also included in FSL.
Whole-brain WM integrity was analyzed using non parametric T tests and permutations, and it showed no significant difference between groups, defined as $p<0.05$ after correction for multiple comparisons across regions using the Threshold-Free Cluster Enhancement Tools developed for Tract Based Spatial Statistics (TBSS) by FSL. However, when analyzing specific tracts individually, widespread structural differences were identified in prefrontal and thalamic fibers. In addition, significant interactions between age and performance on psychometric tests were noted across predominantly frontal tracts for WM integrity indices.
Our results extend those findings on the effects of regular marijuana use on the brain's WM, supporting the idea that exogenous cannabinoids induce microstructural differences on several tracts by altering normal neural development and maintenance processes. Also we provide evidence that WM structural integrity measures relate to cognitive performance when assessed by different neuropsychological tests.

Disclosures: G.P. Monteverde: None. **A. Angulo:** None. **L. Nava:** None. **S. Alcauter:** None.

Poster

602. Cannabinoids: Neural Mechanisms

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Program #/Poster #: 602.07/HHH1

Topic: G.08. Drugs of Abuse and Addiction

Support: CONICYT-PCHA Fellowship Doctorado Nacional 2015-21150450
FONDECYT N° 1141088
Proyecto de Internacionalización PUC1566

Title: Adolescent cannabinoid exposure increases nigrostriatal dopaminergic transmission

Authors: *E. PÉREZ[1], A. A. GRACE[3], M. E. ANDRÉS[2], J. A. FUENTEALBA[1]
[1]Ctr. interdisciplinario de Neurociencia, [2]Pontificia Univ. Católica De Chile, Santiago, Chile; [3]Univ. of Pittsburgh Dept. of Neurosci., Pittsburgh, PA

Abstract: Adolescence is a period characterized by gradual behavioral and physiological transition from childhood to adulthood. During adolescence, critical neuronal circuits changes to respond to physiological changes and to adapt to environmental stimuli (Sturman and Moghaddam 2011). In particular, nigrostriatal dopaminergic (DA) pathways are in constant change during the development of animals (McCutcheon and Marinelli 2009). In early

adolescence, the DA activity is lower compared to adulthood, but during the middle and late adolescence, the DA activity is higher than in adults (McCutcheon and Marinelli 2009; Naneix et al 2012). The dynamic changes observed in DA circuits suggest that adolescence is a period of high vulnerability to the long-term effects associated to drugs of abuse (Schneider 2008). The most common illegal drug of abuse used in Chile by teenagers is cannabis (Servicio Nacional para la Prevención y Rehabilitación del Consumo de Drogas y Alcohol, http://www.senda.gov.cl/observatorio/estadisticas). Evidence suggest a relationship between an early use of cannabinoids and psychiatric disorders with abnormal DA system, such as schizophrenia, depression and addiction (Renard, Rushlow, and Laviolette 2016). However, it remains unclear the impact of adolescent exposure to cannabinoids on nigrostriatal DA pathways in adulthood. We hypothesize that repeated treatment with the CB1/2 agonist, WIN55212-2, during adolescence produces a long-lasting increase in the DA activity of nigrostriatal pathways mediated by changes of neurotransmitter in DA somatodendritic region. Male rats were treated with 1.2 mg/kg WIN 55212-2 daily during the adolescence period (postnatal day 40 - 65, 25 injections), then DA electrophysiological activity in Substantia Nigra (SN), microdialysis of GABA and glutamate in SN, and microdialysis No-Net flux of DA in Dorsolateral Striatum (DLS) were carried out during adulthood (Postnatal day 72 - 78). The results show an increase in extracellular levels and releases of DA accompanied by an increases of population activity of DA neurons and a decrease of GABA extracellular levels without changes in the firing burst pattern nor extracellular levels of glutamate. These results suggest that adolescent treatment with WIN55212-2 produce a long-lasting increase of DA transmission by changes on GABAergic input, that modulate the population activity, without modify glutamatergic input, that modulate firing burst pattern (Floresco et al. 2003; Gomes, Rincón-Cortés, and Grace 2016; Steiner and Tseng 2017).

Disclosures: E. Pérez: None. **A.A. Grace:** None. **M.E. Andrés:** None. **J.A. Fuentealba:** None.

Poster

602. Cannabinoids: Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 602.08/HHH2

Topic: F.10. Food Intake and Energy Balance

Support: NIH DK092651
WSU ADARP-Dmac
WSU ADARP-Predoctoral

Title: Effects of exogenous cannabinoids on vagal afferent signaling

Authors: *C. W. KOWALSKI[1], F. J. SHAFFER[2], J. E. M. LINDBERG[2], B. PETERSON[2], J. H. PETERS[2]
[2]IPN, [1]Washington State Univ., Pullman, WA

Abstract: Following legalization of cannabis in some U.S. states, its use among adults over 50 years of age has increased more than twice as fast as any other age group. This population frequently suffers chronic diseases including type-II diabetes, metabolic syndrome, and cardiovascular disease; conditions influenced by both vagal afferent signaling and cannabis. Although the majority of cannabis research has focused on cannabinoid receptor 1 and 2 signaling due to its ubiquity, TRP channels have recently emerged as an excitatory target of cannabinoids. Since transient receptor potential (TRP) channels are abundantly expressed in vagal afferents where they robustly influence glutamate release, they constitute a novel and direct excitatory mechanism for cannabis to influence autonomic function. We have investigated the direct effects of the primary cannabinoids Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) acutely and after chronic cannabis vapor exposure in cultured dissociated nodose ganglia and brainstem slices containing the NTS and vagal afferent terminals. With calcium imaging, we found that CBD and THC increased intracellular calcium primarily in cultured nodose neurons expressing TRPA1. This effect was blocked by removal of extracellular calcium, the nonspecific TRP channel blocker ruthenium red, the TRPA1 antagonist A967079, and genetic knockout of TRPA1. Using patch-clamp electrophysiology, we recorded CBD provoked currents in dissociated nodose ganglia consistent with a TRP-channel mediated conductance, in afferents expressing TRPA1. Both CBD and THC inhibited potassium conductance, while CBD also inhibited voltage-activated sodium channels selectively in A-fibers but not C-fibers. Using patch-clamp recordings in brainstem slices, we found that CBD approximately doubled spontaneous release frequency from NTS neurons containing TRPA1; genetic deletion of TRPA1 prevented this effect, with CBD instead slightly reducing spontaneous release frequency. The acute and chronic autonomic effects of cannabis are biphasic and generally opposed, but an explanation for this change is lacking. We investigated the effects of chronic cannabis vapor exposure on CBD and THC provoked calcium and current responses in dissociated nodose ganglia, on glutamate release in brainstem slices, and on expression of TRPA1, TRPV1, and CB1 in nodose ganglia and the NTS. Our findings demonstrate that CBD and THC effects on vagal neurotransmission are pleiotropic yet generally excitatory, putatively contributing to the autonomic effects of cannabis.

Disclosures: C.W. Kowalski: A. Employment/Salary (full or part-time):; Washington State University. **F.J. Shaffer:** A. Employment/Salary (full or part-time):; Washington State University. **J.E.M. Lindberg:** A. Employment/Salary (full or part-time):; Washington State University. **B. Peterson:** A. Employment/Salary (full or part-time):; Washington State University. **J.H. Peters:** A. Employment/Salary (full or part-time):; Washington State University.

Poster

602. Cannabinoids: Neural Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 602.09/HHH3

Topic: G.08. Drugs of Abuse and Addiction

Title: Differential adaptive properties of mesolimbic and mesocortical dopamine transmission to taste stimuli after repeated exposure to the synthetic cannabinoid JWH-018

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Abstract: Since 2004, herbal mixtures broadly known as Spice/K2, containing synthetic cannabinoids (SC) such as JWH-018, have been marketed as a legal marijuana surrogate. Previous studies of our group showed that JWH-018 has CB1-receptor dependent reinforcing properties and increases dopamine (DA) transmission in the shell of the nucleus accumbens (NAc). Other studied showed that taste stimuli increase extracellular DA in the NAc and in the medial prefrontal cortex (mPFC) of rats. This effect shows single-trial habituation in NAc shell but not in core or in mPFC. However, mPFC 6-OHDA lesions, abolishes habituation of DA responsiveness to taste stimuli in NAc shell but induces it in mPFC. Such findings support the hypothesis of an inhibitory influence of mPFC on NAc DA, and its putative role in the loss of control of the motivational value of stimuli and in impulsivity. In order to test if the repeated administration of JWH-018 is able to modulate the activity of DA terminal areas and is associated to changes in the responsiveness to taste stimuli, rats were administered once a day for 14 consecutive days with JWH-018 (0.25 mg/kg i.p.) or with vehicle. After a week of washout, the DA extracellular levels were measured by *in vivo* brain microdialysis in the NAc shell and core and mPFC of rats either naive or pre-exposed to chocolate (1ml/5min i.o.); behavioral taste reactions were also recorded. JWH-018 administration inhibits the increase of DA in the NAc shell of animals naive to chocolate, abolished habituation of DA responsiveness to repeated chocolate exposure in the same area while induced it in the mPFC. In the NAc core, the treatment with JWH-018 potentiated, delayed and prolonged the stimulatory DA response to taste stimuli of animals pre-exposed to chocolate. No differences in behavioral taste reactivity were observed. Parallel studies of *in vivo* electrophysiology showed that JWH-018 treatment reduces the number of spontaneously active DA neurons of the ventral tegmental area (VTA) and increases their bursting activity. Further studies on neurodegeneration (TH in the VTA; DAT in the mPFC/NAc) produced by repeated JWH-018 administrations are in progress. These data show that JWH-018 is able to change the activity of DA neurons and to induce differential adaptive changes of the responsiveness of DA transmission to taste stimuli in DA terminal areas, similarly to previous results obtained in mPFC 6-OHDA lesioned rats. This study may be useful

to understand if such dysfunctions of cortical-limbic-striatal DAergic circuit can lead to specific detrimental effects of recurring use of Spice/K2 drugs.

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Poster

602. Cannabinoids: Neural Mechanisms

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 602.10/HHH4

Topic: G.08. Drugs of Abuse and Addiction

Support: NSERC Grant 03629
CIHR Grant 137122

Title: Tetrahydrocannabinol-induced hypernausea assessed in the conditioned gaping model in rats

Authors: *M. DEVUONO[1], K. M. HERLJA[1], E. M. ROCK[1], L. SABAZIOTIS[1], A. RAJNA[2], C. L. LIMEBEER[1], D. M. MUTCH[2], L. A. PARKER[1]
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Abstract: The psychoactive component of cannabis, Δ9-tetrahydrocannabinol (THC), is known to produce several aversive effects in human users and experimental animals. THC is a partial agonist of the cannabinoid 1 (CB_1) receptor of the endocannabinoid (eCB) system. Findings in animal, as well as the characterization of "Cannabinoid Hyperemesis Syndrome" in humans suggest that high doses of THC can produce nausea/vomiting. These findings are paradoxical, however, because low doses of THC are known to reduce nausea/vomiting in humans undergoing chemotherapy treatment, and animal models of toxin induced nausea/vomiting. The mechanism responsible for the nauseating effects of THC remains unclear. It is hypothesized that a dysregulation of the eCB system is involved in the nauseating effects of THC.
The conditioned gaping model, a rat model of nausea, was used to examine the nauseating effects of high dose THC. In experiment 1, male Sprague Dawley rats underwent 3 daily conditioning trials where they received an intraperitoneal (i.p.) injection of 0.5, 5, or 10 mg/kg THC, or vehicle (VEH) following an intraoral infusion of a novel saccharin solution for 2 min at a rate of 1 ml/min. The day following the final conditioning trial, rats underwent a drug-free test where they were only exposed to intraoral saccharin. Experiment 2 evaluated the ability of the CB_1 antagonist/inverse agonist, rimonabant (SR141716A; 1 mg/kg i.p.) or VEH 30 min prior to each conditioning trial to interfere with the establishment of conditioned gaping produced by 10 mg/kg THC.

Doses of 5 and 10 mg/kg THC produced conditioned gaping reactions, whereas 0.5 mg/kg THC, a dose known to prevent lithium chloride induced conditioned gaping, and VEH did not. Pre-treatment with 1 mg/kg rimonabant prior to conditioning prevented the establishment of conditioned gaping produced by 10 mg/kg THC. These results suggest that high doses of THC can produce nausea through activation of the CB_1 receptor.
Current experiments are implementing polymerase chain reaction to investigate changes in CB_1 receptor, and eCB related enzymes (fatty acid amide hydrolase, monoacylgycerol lipase, and diacylglycerol lipase) gene expression in nausea related brain regions (interoceptive insular cortex and dorsal vagal complex), thermoregulatory regions (hypothalamus), and control regions following administration of 10 mg/kg THC.

Disclosures: M. Devuono: None. **K.M. Herlja:** None. **E.M. Rock:** None. **L. Sabaziotis:** None. **A. Rajna:** None. **C.L. Limebeer:** None. **D.M. Mutch:** None. **L.A. Parker:** None.

Poster

602. Cannabinoids: Neural Mechanisms

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Topic: G.08. Drugs of Abuse and Addiction

Support: NSERC 92157
CIHR 137122

Title: Cannabinoid/Serotonin interactions in the interoceptive insular cortex in the regulation of LiCl-induced nausea in rats

Authors: *L. A. PARKER, C. L. LIMEBEER, E. M. ROCK
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Abstract: Nausea and vomiting are distressing side effects of chemotherapy treatment for cancer. Currently available first-line anti-emetic therapies (in particular 5-HT3 antagonists) have been critical in reducing these side effects, however, nausea in particular is more resistant to treatment than is vomiting. Considerable recent evidence indicates that cannabinoids and manipulations that enhance the functioning of the natural endocannabinoid system may be promising treatments for nausea. Although the neurobiology of vomiting is well understood, much less is understood about that of nausea, because of the lack of selective and reliable animal models of nausea. Rats do not vomit, but they display conditioned gaping reactions (Grill & Norgren, 1978) to flavors previously paired with a nauseating treatment, such as lithium chloride (LiCl). Conditioned gaping is a more selective and specific model of nausea than is that of conditioned taste avoidance learning in rats, as anti-emetic drugs prevent gaping, but not conditioned taste avoidance. Reduced forebrain 5-HT availability, as well as enhanced

endocannabinoid availability interfere with the establishment of LiCl-induced conditioning gaping in rats, without interfering with conditioned taste avoidance. Endocannabinoids act on presynaptic cannabinoid-1 (CB_1) receptors to reduce neurotransmitter release, including that of 5-HT. Using the conditioned gaping model, we have identified the interoceptive insular cortex as a central site of action of the nausea-inducing effects of 5-HT and the anti-nausea effects of endocannabinoid manipulations. At this site, recent evidence suggests that selective depletion of 5-HT prevents the establishment of LiCl-induced conditioned gaping reactions, but not taste avoidance. As well, elevation of the endocannabinoid 2-arachidonyl glycerol (2-AG) by inhibition of its degradative enzyme, monoacylglycerol lipase (MAGL), reduces LiCl-induced conditioned gaping reactions. Using in-vivo microdialysis, we found that LiCl triggers the release of 5-HT in this region and elevation of 2-AG prevents this LiCl-induced elevation of 5-HT, presumably by its action on the CB_1 receptor. As well, the phytocannabinoid, cannabidiol, reduces LiCl-induced elevation of 5-HT, most likely by its action on somatodendritic $5\text{-}HT_{1A}$ autoreceptors in the dorsal raphe nucleus. Finally, intraoral exposure to LiCl-paired saccharin also conditionally elevates 5-HT in the interoceptive insular cortex. Understanding the neural mechanisms regulating nausea may result in the development of better treatments to control this distressing disorder.

Disclosures: L.A. Parker: None. **C.L. Limebeer:** None. **E.M. Rock:** None.

Poster

602. Cannabinoids: Neural Mechanisms

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Program #/Poster #: 602.12/HHH6

Topic: G.08. Drugs of Abuse and Addiction

Support: Nipissing University

Title: THC and stress exposure during adolescence alters behaviour in adult male Wistar rats

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Abstract: The legalization and increased use of cannabis has enhanced research interest related to the potential interactions of cannabis use and other psychological conditions. Adults and adolescents often report using cannabis as a way of coping with stress and anxiety. While the effects of cannabis use on the adult brain are fairly well understood, less is known about chronic use during adolescence. This study assessed anxiety levels, spatial memory and social interactions in adult rats after chronic stress and Δ9-tetrahydrocannabinol (THC; the main psychoactive constituent in cannabis) during the adolescent phase of development. Four

experimental groups included: stress/THC, no stress/THC, stress/vehicle, and no stress/vehicle. Following 15 days in the housing condition (stress or no stress; PND 30 to 45), daily THC or vehicle injections were carried out for 10 days (PND 45 to 55). An open field test was completed after the stress housing period to assess pre-THC anxiety levels. Following the injections, animals were allowed to age to adulthood or PND 63. An elevated zero maze task, a water maze and a social interaction task were then used to assess anxiety/fear, spatial memory, and social behavioral changes between the groups. The behavioral results suggest that chronic adolescent THC administration following stress caused adult rats to become emotionally dysregulated but not more fearful. Specifically, the elevated zero maze showed changes in the initial phases of the task. No significant changes were found in the acquisition of the water maze task but differences were observed in the probe trial where the stress/THC rats spent less time in the established location of the escape platform. The social interaction results indicated that the stress/THC rats groomed and mounted other rats less frequently. Following the behavioral tests, the rat were also perfused for synaptic analysis using electron microscopy. Pilot data from this analysis related to changes in synaptic ultrastructure in the amygdala will be presented.

Disclosures: A.C. Weeks: None. **H. Quigley:** None. **B. Pulyk:** None. **T. McCharles:** None. **H. Domsy:** None. **S. Kilby:** None. **T. Bent:** None. **B. Reimer:** None. **A. Stillar:** None.

Poster

602. Cannabinoids: Neural Mechanisms

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Program #/Poster #: 602.13/HHH7

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant GM100829

Title: Cannabinoid pre-exposure does not induce sensitization or conditioned activity in adolescent rats

Authors: M. J. STONE, B. C. ADAME, B. L. OLIVER, D. O. SANCHEZ, *C. A. CRAWFORD
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Abstract: Cannabis use during adolescence has increased due to enhanced availability and greater societal acceptance. This increased usage is problematic because individuals who experiment with cannabis during adolescence, when compared to adulthood, are more likely to become chronic cannabis users and experiment with other drugs. The reason why early cannabis exposure increases the susceptibility to later drug use is unclear, but it may result from altered drug sensitivity. To assess this issue, we examined the behavioral response of early adolescent rats to CP-55,490 (a cannabinoid agonist) or cocaine (a psychomotor stimulant) two days after

repeated CP-55,490 administration. In the first experiment, 137 male and female adolescent rats were given vehicle (50% DMSO/H_2O) or CP-55,490 (4, 16.5, or 40 µg/kg, IP) once daily for five consecutive days (PD 30-PD 34). On each day, locomotor activity was measured for 1 h after drug injection. After a 48 h abstinence period (i.e., on PD 36), rats were injected with CP-55,490 (4 or 16.5 µg/kg, IP) and locomotor activity was monitored for 2 h. In the second experiment, 146 male and female adolescent rats were tested using the same protocol as in Experiment 1, except rats were given vehicle (saline or 50% DMSO/H_2O), CP-55,490 (16.5 or 40 µg/kg) or cocaine (20 mg/kg) for five days and then challenged with saline or cocaine (10 mg/kg) after 48 h. In Experiment 1, CP-55,490 did not alter locomotor activity on the five pre-exposure days or cause enhanced locomotor activity on the test day (i.e., behavioral sensitization was not evident). In Experiment 2, cocaine pretreatment led to both behavioral sensitization and conditioned activity when rats were challenged with cocaine or saline, respectively, on the test day. In contrast, CP-55,490-treated rats did not show enhanced locomotor activity when injected with cocaine or saline on the test day, thus indicating that CP-55,940 did not induce behavioral sensitization or conditioned activity. These data show that repeated CP-55,490 exposure does not cause a change in drug sensitivity nor does this cannabinoid agonist act similarly to a psychostimulant drug. The failure to detect cannabinoid-induced behavioral sensitization, cross-sensitization, or conditioned activity during adolescence suggests that cannabinoid exposure may not alter drug sensitivity in a way that causes an increase in later cannabinoid or cocaine use.

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Poster

602. Cannabinoids: Neural Mechanisms

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA041563
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Title: Working memory training reduces drug-seeking for the cannabinoid WIN 55,212-2

Authors: *S. J. STRINGFIELD[1,2], E. K. KIRSCHMANN[4], M. M. TORREGROSSA[1,2,3]
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Abstract: Evidence from clinical and preclinical studies suggests that cognitive training may promote resistance to the development of problem drug use or dependence. Cognitive training

may also serve as a treatment option to promote continued abstinence in individuals with several substance use disorders and is the subject of ongoing research. In rodent models of drug self-administration, rats will self-administer the synthetic cannabinoid WIN 55,212-2 (WIN), show cue-induced reinstatement of WIN-seeking, and show incubation of WIN craving after 30 days of abstinence. We hypothesized that cognitive training on a working memory task prior to WIN exposure would blunt this elevation of drug-seeking during abstinence. To test this hypothesis, adult male Sprague-Dawley rats (n=42) were trained on a delayed-match-to-sample working memory task. During this task, rats learn to nose poke into one of 5 illuminated sample ports to receive a sucrose pellet reward. After the rat nosepokes into a specific sample port, 3 adjacent ports are presented and the rat must choose the originally sampled port. Rats in the experimental group (WM) completed a cognitively taxing version of the task that engaged their working memory during a 0 - 24s delay period before the choice phase. Animals in the control (CON) group did not experience a delay before the choice phase. Next, all rats were trained to self-administer WIN (12.5µg/kg/infusion) during 2-hour sessions for 14 days. Rats were then tested in abstinence for working memory performance and WIN-seeking over 35 days. Rats were classified into high- and low-drug taking groups for further analysis based on WIN intake during self-administration. We found that CON rats took significantly more WIN than WM animals, and showed increased WIN seeking in abstinence. This effect was most pronounced in CON animals that stably self-administered higher amounts of WIN throughout self-administration. Both WM and CON animals showed decreases in working memory or control task accuracy when tested in abstinence after WIN self-administration. Thus, cognitive training on a working memory task prior to WIN self-administration has a protective effect against the subsequent expression of high levels of drug-seeking. Ongoing studies will continue to investigate the involvement of the prefrontal cortex in mediating this effect.

Disclosures: S.J. Stringfield: None. **E.K. Kirschmann:** None. **M.M. Torregrossa:** None.

Poster

602. Cannabinoids: Neural Mechanisms

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Program #/Poster #: 602.15/HHH9

Topic: G.08. Drugs of Abuse and Addiction

Title: Cocaine-induced increases in motivation require CB1 receptor activation

Authors: *V. M. AYVAZIAN[1], J. M. WENZEL[2], N. E. ZLEBNIK[2], A. BOWS[2], J. F. CHEER[2]
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Abstract: A large body of evidence supports an integral role for mesolimbic dopamine in motivation. Indeed, drugs that increase dopaminergic transmission, such as cocaine, increase motivation for a number of reinforcers as measured by increased breakpoints in a progressive

ratio (PR) schedule of reinforcement. Conversely, dopamine receptor antagonism decreases PR breakpoints. Our laboratory and others have shown that phasic mesolimbic dopamine is under the control of midbrain endocannabinoids (eCBs). Relatedly, antagonism of cannabinoid type-1 (CB1) receptors decreases PR breakpoints for food reinforcers, likely through downstream effects on dopaminergic function. However, it remains unclear if drugs that increase dopamine effectively increase motivation through eCB-dependent processes. To test this, we trained female rats on a PR task for food. Once breakpoints stabilized, each rat underwent a series of test days in which they were pretreated with either cocaine (10mg/kg, IP), one of two doses of the CB1 receptor antagonist rimonabant (1, 3mg/kg, IP), or both cocaine and rimonabant. Each test session was interleaved with non-drug baseline sessions until breakpoints re-stabilized. As expected, acute cocaine increased breakpoints, while rimonabant dose-dependently decreased breakpoints. Importantly, rimonabant, at a dose that on its own did not decrease breakpoints, blocked the ability of cocaine administration to increase breakpoints. These data suggest that cocaine-induced gains in motivation are dependent upon eCB signaling, and add to a growing body of evidence supporting the eCB system as a key modulator of dopaminergic function. We are currently utilizing fast-scan cyclic voltammetry to further understand the neurobiological mechanisms underlying our behavioral observations.

Disclosures: V.M. Ayvazian: None. **J.M. Wenzel:** None. **N.E. Zlebnik:** None. **A. Bows:** None. **J.F. Cheer:** None.

Poster

602. Cannabinoids: Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 602.16/HHH10

Topic: G.08. Drugs of Abuse and Addiction

Title: Cell type and pathway-specific imaging of cannabinoid receptor modulation at cholinergic terminals

Authors: *E. HERNANDEZ, D. P. COVEY, J. F. CHEER
Dept. of Anat. and Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: The hippocampus is well-known as a center for memory consolidation and spatial navigation, as it receives input from and is connected to a wide array of neuronal circuits. Acetylcholine input to the hippocampus is critical during memory formation, with abnormal cholinergic activity exhibiting major implications in memory-related disorders, such as Alzheimer's disease. The endocannabinoid (eCB) system, known for its modulation of marijuana's psychoactive and reinforcing aspects in the brain, is a wide neurochemical network that also plays a major role in memory consolidation. Indeed, we have recently found dense expression of the cannabinoid type 1 (CB1) receptor on medial septal cholinergic neurons that

innervate the hippocampus, suggesting an important role of this pathway in mnemonic processes. In order to harness this anatomical framework, we used our recently-published transgenic mouse line bearing a selective deletion of CB1 receptors on cholinergic neurons. We tested short-term memory in these animals and showed an increase in function in a delayed non-match to sample task. Additionally, we monitored short-term spatial memory consolidation in a novel object recognition task. This particular test allows for habituation to the environment and familiarization to a set of objects before the introduction of a novel object. Spatial memory capacity was quantified by measuring the rodents' innate tendency to recognize novel objects familiar objects within an open field. To further understand cholinergic signaling in the hippocampus, we employed *in vivo* calcium imaging through miniature endoscopes to visualize longitudinal patterns of activity at CA1 pyramidal neurons, which were contrasted between experimental and control animal cohorts of both sexes. These results will help elucidate the extent to which the eCB system regulates memory function within the hippocampus and may offer insight into abnormal cholinergic activity associated with memory-related symptoms during cannabis use, as well as in disorders such as dementia.

Disclosures: E. Hernandez: None. **D.P. Covey:** None. **J.F. Cheer:** None.

Poster

602. Cannabinoids: Neural Mechanisms

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Intramural Research Program (IRP)

Title: Chronic D9-tetrahydrocannabinol (THC) causes pathway-specific synaptic plasticity in the nucleus accumbens

Authors: *E.-K. HWANG, C. R. LUPICA
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Abstract: Cannabis is the most widely used illicit drug and its chronic use is associated with cannabis use disorder (CUD). THC is an agonist of CB1, and CB2 cannabinoid receptors that are found throughout the brain. The nucleus accumbens shell (NAcs) is important for reward and motivated behavior, receives glutamate inputs from a wide array of brain regions, including the ventromedial prefrontal cortex (vmPFC), the ventral hippocampus (vHipp) and the basolateral amygdala (BLA). These divergent glutamatergic inputs are integrated in the NAcs to regulate the activity of medium spiny neurons, and dysfunction of these pathways contribute to drug reward and addiction. The axons of dopamine (DA) neurons originating in the ventral tegmental area (VTA) also densely project to neurons in the NAcs. Many VTA DA neurons projecting to NAcs

also co-release glutamate, and the consequences of this are not fully understood. Brain imaging studies in CUD subjects show that corticolimbic circuits, including the NAcs, are altered, and this is associated with dysregulated cognition, learning, and emotion. However, our understanding of the mechanisms underlying these neuronal changes is limited. Here, we examine functional consequences of chronic exposure to the psychoactive constituent of cannabis (THC) on glutamatergic afferents to the NAcs arising in the vmPFC, vHipp, BLA and VTA using optogenetics. We find that chronic THC weakens glutamatergic vmPFC-NAcs synapses via diminished glutamate release probability. In contrast, synaptic strength in vHipp-NAcs synapses is strengthened by chronic THC, and this is mediated by AMPA receptor subunit changes. Moreover, BLA-NAcs synapses are strengthened by chronic THC, and, although overall glutamate synaptic strength is not altered by chronic THC in the VTA-NAcs pathway, both the proportion of AMPA receptors lacking GluR2 subunits and the probability of glutamate release increase, suggesting homeostatic reorganization. We also find that group I mGluR-dependent long-term depression is prevented by chronic THC at both vmPFC-NAcs and vHipp-NAcs synapses. In conclusion, chronic THC exposure results in altered glutamate synapse function in several corticolimbic pathways impinging on the NAcs, and we suggest that this relates to the pattern of cognitive and emotional changes observed in chronic cannabis users.

Disclosures: E. Hwang: None. **C.R. Lupica:** None.

Poster

602. Cannabinoids: Neural Mechanisms

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Topic: G.08. Drugs of Abuse and Addiction

Support: Intramural Research Program of the NIH/NIDA
University of Genoa

Title: Acute THC impairs voluntary locomotor activity through both CNR1 and non- CNR1 ammonia-mediated mechanisms

Authors: *M. ZUCCOLI[1,2], S. SARSFIELD[1], L. WHITAKER[1], O. A. ABULSEOUD[1], Y. APONTE[1]
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Abstract: Substantial evidence indicates inhibitory effects of THC on locomotor activity. The dorsolateral striatum (DLS) has been broadly studied for its role in mediating these locomotor effects. It is widely accepted that these effects occur through presynaptic inhibition of neurotransmitter release of striatal outputs mediated by cannabinoid type 1 receptor (CNR1). However, we previously demonstrated that a single administration of THC to naive mice induced

a 10-fold increase in striatal ammonia concentration, suggesting its potential role in locomotor activity suppression. Excess ammonia decreases spontaneous locomotor activity in mice and alters neuronal firing patterns, glutamatergic signaling, and production of nitric oxide (NO). Moreover, a recent study showed a decrease in exploratory activity and reduced magnitude of long-term potentiation in corticostriatal synaptic transmission under hyperammonemia. These findings suggest that THC might alter the excitability of neuronal circuits in the striatum, not only through the activation of CNR1, but also by inducing a transient increase in ammonia concentration. Here we report the effects of single administration of THC or ammonium acetate (NH_4Ac) on locomotor activity tested by open field (OF) and running wheel (RW), neuronal activity within the DLS using *in vivo* two-photon endomicroscopy, and glutamatergic transmission using brain slice electrophysiology. Moreover, we tested the efficacy of sildenafil citrate (SILD), a phosphodiesterase inhibitor involved in the NO pathway, to reduce THC-induced hypolocomotion. Our results show that both THC and NH_4Ac cause significant reduction in OF (THC: $p<0.0001$; NH_4Ac: $p<0.0001$, $n=8$/group) and RW (THC: $p<0.0001$; NH_4Ac: $p<0.0001$, $n=4$/group). These behavioral changes correlate with a significant attenuation in DLS neuronal activity. In addition, electrophysiological recordings reveal fluctuations in NMDAR function following THC and NH_4Ac administration. Neutral CNR1 antagonist NESS 0327 successfully reversed the overall locomotor effects of THC ($p<0.0001$, $n=7$). However, NESS 0327 did not reverse the THC effect within the first 5 minutes post-injection, suggesting that during this early time window hypolocomotion is not mediated by CNR1. Remarkably, pretreatment with SILD blocked the overall effect of THC on locomotor activity ($p<0.0001$, $n=8$), and unlike NESS 0327, SILD blocked this activity within the first 5 minutes ($p=0.003$). In summary, these results are the first to report effects of THC mediated by ammonia and indicate sildenafil citrate as a potential treatment for some cannabis-related side effects.

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Poster

602. Cannabinoids: Neural Mechanisms

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Topic: G.08. Drugs of Abuse and Addiction

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Title: The cognitive effects of delta-9-tetrahydrocannabinol and cannabidiol in the ventral hippocampus are mediated through differential modulation of the c-jun-n-terminal kinase pathway and prefrontal cortex neuronal activity

Authors: *R. M. HUDSON[1], W. RUSHLOW[2], S. R. LAVIOLETTE[2]
[1]Neurosci., [2]Anat. and Cell Biol., Univ. of Western Ontario, London, ON, Canada

Abstract: Adaptive behaviours and cognition require accurate processing of the endless barrage of sensory information entering the brain. In particular, the brain must appropriately discern this information to engage in contextually appropriate and adaptive memory formation that enables ongoing environmental interactions. Evidence suggests that the phytocannabinoids delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) induce distinct actions on attention, memory acquisition, and psychiatric risk. In fact, chronic cannabis users exhibit volumetric reductions in cortical and subcortical brain regions, and large doses of THC induce neuronal apoptosis via activation of the c-Jun N-terminal kinase (JNK) signaling cascade. However, the contributions of specific neurocircuitry to THC-induced cognitive disturbances, and whether CBD co-administration can counteract these processes remain unknown. Given that direct projections between the ventral hippocampus (VHipp) and medial prelimbic prefrontal cortex (mPFC) facilitate contextually-relevant attentional processing and subsequent memory formation, we explored the hypothesis that VHipp THC and CBD elicit opposing effects on mPFC neuronal activity to influence attention and memory processing via distinct actions on the JNK signal transduction pathway. We examined the effects of VHipp THC, CBD and their combination on attentional processing and memory formation, VHipp JNK phosphorylation, and mPFC neuronal and oscillatory activity. VHipp THC induced deficits in social interaction, exploratory behaviours, and spatial memory assays that were rescued by CBD co-administration, or inhibition of VHipp JNK phosphorylation. Although THC considerably increased startle responses during pre-pulse facilitation, this effect was inverted into startle inhibition by CBD co-administration, or JNK inhibition. Sensorimotor gating was enhanced by the THC+CBD combination. Whereas intra-VHipp THC increased mPFC gamma oscillations and decreased mPFC phasic bursting activity via activation of VHipp JNK phosphorylation, CBD increased mPFC bursting activity via decreased VHipp JNK phosphorylation. In contrast, combined THC+CBD inhibited the actions of either drug on mPFC single unit and oscillatory activity. Our findings indicate the VHipp JNK signaling pathway as a critical molecular signaling mechanism by which THC dysregulates attention and memory processing, and suggest important implications for specific cannabis compounds in psychiatric disorders.

Disclosures: R.M. Hudson: None. **W. Rushlow:** None. **S.R. Laviolette:** None.

Poster

602. Cannabinoids: Neural Mechanisms

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH DA026430
NIH NS098777

Title: Measuring 2-arachidonoylglycerol hydrolyzing activity by ABHD6

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Abstract: α/β Hydrolase domain containing 6 (ABHD6) is a serine hydrolase that cleaves 2-arachidonoylglycerol (2-AG), the most abundant endocannabinoid (eCB) in the brain, into arachidonic acid and glycerol. Inhibition of ABHD6 may produce potential therapeutic benefits, including reducing the severity and frequency of seizures that are triggered by various devastating neurological diseases. To date, we have a very limited understanding of the molecular mechanism behind ABHD6 function in neurons and how it controls 2-AG levels and eCB signaling.

To study the molecular mechanism involved in 2-AG hydrolysis by ABHD6, we implemented and validated an enzyme-linked fluorescent assay that measures the production of glycerol resulting from 2-AG hydrolysis. In this assay, glycerol enters a series of enzymatic reactions that produces the fluorescent product resorufin, which is excited at 530 nm to detect a 590 nm emission. To validate this assay, we selected a cell line that expressed high levels of ABHD6 mRNA (SK-MEL-2, a melanoma cell line) and low levels of ABHD6 mRNA (U251 cells, a glioblastoma cell line). We found that SK-MEL-2 exhibits higher 2-AG hydrolyzing activity than U251 (rate of glycerol production over the course of 60 minutes was 0.75 pmol/min and 0.53 pmol/min, respectively, for 30 µg of lysate; n = 2) and that 2-AG hydrolysis in SK-MEL-2 cells showed greater inhibition following treatment with an ABHD6 inhibitor compared to U251 cells (KT-182, 10 µM, 41% and 8.3% inhibition, respectively), confirming a higher ABHD6 activity in the SK-MEL-2 cells. This result was further validated with quantitative-reverse transcriptase PCR of ABHD6 mRNA that showed that SK-MEL-2 cells had on average about five times more ABHD6 mRNA expression than U251 cells.

Our results show that this fluorescent-based assay allows for reliable, precise and relatively cheap measurements of 2-AG hydrolysis in cell lysates. This assay gives us a platform to measure 2-AG hydrolysis by ABHD6 as well as study the molecular mechanism underlying ABHD6 hydrolysis activity and the mechanism of action of ABHD6 inhibitors. Pharmacological

inhibition of ABHD6 activity in the brain represents a promising therapeutic approach to modulate the eCB signaling for the treatment of various diseases, including epilepsy.

Disclosures: S. Singh: None. **N. Stella:** None.

Poster

603. Drugs of Abuse and Addiction: Cocaine Seeking and Reinstatement II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 603.01/HHH15

Topic: G.08. Drugs of Abuse and Addiction

Support: This work was supported by NIDA/NIH.

Title: Sex differences in the potentiation of intermittent self-administration on incubation of cocaine craving: Effect of estrous cycle

Authors: *C. NICOLAS, T. I. RUSSELL[1], A. PIERCE[1], A. HOLLEY[2], Z.-B. YOU[1], M. M. MCCARTHY[2], Y. SHAHAM[1], S. IKEMOTO[1]
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Abstract: Cocaine seeking progressively increases during abstinence, a phenomenon termed 'incubation of cocaine craving'. Incubation of craving has been demonstrated using a continuous cocaine self-administration procedure. Recently, Zimmer et al. (2011) introduced an intermittent self-administration procedure in which the rats have access to cocaine for 5 min followed by 25 min of time out, and found that this procedure increases motivation to self-administer cocaine. Additionally, studies report sex differences and a role of ovarian hormone in cocaine relapse. Here, we studied whether intermittent cocaine self-administration would increase incubation of craving in male and female rats, and then we investigated the role of ovarian hormones in this effect. First, we trained male and female rats to self-administer cocaine (0.75 mg/kg/infusion) continuously or intermittently for 12 days (8 h/day). We found, in both sexes and under both training conditions, an escalation of cocaine intake and a higher cocaine seeking in the relapse test after 29 days than 2 days (incubation of craving). Importantly, in both training conditions, female rats showed an increase of drug seeking on both day 2 and 29 compared to males. However, potentiation of incubation of craving was observed exclusively in females after intermittent self-administration. Next, by monitoring the estrous cycle in females, we found that only the rats in estrus showed an incubation of cocaine craving. Our results demonstrate that intermittent cocaine access potentiates the incubation of craving and suggest a critical role of ovarian hormones in this effect in female rats, highlighting the importance of the therapeutic window in the treatment of addiction in women.

Disclosures: T.I. Russell: None. **A. Pierce:** None. **A. Holley:** None. **Z. You:** None. **M.M. McCarthy:** None. **Y. Shaham:** None. **S. Ikemoto:** None.

Poster

603. Drugs of Abuse and Addiction: Cocaine Seeking and Reinstatement II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 603.02/HHH16

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA/NIH

Title: Neural encoding of reward seeking in the medial prefrontal cortex

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Abstract: The search for better therapeutic strategies for drug addiction raises the challenge to diminish motivation for drug without decreasing that for natural rewards. While the medial prefrontal cortex (mPFC) is important for reward seeking, how prefrontal neural activities code reward seeking remains unknown. Here, we employed miniScope, a custom miniature fluorescence imaging system, together with detailed computational analysis, to simultaneously track calcium activities from hundreds of neurons longitudinally, at the single cell resolution in the mPFC during mice food and cocaine self-administration. We found that different subgroups of neurons showed increased activity around distinct behavioral events (i.e. house light on, lever extend into behavior chamber, lever press/cue presentation, and food retrieval). More neurons were active during lever press/cue presentation and food retrieval. We further observed that distinct subsets of neurons were active during lever press/cue presentation for food and cocaine, respectively. Our results suggest distinct and dynamic neural population codes for natural reward and drug reward seeking in the mPFC and pave the way for future efforts in targeting specific neural codes for drug reward seeking as novel therapeutic strategies for drug addiction.

Disclosures: Y. Zhang: None. **G. Barbera:** None. **L. Zhang:** None. **B. Liang:** None. **Y. Li:** None. **Y. Shaham:** None. **D. Lin:** None.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

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Research and Education Initiative Fund, a component of the Advancing a Healthier Wisconsin Endowment at the Medical College of Wisconsin
Neuroscience Research Center, Medical College of Wisconsin

Title: Reward- and context-specific ensembles following sucrose or cocaine self-administration in mice

Authors: *M. SLAKER[1], N. N. NAWARAWONG[1], C. M. OLSEN[2]
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Abstract: Neuronal ensembles are small sets of neurons whose activity is required for manifestation of a behavior. Previous studies have identified ensembles in fear and reward memory that are sufficient to drive behavior. The TetTag mouse model is a unique reporter line that allows the study of neuronal ensembles over time. The TetTag mouse model is a Tet-Off reporter mouse that uses the c-Fos promoter to drive expression of a long-lasting EGFP-histone-2B fusion protein tag. Our lab has previously identified an ensemble within the medial prefrontal cortex (mPFC) whose reactivation correlated to the persistence of cocaine seeking across two drug seeking sessions separated by 2 weeks abstinence. We found that the greater proportion of an ensemble activated during a seeking session on abstinence day 7 that was reactivated on day 21 was correlated to a higher degree of cocaine-seeking behavior. Most human drug use, however, does not occur in one environment, but instead spans multiple settings. Contextual cues can reinstate drug seeking even when operant responding has been extinguished in a different environment, and a 2014 study identified a specific ensemble within the nucleus accumbens shell that mediated context-induced reinstatement of cocaine seeking. Therefore, in this study, we examined the association between cocaine-seeking ensembles and contexts within the mPFC and nucleus accumbens. TetTag mice were trained to self-administer cocaine (0.5mg/kg/infusion) in two distinct contexts. Seeking on abstinence day 7 was tested in one context and tagged with EGFP, while seeking on abstinence day 21 was tested in the other context and assessed for c-Fos. In both contexts, mice were able to learn self-administration and show elevated levels of seeking. We also examined the specificity of the context ensemble to an additional rewarding modality, 10% sucrose. TetTag mice learned to self-administer a sucrose solution in two contexts with robust responding during seeking tests. Future analysis will examine the similarities and differences between populations of ensemble cells identified in each context, as well as those

labeled by both contexts. These studies provide insight into the ability of contextual cues to trigger seeking behaviors at a neuronal level.

Disclosures: M. Slaker: None. **N.N. Nawarawong:** None. **C.M. Olsen:** None.

Poster

603. Drugs of Abuse and Addiction: Cocaine Seeking and Reinstatement II

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Topic: G.08. Drugs of Abuse and Addiction

Support: Natural Sciences and Engineering Research Council (NSERC 92157)
Canadian Institutes of Health Research (CIHR 137122)

Title: Conditioned gaping produced by delayed, but not immediate, exposure to cocaine in rats

Authors: *K. GUENTHER, C. E. WIDEMAN, E. M. ROCK, C. L. LIMEBEER, L. A. PARKER
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Abstract: Wheeler (2008) reported that following several daily pairings of multiple exposures to a saccharin cue with the delayed (30 min) opportunity to self-administer cocaine, rats eventually display conditioned gaping reactions (Grill & Norgren, 1978) during the waiting period, suggesting a conditioned withdrawal effect. In contrast, Parker (1993) demonstrated that following several spaced (72 hr apart) conditioning trials with a 2-min exposure to a saccharin cue immediately followed by a subcutaneous (sc) injection of cocaine, rats did not display conditioned gaping reactions. Here we determined if both effects could be reproduced under similar conditioning protocols (daily conditioning trials) that differed by short single exposure and delayed multiple exposures to saccharin. In Experiment 1, rats were given daily conditioning trials with a 2-min exposure to saccharin which was immediately followed by 5, 10 or 20 mg/kg cocaine sc (1a), cocaine ip (1b) or 50 mg/kg LiCl (1c). In Experiment 2, rats were given daily multiple brief (10 sec) exposures to saccharin over a 30-min period prior to cocaine (20 mg/kg, both sc and ip) or LiCl (50 mg/kg, ip) injections. Experiment 3 evaluated the potential of a context which signals delayed access to cocaine to produce aversive response. Experiment 4 evaluated the potential of another rewarding drug, morphine (10 mg/kg sc) to produce aversive reactions following pairings of delayed access (10 min). Finally, Experiment 5 evaluated the potential of sc cocaine to produce aversive aftereffects (Ettenberg, 1999) using a conditioned floor preference paradigm, with different groups receiving 20 mg/kg, sc cocaine 0, 15, 30 and 60 min prior to placement in the chamber with a distinctive floor. Conditioned gaping reactions and chin rubbing reactions were elicited by saccharin (but not a context) paired with delayed cocaine (sc stronger than ip), but not by immediate exposure to cocaine; however, neither immediate nor

delayed cocaine produced the aversive reactions paw treading. Both cocaine and LiCl produced the ingestion related effects of suppressed tongue protrusions and enhanced passive drips consistent with previous reports (Parker 1993). When injected sc, but not ip, cocaine also elicited the potential withdrawal reactions of yawning. When administered sc, 20 mg/kg cocaine did not produce a conditioned floor preference or aversion (as reported by Mayer & Parker, 1993) at any post-cocaine interval. The results are consistent with Wheeler et al (2008) that when paired with delayed access to cocaine, the flavour triggers an negative affective state that is revealed by gaping.

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Poster

603. Drugs of Abuse and Addiction: Cocaine Seeking and Reinstatement II

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA042475

Title: Alpha$_{2a}$-adrenergic hetero-receptors are necessary for stress and agonist regulation of BNST activity and stress-induced reinstatement of cocaine-associated behaviors

Authors: *R. PEREZ[1], N. HARRIS[2], B. NABIT[3], S. FLAVIN[1], K. MERKEL[2], R. GILSBACH[4], L. HEIN[4], D. WINDER[2]
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Abstract: Stress is often cited as a precipitating factor for relapse of cocaine use. Therefore, clinically available alpha$_{2a}$-adrenergic receptor (A_{2a}-AR) agonists, have been investigated as potential treatments for stress-induced craving and relapse. While A_{2a}-AR agonists decrease stress-induced craving, they have not reduced relapse rates. It has been postulated that A_{2a}-AR agonists reduce craving by engaging auto-receptors and blunting norepinephrine (NE) release in brain regions critical for stress-induced drug seeking, such as the bed nucleus of the stria terminalis (BNST). However, using a genetic strategy in which A_{2a}-ARs are re-expressed in NE-producing cells of A_{2a}-ARs knockout mice (hetero-receptor KOs), many of the pharmacological functions originally ascribed to A_{2a}-AR auto-receptors, such as analgesia and sedation, have been suggested to be mediated by hetero-receptors. The relative role of these receptor pools in the regulation of drug seeking behavior has not been investigated. Thus, we aimed to determine the role of A_{2a}-AR hetero-receptors in stress induced reinstatement of cocaine-associated behaviors and in regulation of BNST activity. We evaluated the role of hetero-receptors in stress-induced

reinstatement using the cocaine conditioned place preference (CPP) paradigm. We found that in wild-type, 6 minutes of forced swim stress reinstates previously extinguished cocaine CPP. However, stress failed to reinstate CPP in full or hetero-receptor KO mice. We also evaluated changes in BNST activity following stress by measuring levels of cFOS, a neuronal activity marker, following 30 minutes of restraint stress in wild type, full and hetero-receptor A_{2a}-AR KO mice. We found that stress significantly increases the number of cFOS positive neurons in wild-type mice but not in full or hetero-receptor KO mice. We also evaluated whether A_{2a}-AR hetero-receptors are necessary for agonist regulation of excitatory transmission within the BNST using an *ex vivo* patch-clamp electrophysiology approach combined with application of the A_{2a}-AR agonist guanfacine. We found that guanfacine depresses excitatory transmission within the BNST of wild-type mice, however, this suppression does not occur in full or hetero-receptor KO mice. These findings suggest that following stressful stimuli, A_{2a}-AR hetero-receptors regulate the activity of the BNST to induce reinstatement of cocaine seeking.

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Poster

603. Drugs of Abuse and Addiction: Cocaine Seeking and Reinstatement II

Location: SDCC Halls B-H

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Program #/Poster #: 603.06/HHH20

Topic: G.08. Drugs of Abuse and Addiction

Support: DA 08227

Title: Novelty place preference predicts addiction-like behavior in C57BL/6J mice self-administering cocaine

Authors: *D. GUZMAN, K. LINDQUIST, S. G. BIRNBAUM, D. W. SELF
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Abstract: Identification of premorbid genetic vulnerability to drug addiction has been hindered by an absence of large scale human genetic data to specific drug classes (e.g., opiates, psychostimulants). One approach is to focus on intermediate phenotypes related to vulnerability to drug addiction in both pre-clinical and clinical studies. For example, novelty-seeking traits have been associated with drug abuse disorders in humans and have been found to predict initiation of drug use. In rats, a high Novelty Place Preference (NPP) predicts the development of an addictive-like phenotype with prolonged cocaine self-administration. The aim of the current study is to confirm that NPP also predicts cocaine addictive-like behaviors in mice, which would allow for high throughput screens of NPP in mutagenesis studies to identify genes involved in novelty seeking and vulnerability to addiction. We measured the preference for novel versus

familiar side of a 3-compartment test chamber in male C57BL/6J mice. The mice were first confined to one side for a 20-min period (i.e., becomes the “familiar” side). The mice were then removed and placed in a holding cage for 2-min, and then placed into a center compartment and given free access to both the familiar and novel sides for a 10-min test period. As expected, animals averaged over 60% of the time in the novel side during the test. We also measured the difference in time spent between the novel and familiar side as a “novelty score”. 24 mice were grouped by a median split and upper and lower quartiles and designated as “HIGH” or “LOW” NPP responders and were subsequently trained to self-administer cocaine (CSA). Once animals reached acquisition criteria, we tested them using two measures of addictive-like self-administration and relapse behaviors. We found that the HIGH NPP group (n = 6/upper quartile) exhibited a vertical shift in the dose-response for CSA (fixed ratio schedule) compared to the LOW group (n = 6/ lower quartile). Second, following a 7-10 day withdrawal period and 5-hr extinction session, the HIGH group also exhibited a significantly higher level of cue-induced reinstatement compared to the LOW group. Finally, in a separate group of 6 mice, HIGH NPP responders (n=3/upper median) worked harder to self-administer cocaine compared to the LOW NPP responders (n=3/lower median) when tested using the progressive ratio reinforcement schedule. These data validate the association between a high NPP phenotype and cocaine addiction-like behaviors, and support the use of NPP as a screen in mutagenesis studies to identify genes that convey vulnerability to cocaine addiction.

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Poster

603. Drugs of Abuse and Addiction: Cocaine Seeking and Reinstatement II

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R00DA038110 (J.A.L.)

Title: Effects of chronic stress exposure during withdrawal on the incubation of cocaine craving in adult female rats

Authors: C. M. CORBETT[1], E. BABENKO[2], *J. A. LOWETH[2]
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Abstract: Although clinical studies indicate sex differences in both cocaine addiction and stress and anxiety disorders, the majority of preclinical studies on cue- and stress-induced relapse vulnerability have been conducted with male rats. Our own recent studies with adult male rats

indicate that chronic stress exposure during early withdrawal from extended access cocaine self-administration accelerates the time-dependent intensification or incubation of cue-induced cocaine craving that occurs during the first few weeks of withdrawal. This enhanced cue-induced seeking behavior observed following chronic stress exposure may make rats more vulnerable to cue-induced relapse during this period. We are currently conducting studies to assess whether similar effects of chronic stress exposure on incubation of cocaine craving are observed in adult female rats and to characterize the time course of these effects. Similar to our previously published studies with male rats, freely cycling female rats will self-administer cocaine under extended access conditions (6 hours per day for 10 days). During the first two weeks of withdrawal, rats will receive repeated restraint stress exposure or control conditions and changes in cue-induced seeking behavior will be assessed at different time points during withdrawal. Prior to all seeking tests, vaginal smears will be performed to determine estrous cycle stage and preliminary analyses will be conducted to determine whether estrous cycle influences stress-induced changes in cocaine seeking behavior. These findings will identify whether sex differences exist in cue- and chronic stress-induced relapse vulnerability and may pave the way for subsequent studies to study the interaction of cocaine and stress in female rats.

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Poster

603. Drugs of Abuse and Addiction: Cocaine Seeking and Reinstatement II

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA015215
NIH Grant T32 DA028874

Title: DBS-like optogenetic stimulation of accumbens dopamine D2 receptor-containing neurons attenuates cocaine reinstatement

Authors: *P. J. HUFFMAN[1], M. C. KNOUSE[1], S. E. SWINFORD-JACKSON[1], A. U. DEUTSCHMANN[2], A. S. THOMAS[1], L. A. BRIAND[2], R. C. PIERCE[1]
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Abstract: Previous work indicated that deep brain stimulation (DBS) of the nucleus accumbens (NAc) shell attenuated reinstatement of cocaine-seeking in rats. However, the potential differential impact of DBS on specific populations of neurons to drive the suppression of cocaine-seeking is unknown. Medium spiny neurons in the NAc are differentiated by the expression of dopamine D1 receptors (D1DRs) or dopamine D2 receptors (D2DRs), activation of

which promotes or inhibits cocaine-seeking behavior, respectively. We used recently-developed transgenic rat lines that express Cre recombinase selectively in D1DR-containing or D2DR-containing neurons in combination with a Cre-dependent adeno-associated viral vector expressing channelrhodopsin (ChR2) to deliver high frequency optogenetic stimulation selectively to each population of neurons in the NAc shell. Rats were trained to self-administer cocaine, and this behavior was extinguished prior to the cocaine-primed reinstatement sessions. Intra-NAc shell DBS-like optogenetic stimulation or no stimulation was administered throughout the reinstatement session. DBS-like optogenetic stimulation of D2DR-containing neurons attenuated reinstatement of cocaine seeking, whereas DBS-like optogenetic stimulation of D1DR-containing neurons did not alter cocaine-primed reinstatement. Electrophysiology experiments to further explore the effect of DBS-like optogenetic stimulation on cell firing indicate that high frequency stimulation of D2DR-containing accumbens MSNs results in virtually instantaneous neural silencing. Collectively, these results suggest that DBS of the NAc attenuates cocaine-primed reinstatement through the selective inactivation of D2DR-containing neurons.

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Poster

603. Drugs of Abuse and Addiction: Cocaine Seeking and Reinstatement II

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIHMH97988
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P30 RR032135

Title: Stress-induced reinstatement requires PAC1 receptor endosomal MEK signaling

Authors: *O. MILES[1], S. BRAINARD[1], V. MAY[2], M. E. BOUTON[1], S. E. HAMMACK[1]
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Abstract: A primary challenge in the treatment of substance abuse is the tendency of users to relapse following acute or extended periods of abstinence; on average, over 60% of substance abusers will return to drug use within a year of receiving treatment, many relapsing following stressful life events. Central to the successful treatment of drug addiction is understanding the cellular mechanisms by which relapse episodes occur. We have previously demonstrated that in rats that learned to lever press for intravenous cocaine, and subsequently had that behavior

extinguished, a bilateral intra-bed nucleus of the stria terminalis (BNST) infusion of pituitary adenylate cyclase-activating peptide (PACAP) caused robust reinstatement of drug seeking on the lever previously associated with cocaine delivery. Furthermore, the bilateral intra-BNST infusion of PACAP type 1 receptor (PAC1-R)/vasoactive intestinal peptide type 2 receptor (VPAC2) antagonist PACAP6-38 blocked reinstatement of cocaine seeking following stressor exposure. In the current studies, we used immunohistochemical procedures, pharmacological treatments, and a behavioral model of stress-induced relapse to evaluate PACAP and PAC1-R signaling in stress-induced reinstatement to cocaine seeking. BNST infusions of PAC1-R selective agonist, maxadilan, reinstated drug-seeking on the lever previously associated with cocaine delivery, specifically implicating PAC1-Rs (rather than VPAC2-Rs) in reinstatement to drug-seeking. Moreover, footshock stress increased BNST phosphorylated extracellular signal-related kinase (pERK) expression in cocaine-experienced rats, and this increase was blocked by BNST PACAP receptor antagonism. Recent studies have suggested that PAC1-R activation may lead to endosomal signaling and MEK activation that influence intracellular events. Here, we show that footshock-induced reinstatement and subsequent PACAP receptor-dependent pERK signaling is abrogated by BNST pretreatment with mitogen activated protein kinase-ERK (MEK) inhibitor, PD98059, or an endocytosis inhibitor, Pitstop2, that blocks extracellular signal-related kinase (ERK) signaling. These data suggest that the activation of PAC1-R endosomal MEK/ERK signaling is a key event underlying stress-induced reinstatement. Furthermore, this data suggest that there may be long term changes in the BNST PACAPergic system (i.e. sensitization) following cocaine exposure.

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Poster

603. Drugs of Abuse and Addiction: Cocaine Seeking and Reinstatement II

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Program #/Poster #: 603.10/HHH24

Topic: G.08. Drugs of Abuse and Addiction

Title: Impact of endogenous ghrelin on the maintenance and reinstatement of cocaine addiction-like behaviors in self-administration trained rats

Authors: *Z.-B. YOU[1], B. WANG[2], G.-H. BI[1], F. ALEN[1], Z.-X. XI[1], R. A. WISE[2], E. L. GARDNER[1]
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Abstract: Appetitive hormones are well recognized as important modulators of body mass, food intake and energy homeostasis primarily via their actions on hypothalamus neurons. Most such

hormones also regulate the functions of mesolimbic dopamine system, a brain system critical for the rewarding effects of food and abused drugs. Ghrelin, an orexigenic hormone secreted primarily from stomach and gut, has been recently found not only to stimulate mesolimbic dopamine transmission and modulate food reward, also to be involved in the regulation of the rewarding effects of most abused drugs-including nicotine, amphetamine and cocaine as tested using classical place conditioning. The roles of ghrelin in drug self-administration (S-Ad) remain largely to be investigated. In this study, we systemically investigated the responses of ghrelin in bloodstream to cocaine S-Ad and S-Ad related behaviors, and the effects of ghrelin antagonism on such behaviors using the selective ghrelin receptor antagonist JMV2959 in rats. We found that cocaine S-Ad (1 mg/kg /infusion) is associated with dramatic elevations in plasma ghrelin levels. These elevations are also seen when rats (either cocaine-trained or cocaine-naïve) receive yoked cocaine infusions (cocaine infusions triggered via lever-presses of other trained rats). The elevations of ghrelin by yoked cocaine are significantly less in cocaine-naïve than in cocaine S-Ad trained rats. S-Ad of saline (first saline substitution session in trained rats) also caused significant elevations of ghrelin levels, but these elevations are less prominent than those seen under cocaine S-Ad and are no longer evident during the 14^{th} extinction session. Pretreatment of cocaine trained rats with JMV2959 (0-6mg/kg, i.p.) dose-dependently inhibits animals' responding on the active lever tested either for S-Ad or during extinction. Pretreatment with JMV2959 inhibits reinstatement of cocaine-seeking induced by cocaine (10 mg/kg, i.p.) in cocaine S-Ad trained and subsequently behaviorally extinguished rats. Our findings indicate a significant stimulatory effect of cocaine on ghrelin secretion and that this effect is strengthened in rats following repeated cocaine S-Ad training and can be conditioned to the stimuli associated with cocaine S-Ad. The inhibitory effects of JMV2959 on cocaine S-Ad, non-reinforced extinction "burst" responding and on cocaine-induced reinstatement suggest a contributory role of ghrelin signaling in the maintenance of and in provoking motivation for cocaine. Thus, manipulations of ghrelin systems may represent a feasible approach for psychostimulant addiction treatment. Supported by funds from NIDA-IRP

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Poster

603. Drugs of Abuse and Addiction: Cocaine Seeking and Reinstatement II

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Topic: G.08. Drugs of Abuse and Addiction

Support: R01 DA043988
P30 DA013429

Title: Inhibition of glycogen synthase kinase 3 disrupts cocaine-associated memories in the rat self-administration model

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Abstract: Addictive drugs stimulate associative learning processes, and subsequent exposure to drug-related contexts or cues previously associated with drug elicit conditioned responses that can trigger drug-seeking behaviors that can promote relapse after prolonged periods of abstinence. Therefore, learning and memory processes play an important role in the maintenance of addiction and disruption of the maladaptive associations between environmental cues and the memory of cocaine-induced reward may diminish cue-induced drug seeking behaviors and ameliorate relapse vulnerability. Glycogen Synthase Kinase 3 (GSK3) signaling is critical for the maintenance of cocaine contextual memories. Exposure to an environment previously paired with cocaine activates GSK3, and administration of the selective GSK3 inhibitor SB216763 after exposure to a cocaine-paired environment abolished a previously established place preference. We hypothesized that GSK3 inhibition after recall of cocaine self-administration memories will reduce subsequent cue reactivity. Adult Sprague Dawley rats were trained over 10 days to intravenously self-administer cocaine. After acquisition of stable self-administration behavior, rats underwent cue-induced reactivation followed by GSK3 inhibition (SB216763, 5 mg/kg, ip.). Inhibition of GSK3 immediately following reactivation attenuated previously acquired operant drug-seeking behaviors when tested 24 hours later. Furthermore, reactivation of cocaine cue memory resulted in the stimulation of GSK3 in the nucleus accumbens. These findings indicate that memory for a cocaine-paired stimulus depends critically on GSK3 activity in part within the nucleus accumbens. Current studies are determining the effect of GSK3 inhibition after cue exposure on subsequent cue-induced reinstatement as well as further anatomical substrates for GSK3 signaling important for cocaine cue memory reconsolidation.

Disclosures: X. Shi: None. **E.M. Unterwald:** None.

Poster

603. Drugs of Abuse and Addiction: Cocaine Seeking and Reinstatement II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 603.12/HHH26

Topic: G.08. Drugs of Abuse and Addiction

Support: DA040837

Title: Impact of intra-nucleus accumbens administration of MS-275, a class I HDAC inhibitor, on cocaine-seeking behavior

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Abstract: Recent evidence suggests that histone post translational modifications (PTMs), which can be modified by cocaine use, contribute to the relapse to cocaine addiction. Thus, pan-histone deacetylase (HDAC) inhibitors have been found to attenuate cocaine-seeking behavior in rodent models (Romieu et al. 2008; Romieu et al. 2011), which suggests that histone PTMs are a potential target for pharmacological intervention of cocaine addiction. However, whether specific classes of HDACs differentially influence cocaine-seeking is not fully understood. Class I HDACs are the most abundant HDACs in the brain and the most catalytically active HDAC class. Previous research has suggested that Class I HDACs may regulate histone PTMs which influence cocaine-related behaviors. When paired with repeated administration of non-contingent cocaine, chronic intra-NAc infusion of MS-275, a Class I HDAC inhibitor, altered both acetylation and methylation patterns in the NAc, suggesting that Class I HDACs broadly alter histone PTMs (Kennedy et al., 2013). Here, we expand upon prior studies to explore the role of Class I HDACs in cocaine-seeking behavior. We hypothesized that that MS-275, cocaine, and their combination will differentially alter histone PTMs in the NAc shell, and that repeated intra-nucleus accumbens administration of MS-275 would attenuate reinstatement of cocaine-seeking behavior. In rats that received repeated administration of non-contingent cocaine or saline, bilateral infusions of MS-275 (500 µM) or vehicle (1% DMSO) into the NAc shell differentially altered histone acetylation and methylation at specific histone residues in the NAc, suggesting that cocaine and MS-275 interact to alter histone PTMs. Following cocaine self-administration and extinction training, rats received bilateral infusions (2 µl/side) of MS-275 (500 µM) or vehicle (1% DMSO) into the NAc shell for three consecutive days. Three hours after the last intracranial infusion, rats were pretreated with cocaine (10 mg/kg, i.p.) and reinstatement of cocaine-seeking was assessed. This study advances our understanding of how particular classes of HDACs, specifically Class I HDACs, play a role in cocaine-relapse. Future studies will continue to explore the different epigenetic targets that are modified by cocaine use, such as different classes of HDACs, which may provide insight for future pharmacological intervention for cocaine relapse.

Disclosures: D.K. Fischer: None. **A.S. Thomas:** None. **S.E. Swinford-Jackson:** None. **M.C. Knouse:** None. **R.C. Pierce:** None.

Poster

603. Drugs of Abuse and Addiction: Cocaine Seeking and Reinstatement II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 603.13/HHH27

Topic: G.08. Drugs of Abuse and Addiction

Support: Commonwealth of Pennsylvania CURE Addiction Center of Excellence: Brain Mechanisms of Relapse and Recovery
NIDA U54 DA039002
NIDA R01DA039215

Title: Silence is golden: A “quieter” brain response to 6 sec cocaine video cues is linked to better drug use outcomes

Authors: *A. R. CHILDRESS, K. JAGANNATHAN, P. REGIER, J. J. SUH, Z. MONGE, K. A. YOUNG, S. DARNLEY, E. BERKOWITZ-STURGIS, M. TAYLOR, M. GAWRYSIAK, T. FRANKLIN, R. WETHERILL, D. LANGLEBEN, K. KAMPMAN, C. P. O'BRIEN
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Abstract: Aims: Addicted individuals who are less “triggered” by drug reminder cues may be less vulnerable to relapse. We hypothesized that this advantage might be reflected in a brain that is “quieter”, less reactive, in response to cocaine video cues.
Methods: Prior to outpatient treatment, stabilized cocaine inpatients were scanned with BOLD fMRI during exposure to a quasi-random alternation of 6 sec (Cocaine and NEUTRAL) videos, with instructions to either “WATCH” or try to reduce (“DOWN”) their response to the cocaine videos. The SPM 12 pipeline was used for pre-planned contrasts (e.g., WATCH vs. NEUTRAL, DOWN vs. NEUTRAL, thresholded $2<t<5$) for two clinical outcome subgroups: GOOD (< 30% cocaine urines pos/missing across 12 outpt. weeks; n=9); vs. POOR (>90% cocaine urines pos/missing; n=12). We divided the task trials in half, allowing us to examine “early” and “later” reactivity patterns in the task, as well as any *changes* in reactivity from the first to the second half of the task.
Results: Cocaine inpatients who would proceed to GOOD clinical outcome had low cue reactivity (a “quieter” brain response) to the cocaine video, for both the WATCH and DOWN conditions, and for both “early” and “late” trials. In contrast, patients who would go on to POOR outcome evidenced dramatic brain activations -- including classical motivational circuitry – to the cocaine cues. Interestingly, POOR patients demonstrated increased reactivity (in ventral striatum) to the neutral cues from the first half to the second half of the task (a potential indicator of new learning supported by the cues).
Conclusions: Cocaine patients with a "quieter" brain response to the brief cocaine videos had GOOD drug use outcomes; these patients were a small subgroup. A heightened brain response to the cocaine cues was common in the POOR outcome patients. These results highlight the potential of brain cue-reactivity paradigms for predicting clinical outcome, for screening anti-relapse interventions, and for identifying patients in greatest need of interventions to target their cue-vulnerability.

Disclosures: A.R. Childress: None. **K. Jagannathan:** None. **P. Regier:** None. **J.J. Suh:** None. **Z. Monge:** None. **K.A. Young:** None. **S. Darnley:** None. **E. Berkowitz-Sturgis:** None. **M. Taylor:** None. **M. Gawrysiak:** None. **T. Franklin:** None. **R. Wetherill:** None. **D. Langleben:** None. **K. Kampman:** None. **C.P. O'Brien:** None.

Poster

603. Drugs of Abuse and Addiction: Cocaine Seeking and Reinstatement II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 603.14/HHH28

Topic: G.08. Drugs of Abuse and Addiction

Support: 1T32DA039080-01
5RO1DA029122-04
New York Weill Cornell Center Alumni Council Award
Paul Fund

Title: Prelimbic $Ca_v1.2$ channels mediate stress-induced reinstatement via enhanced projection activity to nucleus accumbens core

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Abstract: Cocaine addiction is a growing problem, but there are currently no approved medications for the treatment of cocaine addiction, and current treatment options, such as rehabilitation, are not successful at preventing relapse. In fact, relapse rates for cocaine addiction are estimated to be around 40-60%, within the range of other chronic illness. Stress can be triggered by a variety of factors, including exposure to stressful life events as well as re-exposure to cocaine itself. Understanding the mechanisms by which these factors elicit relapse is critical in developing better treatment options. In addition to environmental factors, genetic factors can predispose to addiction risk. The gene *CACNA1C*, which codes for the L-type calcium channel $Ca_v1.2$, has been strongly associated with numerous neuropsychiatric conditions, such as bipolar disorder and schizophrenia, which show high comorbidity with addiction. *CACNA1C* has also been shown to influence reward-related behaviors and neural activity in humans, as well as addiction-related phenotypes in rodent models. Additionally, *cacna1c* has been shown to mediate the effects of stress on the brain, suggesting it could be a key mediator of stress-induced addiction-related behaviors like relapse. In the current study, we find that global heterozygous knockout of $Ca_v1.2$, as well as focal deletion of $Ca_v1.2$ within the prelimbic cortex, attenuates stress- and cocaine-induced reinstatement of cocaine conditioned place preference. We are utilizing techniques such as chemogenetics and fiber photometry to explore the $Ca_v1.2$-dependent circuitry downstream of the prelimbic cortex that mediates these behavioral phenotypes.

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Poster

603. Drugs of Abuse and Addiction: Cocaine Seeking and Reinstatement II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 603.15/HHH29

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA R01DA037257
S1-R01DA037257
R21DA044486
NIGMS R25GM09545902

Title: Chromatin remodeler INO80 mediates cocaine craving during prolonged withdrawal

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Abstract: While it is challenging for individuals with cocaine use disorder to achieve abstinence, the greatest challenge is avoiding relapse, which is triggered by drug-associated cues. Cue-evoked cocaine craving intensifies (or "incubates") during abstinence in human cocaine abusers and pre-clinical substance use models that is believed to contribute to persistent relapse vulnerability. Incubated cocaine craving is mediated in part by neuroadaptations in brain regions associated with reward and motivation, including the nucleus accumbens (NAc). While chromatin remodeling regulates drug-induced epigenetic plasticity, the role of multimeric chromatin remodeling complexes is unknown. Following extended-access cocaine self-administration, we found that INO80, a chromatin remodeling complex ATPase subunit, was increased in the NAc of cocaine-treated rats compared to saline controls on withdrawal day (WD)30 but not WD1. Using viral-mediated gene transfer, we determined that INO80 bidirectionally mediates the expression of incubated cocaine craving during prolonged withdrawal. To determine INO80 interactions with DNA, we performed chromatin immunoprecipitation followed by massively parallel DNA sequencing (ChIP-seq). Predicted pathways regulated by INO80 based on enrichment in cocaine-treated rats included cAMP response element binding (CREB) signaling and glutamate receptor signaling, suggesting that INO80 mediates gene expression of pathways that mediate cocaine plasticity. To determine how INO80 is regulated, we examined tripartite motif-containing protein 3 (Trim3), an E3 ubiquitin ligase (E3) in the ubiquitin-proteasome system (UPS) that regulates degradation of INO80. We

also found that Trim3 and polyubiquitinated INO80 were decreased on WD30 in cocaine-treated rats compared with saline controls, indicating that degradation of INO80 is reduced during prolonged withdrawal. Furthermore, viral-mediated gene transfer of Trim3 also bidirectionally mediated incubated cocaine craving. Together, these results demonstrate that INO80-dependent gene expression mediates cocaine-induced behavioral and cellular plasticity during prolonged withdrawal and E3 Trim3 regulates INO80 expression.

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Poster

603. Drugs of Abuse and Addiction: Cocaine Seeking and Reinstatement II

Location: SDCC Halls B-H

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Program #/Poster #: 603.16/HHH30

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant F99NS105217
Brain and Behavior Research Foundation NARSAD 24743
NIH Grant DA042111

Title: Epigenetic priming in the nucleus accumbens underlies relapse of cocaine-associated behaviors

Authors: *A. J. LOPEZ[1,2], M. KUTLU[2], A. R. JOHNSON[2], L. J. BRADY[2], K. C. THIBEAULT[2], E. S. CALIPARI[2]
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Abstract: Substance use disorder, a chronic relapsing neuropsychiatric disease, is characterized by the resilience of drug-seeking even following long periods of abstinence. Drugs of abuse, such as cocaine, are known to cause persistent changes in neuronal function throughout the circuitry regulating motivation, memory, and reward. Underlying these changes in circuit function are maladaptive changes in gene expression and synaptic plasticity caused by repeated drug exposure. Due to the persistence of both drug-seeking behaviors and drug-induced plasticity, the addiction field has implicated various epigenetic mechanisms as targets for drugs of abuse. Of note are the changes in the nucleus accumbens (NAc), a key regulator of cocaine-associated and cocaine-seeking behaviors. Recent work has demonstrated a critical role for histone acetylation in the NAc and has identified key genes critical in the acute and chronic effects of cocaine exposure. Yet, while the role of the NAc in cocaine self-administration has

been extensively studied, few studies have evaluated the long-lasting changes within this region contributing to reinstatement of cocaine-seeking. Recent studies in the Calipari lab have identified a unique gene expression profile in the NAc during cocaine-primed reinstatement, including dysregulation of genes with known roles in synaptic function such as *Oprk1*, *Scn4b*, and *Homer3*. We hypothesize that cocaine self-administration generates a long-lasting epigenetic environment which alters the integration of neural circuit activity within genetically defined cell types in the NAc and, ultimately, driving relapse. To test this, 8-week old c57BL/6J mice were trained to self-administer cocaine or saline for 10d. Following a 30d withdrawal period, animals received an acute saline or cocaine re-exposure (I.P.) and were sacrificed 1 hr later. In the NAc of cocaine re-exposed animals, we identified a subset of genes enriched for epigenetic marks previously shown to be dysregulated during reinstatement of cocaine-associated behaviors, including H3S10 phosphorylation, H3K9 acetylation, and H3K14 acetylation. Moreover, this subset of phosphoacetylation rich genes coincides with genes dysregulated in the NAc during cocaine-primed reinstatement. The results of this study provide evidence for epigenetic alterations leading to gene expression changes that make animals vulnerable to relapse. Future studies will identify a causal link between changes to epigenetic gene regulation and NAc circuit function during relapse-like behaviors.

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Poster

603. Drugs of Abuse and Addiction: Cocaine Seeking and Reinstatement II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 603.17/HHH31

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA IR21DA043150-01

Title: Pairing extinction of cocaine-seeking with vagus nerve stimulation reduces contextual reinstatement and modulates plasticity in extinction networks

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Abstract: Cocaine addiction can cause maladaptive neuroplasticity that persists long after cessation of drug taking. The relative permanence of cue associations formed during drug taking contributes to relapse. These cues and drug-associated environments can trigger relapse to drug use. Breaking the cue/drug association via extinction learning is one approach to preventing

relapse. We use vagus nerve stimulation (VNS) to enhance extinction from drug-seeking, and describe studies which examine the underlying mechanisms. We previously found that giving VNS during extinction of operant drug-seeking reduced the drug-seeking response during extinction and during cued reinstatement. Because we found facilitated extinction learning regardless if VNS was delivered contingently (with the non-reinforced operant response) or non-contingently (at fixed intervals throughout the extinction session), this raised a question: is VNS is also effective for extinguishing context in addition to extinguishing operant response? To answer this question, we used conditioned place preference (CPP) to examine the effect of VNS on contextual extinction in the absence of operant response. Animals which received VNS during extinction showed reduced preference for the cocaine-associated side compared to Sham animals. Additionally, animals that received homecage VNS immediately following drug side extinction sessions also showed reduced drug side preference. These findings suggest that VNS can facilitate the extinction of a drug-paired context. Next, we isolated the effect of VNS on extinction of an operant response by performing self-administration and extinction in two ABA experiments. First, animals were allowed to self-administer in context A, extinguish in context B, and reinstate in context A. Animals that got VNS during extinction in context B showed reduced reinstatement compared to Sham animals, suggesting a generalization of extinction from one context to another, or a strong extinction of the operant response. To further isolate the effect of VNS on extinction of the operant response, a second ABA experiment was conducted in which, in contrast to the first experiment, the levers were absent during extinction and animals were unable to operantly respond. The reciprocal pathways between the basolateral amygdala (BLA) and prefontal cortex (PFC) are involved in consolidating drug reward and expressing extinction learning. We used in-vivo recordings of evoked field potentials to examine changes in the connectivity between the BLA and mPFC, and then used high frequency stimulation to induce LTP. Pairing extinction with VNS altered the synaptic plasticity in this pathway.

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Poster

603. Drugs of Abuse and Addiction: Cocaine Seeking and Reinstatement II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 603.18/HHH32

Topic: G.08. Drugs of Abuse and Addiction

Support: NIMH

Title: Cholinergic receptors in the ventral tegmental area mediate both cue-induced cocaine-seeking and anxiety-related behaviors during cocaine abstinence

Authors: *E. J. NUNES, L. BITNER, S. WALTON, S. HUGHLEY, K. SMALL, L. E. RUPPRECHT, N. A. `. ADDY
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Abstract: Psychiatric co-morbidities, such as anxiety and depression, often accompany symptoms of cocaine craving and cocaine-paired cues to support resumed drug taking. The ventral tegmental area (VTA) to the nucleus accumbens core (NAc) pathway is necessary and sufficient for cue-induced cocaine craving. Furthermore, VTA dopamine (DA) neuron activity has been shown to contribute to depressive and anxiogenic-like behaviors in procedures such as the elevated plus maze (EPM). Cholinergic receptors in the VTA mediate DA neuron activity and phasic DA release in the NAc. We have previously shown that VTA cholinergic receptor blockade decreases cue-induced cocaine seeking on day 3 of abstinence. However, the role of VTA cholinergic receptors following longer cocaine abstinence periods is unknown. Thus, we trained male Sprague-Dawley rats to self-administer cocaine for 10 days followed by a forced abstinence period of 14 days. We show that VTA nicotinic and muscarinic receptor blockade with the non-selective nicotinic receptor antagonists, mecamylamine (10 ug, and 30 ug per side), or the non-selective muscarinic receptor antagonist, scopolamine (2.4 ug, 24 ug per side), decreased cue-induced cocaine seeking after 14 days of forced cocaine abstinence. Next, we sought to determine the role of VTA cholinergic receptor blockade on behavioral responses in the EPM in cocaine naïve rats. VTA blockade of nicotinic and muscarinic receptors was effective to reduce anxiogenic behavior in cocaine-naïve rats at the highest dose only tested on the cue-seeking test. A separate cohort of rats self-administered cocaine or saline for 10 days, followed by 14 days of forced abstinence On the 14^{th} day of abstinence, rats were tested using the EPM. Rats undergoing cocaine abstinence had increased anxiogenic-like responses on the EPM compared to rats that only received saline. Blockade of VTA cholinergic receptors, at the same doses tested above, attenuated this anxiogenic effect of cocaine on the EPM in a separate group of rats. Taken together, VTA nicotinic and muscarinic receptor blockade decreases cue-induced cocaine seeking and the anxiogenic effects of cocaine abstinence. These data point to overlapping roles of VTA cholinergic receptors and the ability to regulate multiple symptoms experienced during periods of cocaine abstinence. This suggests that targeting of VTA cholinergic receptors during periods of cocaine abstinence can reduce both cocaine craving and the anxiety associated with cocaine withdrawal. Future experiments will begin to identify what specific VTA muscarinic acetylcholine receptor subtypes mediate one or multiple symptoms observed during cocaine abstinence.

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Poster

604. Place Cells

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 604.01/HHH33

Topic: H.01. Animal Cognition and Behavior

Support: NSFC 31421003

Title: Item-location representations in the medial temporal lobe of non-human primates during a short-term-retention task

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Abstract: The conventional insight regarding item-location integration processes in the medial temporal lobe (MTL) describes a convergence of ventral and dorsal pathways into the hippocampus (HPC) via the perirhinal cortex (PRC) and the parahippocampal cortex (PHC). However, recent visual neuroscience studies suggest that integration proceeds along the ventral pathway prior to reaching the HPC. In this study, we investigated neuronal process encoding item-location information from the ventral pathway to the MTL. For this, we devised an item-location-retention (ILR) task requiring the subject to retain the identity and location information of a sample stimulus. A trial of the ILR task consisted of an encoding phase and a response phase with an interphasic interval (0.7-1.4 sec). In encoding phase, after presentation of a fixation dot at one of the quadrants on a display, one out of six objects was presented at the same quadrant (i.e., foveal view condition) for 0.3 seconds as a sample stimulus. Following the interphasic interval, the response phase was initiated with a fixation dot presented at the center. Subsequently, one of the six objects was presented in the center for 0.3 seconds as a cue stimulus. After another 0.5 seconds of delay period, five discs were presented as choice: one green disc at center, and four blue discs in the quadrants respectively. When the cue was same as the sample, the subject was required to choose a blue disc in the same quadrant as the sample located. Otherwise, the subject was required to choose the green disc. We recorded single-cell activities from the HPC (n=636), PRC (n= 436), PHC (n=245) in the MTL as well as TE (n =357) of two macaques. We examined item and location selective responses for individual cells during the encoding phase using two-way ANOVA. We found that a substantial number of neurons exhibited significant ($P < 0.01$) item-selective responses in the HPC (25%) as well as in the PRC and TE (18% and 20%), in contrast with the small proportion in the PHC (3%).

Interestingly, both PRC and TE showed as large a proportion of location-selective ($P < 0.01$) neurons (30% and 31%) as the HPC (22%) and PHC (20%) did. The proportion of location-selective neurons decreased in both MTL and TE (HPC:8%, PHC:6%, PRC:10%, TE:7%) when a sample stimulus was presented at a quadrant while the subject fixated at the center of display (i.e., peripheral view condition), though the percentage of item-selective neurons were similar (HPC:27%, PHC:3%, PRC:21%, TE:21%). These results suggest that when a subject encodes an item at a particular location on a background, both the PRC and TE as well as the HPC represent the identity of the item and its location, as viewed by a subject.

Disclosures: H. Chen: None. **Y. Naya:** None.

Poster

604. Place Cells

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 604.02/HHH34

Topic: H.01. Animal Cognition and Behavior

Title: Spatial view cells in the primate hippocampus: Properties demonstrated during active locomotion

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Abstract: Video animations will be presented to illustrate the properties of hippocampal spatial view cells recorded in macaques during active locomotion in a 2.7x2.7 m open field foraging environment located in the middle of a laboratory which provided a rich scene. The place, head direction, eye position, and location being fixated on the walls of the room are displayed every 25 ms during the playback of the neuronal spikes in these new analyses. These neurons recorded in CA3, CA1 and the parahippocampal gyrus (1) respond primarily to a view of space 'out there', with much less information about the place where the monkey is located; (2) have responses that depend on where the monkey is looking, as shown by measuring eye position; (3) can still occur (especially for CA1 neurons) if the view details are obscured with curtains; (4) retain part of their 'space' tuning even in complete darkness, for several minutes (especially in CA1); (5) have an allocentric spatial representation; and (6) utilize independent encoding in that the information about spatial view increases linearly with the number of cells in the representation. A computational model shows that the spatial representation may be different from that of place cells in rats because of the smaller field of view of primates due to the primate fovea. It has also been shown that some hippocampal neurons encode for objects, others for spatial view in a room, and others for a combination of objects and spatial view, while a monkey is performing an object-place memory task in which the place is 'out there' in the room. This task and the one-trial object-place associations formed by these neurons is prototypical of

episodic memory, and provides evidence that the primate hippocampus does associatively link information about objects and allocentric information about places 'out-there'. Recordings were made sufficiently long from 40 of the 708 neurons (5.6%) to provide evidence that they were spatial view neurons in this single environment, and in addition some neurons had place field responses.

Rolls,E.T. (2016) Cerebral Cortex: Principles of Operation. Oxford University Press: Oxford.
Rolls,E.T. and Xiang,J-Z. (2006) Spatial view cells in the primate hippocampus, and memory recall. Reviews in the Neurosciences 17: 175-200.
Rolls,E.T. and Wirth,S. (2018) Spatial representations in the primate hippocampus, and their functions in memory and navigation.

Disclosures: E.T. Rolls: None.

Poster

604. Place Cells

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 604.03/HHH35

Topic: H.01. Animal Cognition and Behavior

Support: CIHR
NSERC

Title: Reference frames for encoding of eye movements: A comparison between lateral prefrontal cortex and hippocampus in non-human primates

Authors: *B. W. CORRIGAN[1], R. A. GULLI[4], G. DOUCET[5], M. ROUSSY[2], R. LUNA[1], J. C. MARTINEZ-TRUJILLO[3]
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Abstract: Primate vision processing is highly developed, with very high acuity at the fovea which requires saccades to explore the visual world in detail. Saccade encoding has been measured area 8A of lateral prefrontal cortex, where neurons also encode several other visual features during attention and working memory (Bichot, Heard, DeGennaro, & Desimone, 2015; Boulay, Pieper, Leavitt, Martinez-Trujillo, & Sachs, 2016). The hippocampus (Hc) has also been reported to encode saccade target locations, and is involved in forming episodic memories(Rolls & Xiang, 2006). We set out to investigate presaccadic tuning for spontaneous saccades in these areas. We had four male macaques (macaca mulatta) perform a cued saccade task, but analysed spontaneous saccades in the intertrial intervals. Two subjects had Utah arrays (Blackrock Microsystems, Utah) in area 8A, and recorded from 520 neurons over six sessions. We used

single electrodes to record from the Hc of the other two NHPs and recorded from 80 neurons over 20 sessions. To analyze spatial selectivity, we binned screen space into 8°x8° bins and calculated firing rates for 200ms before the saccade onset for all saccades that landed in a bin while nothing was on the screen during inter-trial-intervals. And we also calculated this using bins centred at the point of fixation to get retinal coordinates. Using permutation testing on the Hc neurons, we found that 7 (9%) were selective for space in screen coordinates while 2 (3%) were selective for retinal coordinates. We found that of the PFC neurons, 125 (24%) were selective for space in screen coordinates while 191(37%) were selective for space in retinal coordinates. The high percentage of retinal centric target selective neurons in the PFC is expected from previous findings of saccade target encoding (Boulay et al., 2016). Finding more screen coordinate selective neurons might be predicted from the previous results from Rolls and results from the entorhinal cortex (Killian, Potter, & Buffalo, 2015), but the low numbers of neurons encoding spontaneous saccade targets suggests that encoding during active vision may be also encoding target information.

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Poster

604. Place Cells

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 604.04/HHH36

Topic: H.01. Animal Cognition and Behavior

Support: Alberta Heritage Foundation for Medical Research

Title: Spatial information encoding across multiple neocortical regions

Authors: *I. ESTEVES[1], H. CHANG[4], A. R. NEUMANN[2], S. JIANJUN[2], M. H. MOHAJERANI[3], B. L. MCNAUGHTON[5,6]
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Abstract: Hippocampal-cortical interactions are essential to spatial contextual learning processes. Hippocampal place cells (PC) have sparse coding characteristics and, when combined with 'rate remapping' produce experience-unique outputs from the hippocampus to the neocortex. Such patterns are hypothesized to provide an ‘index’ code to link distributed representations of specific experiences over the cortex and may coordinate memory retrieval. Recently it has been shown that superficial Retrosplenial cortex, a major target of hippocampal output, exhibits place-

cell like activity and that this phenomenon depends on an intact hippocampus. We enquire here whether such 'place-cells' can be found more broadly distributed in the cortex. Thy-GCamP6s mice were head-fixed and trained to move a treadmill belt with tactile cues. Cellular calcium imaging was conducted across different cortex regions and hippocampal CA1 region. In five mice a 5 mm craniotomy was made above the dorsal cortex (+1 mm to -4 mm AP and -2.5 mm to 2.5 mm ML) and a coverslip was attached to the skull. For hippocampus imaging, a cranial hippocampal window was performed in one mouse. This window was composed of a 1.5 mm glass cylinder with a 3 mm coverslip attached to one end. Combining the two-photon calcium imaging and the treadmill apparatus, we studied how the neural activity encodes spatial information in different cortical areas. With a systematic survey over the cranial window, we found neurons in multiple neocortical regions that exhibit spatially-localized activity with firing field that are robustly correlated with the animal position on the belt, similar to hippocampal place cells (PC) and Retrosplenial cells measured in the same task. Motor Area and Somatosensory cortex presented greater PC fractions than associational areas such as the Posterior Parietal Cortex and Retrosplenial Cortex in 4/5 of the animals recorded. Thus, there is a substantial population of neurons distributed widely over the cortex whose activity resembles hippocampal place cells. Our results provide support for the hypothesis that the HPF generates a spatiotemporal contextual ‘index’ code to link information distributed across the entire cortex. Furthermore, this study will help to shed light on how the hippocampus and neocortex interaction support both spatial navigation and memory.

Disclosures: I. Esteves: None. **H. Chang:** None. **A.R. Neumann:** None. **S. JianJun:** None. **M.H. Mohajerani:** None. **B.L. McNaughton:** None.

Poster

604. Place Cells

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 604.05/HHH37

Topic: H.01. Animal Cognition and Behavior

Support: NSF Grant 1707408
NIH Grant U01 NS094286-01

Title: Imaging long-term population dynamics of rat hippocampal place cells

Authors: *G. BLAIR[1], A. G. HOWE[2], P. GOLSHANI[4], H. T. BLAIR[3]
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Abstract: Populations of hippocampal place cells are thought to encode spatial memories—or “cognitive maps”—of familiar environments. To implement this population code for space,

individual place cells fire at their own preferred firing locations in a given environment[1]. It was previously assumed that a place cell's spatial tuning function does not change (or changes very slowly on a time scale of many days) across repeated visits to the same familiar environment[2]. But recently, single-unit recording studies in rats[3,4] and calcium imaging studies in mice[5] have suggested that hippocampal place cells exhibit rapid changes in their firing properties across repeated visits to the same familiar environment. This raises new questions about the long-term stability of the hippocampal place code. To address these questions, it is important to understand how place cell stability varies across species (e.g., rats versus mice), and how it might be influenced by methodological confounds (e.g., tissue damage arising from lens implantation in calcium imaging studies). Prior calcium imaging studies of long-term place cell dynamics have been carried out in mice[5], so here, we used the UCLA miniscope to obtain long-term population recordings of hippocampal place cells in freely behaving rats. Rats were injected with 1.0 uL of AAV9-Syn-GCaMP6f into the CA1 pyramidal layer, followed by implantation of a 1.8 mm diameter GRIN lens above the stratum oriens. Four weeks after virus injection, rats were imaged for more than a month, alternating every other day between open-field and linear track environments. Similar to results from mice, population participation within each environment was highly transient across the experimental timeframe. However, place field locations were relatively stable, consistent with the proposed function of rate remapping across time, with relatively little global remapping within the same environment[6]. In future work, we will carry out electrophysiological recording and calcium imaging of place cells from the same rats, to assess how place cell stability depends upon recording methods.

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[2]Lever, C.*et al.* (2002) *Nature*. 416**,** 90.
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[4]Mankin, E. A. *et al.* (2015) *Neuron.* 85**,** 190.
[5]Ziv, Y. *et al.* (2013) *Nat. Neuro.* 16**,** 264.
[6]Leutgeb, J. K. *et al.* (2005) *Neuron* 48**,** 345.

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Poster

604. Place Cells

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Program #/Poster #: 604.06/HHH38

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant NIDA 5R21DA041857-02

Title: Probing neurophysiological substrates of LSD-induced hallucinations in freely behaving rats

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Abstract: The compound lysergic acid diethylamide (LSD) produces visual hallucinations, which are subjective perceptions that occur disjoint from external stimuli. Mounting evidence supports that visual hallucinations arise from activation of 5-hydroxytryptamine-2-A receptor (5-HT2AR) and are behaviorally correlated with the head twitch response (HTR) in rats. By studying disorganized states of consciousness like that induced by LSD, we can better understand how the brain orchestrates the transduction of external inputs and internal signaling to culminate in our subjective experience. To examine the neural substrates of hallucinations, we employed tetrode recordings in the CA1 of hippocampus and the primary visual cortex V1 of freely moving Long-Evans rats as they ran laps on a familiar track. Each rat is recorded twice on the track in the same day with a three-hour rest between: In the first track session, rats are administered saline, and in the second track session they are administered a high or low dose of LSD (.24 mg/kg or .06 mg/kg) or a 5HT2AR antagonist (M10097) prior to LSD administration. Rats demonstrate a significant HTR with LSD administration that is not observed in the LSD and antagonist condition. Further, rats in the LSD conditions have poor lap running behavior and pause often throughout the second track session. Consequently, we observe high voltage spike (HVS) events during immobility in rats in the LSD condition. HVS are high amplitude population activity associated with wake-to-sleep transitioning in rodents. We also see changes in neural population firing rates just prior to the HTR in CA1 and V1 that may correspond to a decoupling of CA1 from the primary sensory area that precedes behavioral expression of hallucination. Further analyses aim to answer whether these false percepts are linked to neural activity associated functionally with a sleep state alongside otherwise wakeful behavior.

Disclosures: C. Domenico: None. **D.C. Haggerty:** None. **X. Mou:** None. **D. Ji:** None.

Poster

604. Place Cells

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Program #/Poster #: 604.07/HHH39

Topic: H.01. Animal Cognition and Behavior

Support: Kaken-hi 17H05939
Kaken-hi 17H05551

Title: Changes in synchronous spike patterns of hippocampal neurons associated with learning of an optimal route in a spatial detour task

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Japan; [2]Precursory Res. for Embryonic Sci. and Technol., Japan Sci. and Technol. Agency, Kawaguchi, Saitama, Japan

Abstract: Animals learn the best spatial navigation strategy to a goal through experiencing multiple possible strategies. During this learning process, activity patterns of brain circuits gradually converge into the most optimal ones by evaluating outcomes resulting from exploratory behavior. Accumulated evidence has shown that the hippocampus plays an important role in spatial learning by representing current and future episodes. Especially, awake hippocampal replay, in which temporally compressed sequential patterns of place cells are reactivated corresponding with running trajectories, have been considered to support memory consolidation and future planning. In this study, we designed a spatial task in which rats learn to take a specific route point to a fixed goal in a two-dimensional task field. In this task, a trial began when the animals performed an active nose poke in a start box where sucrose water was presented for 10 seconds. A start door was then opened so that the rats entered into the field. The rats could obtain reward at a goal box if they correctly stopped at a specific point where a small amount of chocolate milk was placed on the way to the goal box. After the rats continuously performed the same task for 3-4 days, a reward point was changed to a different point. The rats first showed exploratory behavior throughout the field after the rewarding rule was changed but could learn a new optimal route through the trial-and-error exploration. During this learning process, a multiunit recording was performed from hippocampal CA1 neurons. Especially, we focused on synchronous events of neurons, in which instantaneous firing rates were increased to 3 standard deviations above the baseline or more than 4 neurons showed co-firing. The number of synchronous events was increased and event-to-event correlation of synchronous events was increased after the rewarding rule was changed, demonstrating that the frequency of recruiting specific sets of neuronal ensembles within synchronous events was pronouncedly increased associating with a change in behavioral strategy. These results suggest that the contents of hippocampal neuronal reactivation might be prioritized by learning, supporting reinforcement of a specific behavior pattern.

Disclosures: H. Igata: None. **T. Sasaki:** None. **Y. Ikegaya:** None.

Poster

604. Place Cells

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 604.08/HHH40

Topic: H.01. Animal Cognition and Behavior

Support: RIKEN BSI
Howard Hughes Medical Institute
JPB Foundation

Title: Serial cells track the global temporal ordering of discrete episodic events

Authors: *C. SUN[1], W. YANG[2], J. MARTIN[1], S. TONEGAWA[1]
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Abstract: Daily episodic experience occurs as a sequence of events. Hippocampal neurons, essential for episodic memory, have spatial codes, but whether they code the pure temporal order of events in an episode is unknown. Here, we report hippocampal "serial cells" that track the discrete and successive serial order of events, as mice experience a series of materially indistinguishable yet temporally distinguishable events. The serial order code is degraded when the temporal relations between events are disrupted, and the code is remapped when temporal relations between events are globally altered, suggesting that serial cells track the pure temporal order structure of episodic events. Serial cells have place fields but the serial order code is preserved even when place fields globally remap, showing that the serial code is conjunctive with the spatial code but is independent of it. The serial code, tracking the pure serial ordering of events, may be one of the fundamental ingredients by which our brain represents episodic events.

Disclosures: C. Sun: None. **W. Yang:** None. **J. Martin:** None. **S. Tonegawa:** None.

Poster

604. Place Cells

Location: SDCC Halls B-H

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Program #/Poster #: 604.09/HHH41

Topic: H.01. Animal Cognition and Behavior

Support: AcRF Tier 2 (MOE2015-T2-2-035), Ministry of Education, Singapore

Title: Activity-regulated cytoskeleton-associated protein, Arc, is required for the broad tuning of neuronal firing in the hippocampal CA1 area

Authors: L. YUAN, M. FALLAHNEZHAD, Y. WANG, I. ÅMELLEM, C. CHANG, *A. TASHIRO
Nanyang Technological Univ., Singapore, Singapore

Abstract: Tuning of neuronal response is a basic element underlying neural information coding. Two examples of such neuronal tuning are found in principal neurons in the hippocampal CA1 area. First, these neurons are tuned to specific areas in an environment and therefore called place cells. Second, they are tuned to a specific phase of theta oscillations, which is referred to as phase locking. Narrow tuning is beneficial for individual neurons to code specific information while broad tuning allows a group of neurons to function together. Therefore, the breadth of tuning is important for neural circuit functions. However the molecular mechanism by which

neurons achieve a proper breadth of tuning is unknown. Here, we focused on an immediate early gene encoding activity-regulated cytoskeleton-associated protein (Arc, also known as Arg 3.1), which is expressed in response to neuronal activation and regulates synaptic functions. We knocked down Arc gene using virus-mediated RNA interference in a portion of hippocampal CA1 area in rats, and monitored the activity of principal cells in the manipulated area. We found place cell activity both in control and Arc knockdown groups. However, Arc knockdown group showed more specific spatial firing patterns. Further, while firing rate was reduced all over the environment in Arc knockdown group, this reduction was more extensive outside of place fields than inside. This biased reduction outside of place fields resulted in narrower spatial tuning. In addition, we found that higher proportion of firing occurs around a specific phase of theta oscillations in Arc knockdown principal cells, indicating that Arc knockdown principal cells show stronger phase locking to theta oscillations or, in other words, narrower tuning to the specific phase. Next we examined the consequence of Arc knockdown in neuronal network functions. We found that Arc knockdown principal cells cofire less frequently than control. Further, using a computational modeling approach to estimate the animal's position based on the experimental data from groups of neurons, Arc knockdown groups exhibited higher extent of errors. These results support impaired network functions in Arc knockdown group. These effects of Arc knockdown on single-cell and network functions suggest that, under normal conditions, Arc plays a role in establishing the broad tuning of individual neurons. This broad tuning in individual neurons may, in turn, increase the functional interaction among neuronal populations and maximize its information processing capability.

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Poster

604. Place Cells

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Program #/Poster #: 604.10/HHH42

Topic: H.01. Animal Cognition and Behavior

Support: AcRF Tier 2 (MOE2015-T2-2-035), Ministry of Education, Singapore

Title: Long term characterisation of pyramidal cell activity in the hippocampal CA1 area using microendoscopic calcium imaging

Authors: *C. P. KOH[1], L. F. COBAR ZELAYA[2], A. TASHIRO[2]
[1]Nanyang Technological Univ. (NTU), Singapore, Singapore; [2]Sch. of Biol. Sci., Nanyang Technological Univ., Singapore, Singapore

Abstract: Through the use of electrophysiological techniques, it has been shown that pyramidal cells in the hippocampal CA1 area exhibit place cell activity, having place fields that can be stable up to months. However, more recent work using calcium imaging demonstrated that pyramidal cells shift between being active and inactive across days, although they tend to show place cell activity with constant place fields when they are active. Thus, place cell activity seems to be less stable between days than initially thought, but how their activity varies between days and over months is still unclear. Therefore, we performed long-term characterisation of place cell activity to elucidate the day-to-day changes over the course of three months. We injected adeno-associated viral vectors expressing a calcium indicator, GCaMP6s, under the control of the CaMKIIα promoter into the hippocampal CA1 region of mice to express GCaMP6s specifically in pyramidal cells. While these mice ran back and forth in a 1-m long linear track, we carried out calcium imaging using a microendoscope to monitor CA1 pyramidal cell activity. With this method, we performed long-term imaging from the same mice and were able to identify the same set of pyramidal cells over three months. We found that some cells were active on all the days analysed, but others were active only on some days and remained inactive on the other days. For cells that were always active, we observed that their frequency of calcium events differed between imaging days, and a majority of the calcium events occurred when the mouse was stationary at one, but not the other, end of the linear track. In addition, on some of the imaging days analysed, some cells displayed calcium events while the mouse was running. Most of those calcium events began at one end of the track when the mouse started running. These events continued for 0.8-3 sec, over 25-80 cm of the track, and can be regarded as place cell activity. Furthermore, on some imaging days, calcium events occurred at both ends of the track when the mouse started running in both directions. In conclusion, it appears that the activity of CA1 pyramidal cells in freely moving mice, as measured by intracellular calcium levels, shows substantial fluctuations over time.

Disclosures: C.P. Koh: A. Employment/Salary (full or part-time):; School of Biological Sciences, Nanyang Technological 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.; AcRF Tier 2 (MOE2015-T2-2-035), Ministry of Education, Singapore. **L.F. Cobar Zelaya:** A. Employment/Salary (full or part-time):; School of Biological Sciences, Nanyang Technological 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.; AcRF Tier 2 (MOE2015-T2-2-035), Ministry of Education, Singapore. **A. Tashiro:** A. Employment/Salary (full or part-time):; School of Biological Sciences, Nanyang Technological 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.; AcRF Tier 2 (MOE2015-T2-2-035), Ministry of Education, Singapore.

Poster

604. Place Cells

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 604.11/HHH43

Topic: H.01. Animal Cognition and Behavior

Support: Welcome Trust Grant 103896AIA

Title: Place cells represent three-dimensional, volumetric space anisotropically

Authors: *R. GRIEVES, S. JEDIDI-AYOUB, K. MISHCHANCHUK, K. J. JEFFERY
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Abstract: Place cells in the hippocampus represent places by increasing their firing rate when an animal visits specific regions of its environment, these regions of high firing are known as 'place fields'. In flat mazes place fields are typically small and round. However, animals must often navigate complex three dimensional environments. Do place cells represent three-dimensional volumetric space and if so, how?

In freely flying bats place fields have been observed to form spheres, suggesting that these animals can localise themselves equally well in all dimensions. In contrast, fields recorded in rats exploring vertical climbing walls form vertical columns, suggesting that they are less able to localise themselves vertically. Thus, we propose that rats have an anisotropic representation of space; they are less able to localise themselves vertically and their place fields will form elongated vertical columns in three-dimensions. To investigate this we wirelessly recorded the activity of place cells in a three-dimensional volumetric space; a cubic lattice composed of horizontal and vertical climbing bars.

Over the course of 34 sessions (each 60 minutes long) we recorded a total of 428 place cells in this maze from 8 rats. Rats exhibited a strong bias in their style of locomotion; they navigated significantly more frequently and at a faster rate along the horizontal dimensions. Place fields had normal horizontal characteristics but many were elongated vertically. Decoding the position of animals in this maze using only the activity of place cells revealed lower spatial information in the vertical dimension. These results suggest that place cells represent three-dimensional volumetric space anisotropically.

To determine if this is related to the rats' bias in locomotion or the geometry of the maze we also recorded a further 225 place cells from 3 rats in the same lattice maze, rotated to stand on one vertex, so that all horizontal and vertical climbing bars were instead diagonally sloped. Results thus far suggest that in this environment no place fields form vertical columns, they are more likely to form spheres and those that are elongated do so along one diagonal axis of the lattice maze.

Place cells recorded in freely flying bats have been shown to exhibit near spherical place fields,

why might this differ in rats? Our results suggest that the place fields of rats are vertically elongated in a cubic lattice, but that this is dependent on the structure of the lattice and the locomotor constraints on the rat rather than allocentric dimension. Thus, in rats, the representation of volumetric space is shaped by the affordances of the environment for locomotion.

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Poster

604. Place Cells

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Program #/Poster #: 604.12/HHH44

Topic: H.01. Animal Cognition and Behavior

Support: Wellcome Trust
ERC
BBSRC

Title: All-optical manipulation of place cells drives spatially associated behaviour

Authors: *N. T. ROBINSON[1], L. A. L. DESCAMPS[1], L. E. RUSSELL[1], C. SCHMIDT-HIEBER[2,1], M. HAUSSER[1]
[1]Univ. Col. London, London, United Kingdom; [2]Inst. Pasteur, Paris, France

Abstract: Hippocampal place cells fire when an animal occupies a specific location in an environment and are thought to play an important role in memory formation and spatial navigation. However, the causal role of place cell firing in driving decision making during spatial navigation has remained elusive. Addressing this question requires targeted manipulation of specific place cells in navigating animals. Here we have used an “all-optical” approach for targeted manipulation of place cell activity in head-fixed mice navigating a virtual reality environment. Using simultaneous two-photon calcium imaging and two-photon optogenetic holographic stimulation, we functionally define and manipulate the activity of populations of place cells with a specific firing field location and examine the impact on behaviour. Mice were trained to stop and lick for reward at a location on a linear track. Place cells were identified and grouped into neurons which fired either at the rewarded zone or an equivalent area near the start of the track. We then selectively activated either the reward zone or non-reward zone place cells at a separate equidistant location on the virtual track to test whether the behavioural output associated with the originally encoded location can be retrieved. Our preliminary results suggest that activation of appropriate place cells can drive reward-associated behaviour. This approach

enables us to probe the causal relationship between hippocampal neural activity patterns and memory retrieval to guide behaviour.

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Poster

604. Place Cells

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Support: NIH Grant R01-MH101297
NSF Grant NSF/CRCNS-1516235
McKnight Foundation

Title: Investigating multisensory integration by place cells in visual + olfactory virtual reality

Authors: *B. A. RADVANSKY[1], D. A. DOMBECK[2]
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Abstract: Mammals are believed to discern their locations in their environments using a cognitive map - a stored internal representation of space. Yet, the brain has no mechanism to directly detect “space.” Rather, it draws upon sensory perceptions of sights, sounds, smells, etc. to *construct* the cognitive map of space. Therefore, the mapping of space must ultimately be rooted in the senses. The overwhelming sensory complexity of the real world, however, has made it difficult to investigate how individual senses, or combinations of senses, contribute to and are represented by the cognitive map. Here, we address this challenge by using virtual reality to control a multisensory visual + olfactory landscape. We trained head-fixed mice on a spherical treadmill to navigate a multisensory linear track comprised of numerous proximal and distal visual cues overlaid with two olfactory gradients. The sensory cues making up the linear track were designed such that each location was uniquely defined using either visual or olfactory cues (or both). We established a multisensory-guided spatial behavior in which mice attended to both visual and olfactory spatial features during virtual navigation. To determine the sensory composition of the cognitive map engaged during this behavior, we systematically manipulated visual vs. olfactory features while recording from “place cells” in hippocampal region CA1 using 2-photon calcium imaging. We deconstructed the spatial tuning of each neuron into visuospatial and olfacto-spatial components based on responses to each sensory feature manipulation. From the population responses of these neurons, we determined how the different sensory sub-components of the environment were represented by the cognitive map.

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Poster

604. Place Cells

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Topic: H.01. Animal Cognition and Behavior

Support: MSCA-IF H2020 709328
ERC 692692

Title: CA3 population activity during free running on a virtual circular track

Authors: *B. A. SUTER, C. BORGES-MERJANE, Y. BEN SIMON, P. JONAS
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Abstract: Hippocampal region CA3 is believed to implement pattern completion, in particular the CA3a region (Lee et al, 2015), which is densely innervated by the dentate gyrus (DG) via the mossy fiber tract. Recurrent connectivity between CA3 principal neurons is sparse, yet finely tuned to optimize information storage (Guzman et al, 2016). Mossy fibers from the DG form powerful synapses onto CA3 pyramidal neurons, capable of one-to-one action potential transmission in vitro (Vyleta et al, 2016), however DG granule cells exhibit very sparse activity in vivo (Danielson et al, 2016; Pilz et al, 2016). Recently, our lab found that the small fraction of active DG granule cells exhibit a diversity of activity types, from single action potentials to super-bursts during head-fixed locomotion. We are interested in determining the overall activity pattern of CA3 at the population level, and as it relates to this sparse yet diverse input from the DG, in order to understand how information is shaped along the hippocampal axis.
To this end, we aim to quantify the distribution of firing rates of CA3 pyramidal neurons in the awake, behaving animal. Extracellular recordings report high firing rates, including bursting, in individual CA3 pyramidal neurons, but may be unable to detect silent and sparsely firing neurons. We therefore use concurrent two-color, two-photon imaging in the head-fixed mouse to record neural activity while simultaneously visualizing anatomical landmarks and individual neurons in the second, structural channel. This permits us to count CA3 pyramidal neurons in an unbiased manner, and quantify their activity patterns during behavior on a circular virtual reality track.
In order to image identified CA3 pyramidal neurons, we used transgenic mice expressing a red fluorophore in the DG with the labeled mossy fiber tract as a landmark for the CA2/CA3 border. We targeted viral injections into CA3 to transfect neurons with a red fluorophore in the nucleus, to allow for unbiased detection of neurons independent of their activity, while co-expressing the most sensitive calcium indicator currently available (GCaMP6s). Using the red (structural) channel to identify a dense sample of 89 CA3 pyramidal neurons, we manually defined somatic

ROIs and extracted time-varying Calcium traces from the green channel, restricting our analysis to periods of forward locomotion (speed > 2 cm/s). We used MLSpike (Deneux et al, 2016) to estimate the underlying spike trains and found a broad range of firing rates: average 0.58 Hz, standard deviation 0.53 Hz. The highest average rate across the population was 2.4 Hz, while 2 (of 89) neurons never fired during the duration of the acquisition.

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Poster

604. Place Cells

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Title: Spatial and temporal stability deficits in hippocampal place cells following moderate prenatal alcohol exposure

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Abstract: Spatial memory and navigation impairments are common following prenatal alcohol exposure (PAE) in humans and in animal models. Hippocampal neurons, some of which are highly modulated by environmental locations i.e. place cells, display significant synaptic and structural alterations after PAE. Each hippocampal place cell fires in a unique environmental location indicating that a large population of these cells covers the spatial layout of each environment encountered by the animal. It is currently unknown whether the spatial and temporal coding characteristics of hippocampal place cells are altered in PAE. Thus, we performed electrophysiological recordings from the hippocampus (CA1 and CA3) of adult male rats exposed to either moderate amounts of ethanol or saccharin prenatally. Hippocampal neural activity was monitored in two behavioral paradigms in which rats performed laps to each end of a narrow linear track (120 x 9cm) or while randomly foraging in a circular open field (76cm in dia). Each recording session on the track or in the cylinder was ~20 min in duration. Similar numbers of hippocampal place cells were identified in both PAE and saccharin exposed animals. However, place cells recorded in PAE animals exhibited larger field sizes in both the linear and

circular environments. Further, place cells recorded in PAE animals on the linear track displayed inconsistent firing as they progressively ran laps and often took several laps on the linear track to initiate firing. In contrast, place cells from control animals displayed stable firing throughout all laps. Finally, place cells are known to change their spike timing in such a way that cells fire at progressively earlier phases of the extracellular theta rhythm as the animal passes through their respective place fields. This phenomenon is known as theta-phase precession, and is thought to be supported by medial entorhinal cortex input. Importantly, while place cells from PAE animals had deficits in stable firing, they did not show deficits in theta-phase precession relative to control place cells. Collectively, the broader tuning and instability of hippocampal place cells provides a potential mechanism to explain spatial memory impairment after PAE.

Disclosures: R.E. Harvey: None. **L.E. Berkowitz:** None. **D.D. Savage:** None. **D.A. Hamilton:** None. **B.J. Clark:** None.

Poster

604. Place Cells

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 604.16/HHH48

Topic: H.01. Animal Cognition and Behavior

Support: R01NS105472
R01MH099128

Title: Neurobiology of learning to learn; long-lasting, input-specific synaptic circuit function changes in hippocampus

Authors: *A. CHUNG, A. A. FENTON
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Abstract: Cognitive behavior therapy (CBT) improves learning and memory, such that the brain learns to learn. The dominant CBT-neuroplasticity hypothesis asserts that CBT causes neural plasticity to change brain function but predicted evidence of 1) CBT-induced 2) long-lasting, and 3) memory-independent changes in synaptic circuit function is lacking. We previously reported persistent *ex vivo* and anesthetized *in vivo* electrophysiological changes in GABAergic-sensitive hippocampus synaptic function after learning an active place avoidance task that requires intact hippocampal activity, and persistent PKMzeta-mediated long-term potentiation (LTP) of synaptic function. Here, we test the three predictions of the CBT-neuroplasticity hypothesis in freely-behaving mice. Adult mice were implanted with sets of stimulating electrodes in the perforant path entorhinal cortex (EC) input to DG and CA1 along with 32-site recording electrodes that spanned the somatodendritic axis of dorsal hippocampus. Evoked potential responses were measured in DG in response to 0-250 μA test pulses before and 2h after each

training session in either active place avoidance or control tasks with minimal cognitive demand. Initial training reduced the fEPSP slope in the molecular layer of the supra-pyramidal blade of DG (supDG); changes were minimal in the hilus population spike and at the infra-pyramidal blade (infDG). Current source density analysis showed that training reduced the sink at the inner molecular layer of supDG where medial EC axons terminate. These changes persisted at least 60 days without further training. Additional training to acquire a new place memory in the same environment, or to learn a new place avoidance in a novel environment showed that subsequent learning was improved and that the initial training-induced changes occluded further changes of synaptic function. DG expression of PKMzeta increased after training compared to the control groups. Specifically, somatostatin (SST) expressing GABAergic neurons showed increased PKMzeta expression, particularly in supDG where interneurons predominate. Optogenetic manipulations of mice expressing ChR2 in Gad2 expressing neurons were studied to localize the training-induced inhibitory synaptic function changes. These findings confirm predictions of the CBT-neuroplasticity hypothesis, demonstrating that cognitive training during which mice learn to learn, can cause persistent hippocampus synaptic circuit function changes coincident with increased plasticity-protein expression at specific post-synaptic interneuron subtypes, independent of synaptic changes that may encode memories.

Disclosures: A. Chung: None. **A.A. Fenton:** None.

Poster

604. Place Cells

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Topic: H.01. Animal Cognition and Behavior

Support: R01NS105472
R01MH099128

Title: Remapping 2.0 : Ensemble coding in the hippocampus

Authors: *E. R. LEVY, E. PARK, A. A. FENTON
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Abstract: Action potential discharge in rodent hippocampus is thought to encode spatial information using an across-cell - “ensemble” - place code, such that each environment is represented by a set of ensemble discharge patterns - “activity vectors” - each of which is characteristic for a particular environmental location. Activity vectors typically change smoothly from one location to the next, but the set of activity vectors can also change abruptly, a phenomenon called “remapping.” Remapping is most reliably observed between distinct environments as the sets of activity vectors tend to be unrelated between environments. Instead

of using activity vectors, remapping is standardly depicted as a change in cell-specific "place fields", which describe location tuning of a single cell. Place fields are quantified using firing rate maps, each of which describes the activity of a single cell as a function of location. Place field remapping necessarily implies ensemble remapping. However, because ensemble remapping can occur in a single environment, without place field remapping, the relationship between activity vectors and environments is uncertain (e.g. Kao et al., 2017, J. Neurosci. 37:12031; Fenton et al., 2010, J Neurosci. 30:4613). Here we evaluate the hippocampus ensemble place code across different environments to investigate how activity vectors differ between distinct environments. We recorded the activity of mouse CA1 neurons expressing virally-induced GCaMP6f with a bright-field miniature microscope placed above a surgically implanted GRIN lens. In a "physically distinct" paradigm, freely-behaving mice alternated between exploring two visually and geometrically distinct environments 3 days a week, for 3 weeks. In a "behaviorally distinct" paradigm, mice alternated between two similar environments: in one of them they learned to avoid an unmarked shock zone in a hippocampus-dependent active place avoidance task; in the second environment mice did not show avoidance behavior because a clear Perspex floor prevented shock. We find that although activity vectors in the same environment significantly reproduce across days, they become more distinct with longer intervals. Ensemble activity vectors are more distinct (but not independent) between different environments than across days in the same environment and these patterns maintain whether or not cells with place fields are included in the ensemble comparisons. The properties of remapping are compared as evaluated by 1) correlating single cell firing rate maps, 2) correlating ensemble activity vectors, and 3) decoding position information from ensemble activity.

Disclosures: E.R. Levy: None. **E. Park:** None. **A.A. Fenton:** None.

Poster

604. Place Cells

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant DC012630
NIH Grant AA024983

Title: Linear self-motion cues contribute to hippocampal place cells: Functional implications

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Abstract: The vestibular system contributes to the activity of hippocampal place cells and navigational performance. We tested whether the vestibular contribution to place cells includes linear self-motion signals originating in the otolith organs by recording hippocampal place cells from otoconia-deficient *tilted* mice and littermate controls, across five consecutive recording sessions [standard, 90° cue rotation, standard, darkness, and standard]. This procedure enabled evaluation of place cells' basic firing characteristics, stability within and across trials, response to cue rotation, and reliance on visual information. *Tilted* mice's place cells showed reduced coherence across all testing sessions, regardless of changes in cue position or room lighting. *Tilted* place cells also showed reduced intra-session stability, indicating impairments in the ability to represent location across short time scales. Following cue rotation, *tilted* place cells showed less accurate rotations with the cue, suggesting deficits in landmark control over place fields. *Tilted* place cells also represented locations closer to environmental boundaries, relative to control place cells, suggesting an increased reliance on tactile cues. These place cell deficits suggest that putative associated functions (e.g., place recognition) crucially rely on signals from the otolith organs. We tested this prediction by evaluating *tilted* mice's search strategy and place recognition on two versions of a Barnes maze: a 69 cm maze with 8 days of training, and a 120 cm maze with 4 days of training; for both mazes, a probe trial was conducted one day after the end of training. Control mice, but not *tilted* mice, preferentially used a directional search strategy by the 8th day of training on the small maze. Control mice also preferentially used a directional search strategy by the 2nd day of training on the large maze, whereas *tilted* mice failed to show a significant preference by the last day of training. Somewhat surprisingly, both groups clearly showed a preference for the former goal quadrant on the subsequent probe trial for both mazes, suggesting intact place recognition. Overall, our recordings suggest that otolith signals contribute to place cells' ability to represent locations distant to walls, possibly via the head direction signal or associated brain signals such as the grid cell or boundary vector cell signal. Our behavioral tests suggest that otolith signals contribute to the use of a directional search strategy, but are not required for accurate place recognition.

Disclosures: R.M. Yoder: None. **R.E. Harvey:** None. **S.A. Rutan:** None. **L.C. Carstensen:** None. **G.R. Willey:** None. **C.A. Terry:** None. **J.J. Siegel:** None. **B.J. Clark:** None.

Poster

604. Place Cells

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Program #/Poster #: 604.19/HHH51

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01NS105472
NIH Grant R01MH099128

Title: Dentate spike modulation of hippocampal activity

Authors: *D. DVORAK[1], A. CHUNG[2], N. HUSSAIN[2], A. A. FENTON[1]
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Abstract: Dentate spikes (DS) are large amplitude, short duration field potentials that can be localized to the hilar region of the dentate gyrus, and may play a role in learning, memory consolidation and stabilization of the hippocampal circuit through anti-excitation. While the exact roles of DS are unknown, prior observations suggested the hypothesis that excessive DS may underlie cognitive flexibility deficits in Fmr1-null mice that model the genetic defect in Fragile X Syndrome (FXS) and express representational inflexibility of hippocampus place memory representations (Dvorak et al., PLoS Biol. 16: e2003354).We recorded local field potentials (LFPs) from freely-behaving wild-type and Fmr1-null mice during sleep and open-field exploration, as well as during aversively-motivated active place avoidance tasks, two unreinforced object spatial novelty tasks, and the three-chamber social novelty tests. LFP recordings using 32-channel linear silicon probe arrays that spanned the dentate gyrus and CA1 of dorsal hippocampus allowed current source density analysis and identification and classification of DS events.We find that Fmr1-null mice are impaired when behavior requires cognitive control to judiciously use relevant and ignore irrelevant information in memory. Although conditioned-place learning and memory of Fmr1-null mice are both normal for the initial location of an avoidable shock, the mice are impaired on conflict trials when the shock is relocated opposite to the initial location. Unlike wild-type, Fmr1-null mice fail to show the normal early preference for novelty in an object/place mismatch when the locations of two of four familiar objects are exchanged in the same environment and in an object/context mismatch when two of four objects are exchanged between environments. While Fmr1-null mice express a normal preference for mice over inanimate objects, their preference for novel mice over familiar mice is weaker than the wild-type's.We detected exaggerated rates of DS events in Fmr1-null mice. Whereas wild-type DS rates decrease dramatically during conflict trials, Fmr1-null DS rates remain high. Fmr1-null DS rates are also exaggerated compared to wild-type during exploration of place-mismatched and context-mismatched familiar objects, and during exploration of novel but not familiar mice during social discrimination.These findings point to a possible role for dentate spikes in maintaining hippocampal representations, and that the cognitive representational inflexibility of FXS-model mice is indexed by inability to attenuate DS rates in response to diverse types of environmental novelty.

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Poster

604. Place Cells

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant P50AA022534
NIH Grant R21AA024983

Title: The effect of moderate prenatal alcohol exposure on object discrimination by adult rats

Authors: *L. M. SANCHEZ[1], S. D. BENTHEM[1], S. A. JOHNSON[3], S. M. TURNER[3], D. D. SAVAGE II[5], S. N. BURKE[4], B. J. CLARK[2]
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Abstract: Fetal Alcohol Spectrum Disorder, which includes Fetal Alcohol Syndrome (FAS), partial FAS, and Alcohol related Neurodevelopmental disorder, are a major public health concern in the United States impacting approximately 2-5% of children especially since it is an avoidable public health concern. While a great deal of research has been done to understand the effects of high dose prenatal alcohol exposure (PAE), there is increasing evidence that moderate PAE is much more common and can also have a long-lasting impact on cognition and behavior. One of the most striking behavioral abnormalities after PAE are deficits in learning and memory which can have serious repercussions for scholastic performance. While a large body of research has been focused on the effects of PAE on cognitive processes such as spatial learning and memory, the impact of PAE on high-order sensory representations such as the perception of complex objects is currently unknown. In the present study, we tested a moderate PAE rat model (Savage et al., 2010) in an object discrimination task (PAE: n = 25; Saccharin control: n = 22). In brief, the task is composed of training rats to discriminate between a pair of toy objects that differed in size, shape, and color. In Experiment 1, rats were given a total of 20 trials per day with each trial ending once the rat pushed one of two objects to uncover a piece of food. The same object was rewarded on each trial throughout training and the position of the object within the pair varied across trials. Thus, animals were required to select a particular object on the basis of its perceptual features. In Experiment 2, rats were given 10 trials per day throughout training. Overall, PAE and control rats expressed a similar rate of task acquisition in each experiment, with rats reaching criterion (two consecutive days >80% correct) within 4 days of training in Experiment 1, and within 8 days in Experiment 2. In addition, PAE and control rats exhibited similar performance as measured by the percentage of correct trials across testing ($p > 0.05$). Although these findings suggest that moderate PAE does not impair object discrimination, the objects used in these experiments were distinct. Thus, in Experiment 3, rats will be tested in a discrimination task in which the degree of feature overlap between the object pairs is systematically increased. The results of this study will be discussed in relation to the hippocampal-parahippocampal basis of object discrimination learning and the effects of moderate prenatal ethanol on this neural circuitry.

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Poster

604. Place Cells

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Topic: H.01. Animal Cognition and Behavior

Support: R01AG043688
R01NS105472

Title: Cognitive control and the dynamic grouping of spatial frame-specific hippocampus discharge in the absence and presence of task demands

Authors: *A. A. FENTON, Z. TALBOT, M. VAN DIJK
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Cognitive control describes the ability to judiciously use task-relevant information while ignoring task-irrelevant information. How can a single neural system, able to process more than one class of information selectively represent only the most currently relevant information, and how should the selection reverse when what is task-relevant changes? One solution is "dynamic functional grouping," whereby the ensemble discharge of a population of neurons transiently organizes into same-function groups of co-active cells to process one class of information at the exclusion of other classes. Dynamic functional grouping in the discharge of hippocampus place cells is observed as rats solve a two-frame active place avoidance task on a rotating arena with two concurrent goals: 1) avoid shock in a stationary location defined by static room cues, and 2) avoid shock in a rotating location defined by rotating arena cues. Every few seconds, place cells alternate between an ensemble-level preference for signaling locations in either the room frame or the arena frame, but rarely both. Here, to investigate features of cognitive control independent of overt behavior, we use this spatial-frame ensemble preference (SFEP) on a rotating arena as a neural expression of cognitive control. We ask whether 1) cognitive control is expressed in the absence of an explicit task; and 2) whether explicitly reinforcing one class of information (room places) as useful, causes preferential representation of the relevant information. Hippocampus principal cells were recorded while mice explored a continuously (1 rpm) rotating arena during pretraining (before) and (during/after) active place avoidance training to avoid a room shock zone. Ensemble discharge alternatingly represented room places and arena places, with a preference for room information during pretraining. Surprisingly, this room-dominant spatial-frame ensemble preference reduced with training to avoid the room shock zone; hippocampus discharge changes to represent room locations and arena locations with equal frequency. With training, room-preferring discharge was more likely near the shock zone and arena-preferring discharge was more likely away from it. These findings indicate that internal cognitive variables may not be accurately inferred from overt behavior or

task contingencies, and point to the hippocampus opting for greater representational flexibility than representational compression in solving this hippocampus-dependent cognitive task, more consistent with model-based than with model-free learning systems.

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Poster

604. Place Cells

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Topic: H.01. Animal Cognition and Behavior

Support: Simons Foundation
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Office of Naval Research Young Investigator Program
New York Stem Cell Foundation

Title: Investigating task-adaptive position coding in MEC neurons

Authors: *K. HARDCASTLE, W. N. BUTLER, L. M. GIOCOMO
Neurobio., Stanford Univ., Stanford, CA

Abstract: Accurate navigation requires that animals maintain an internal representation of their current location. Medial entorhinal cortex (MEC) likely supports this representation, as MEC neurons have been shown to modulate their activity with the animal's position, head direction, or running speed. Further, recent work has shown that the position and head direction tuning of MEC neurons is also modulated by the running speed of the animal; a greater number of cells encode position and head direction information at fast running speeds, and the quality of encoding also increases (Hardcastle et al., 2017). However, it is unclear whether this change in navigationally-relevant information encoding is due to locomotion alone, or rather reflects an increase in attention to navigation-specific information, which has previously been shown to improve encoding in hippocampal place cells (Kentros et al., 2004). To probe this distinction, we recorded MEC neurons while rats performed two separate tasks in two distinct environments. In environment 1, animals foraged for randomly scattered food rewards. In environment 2, animals were trained to navigate to an unmarked goal location in response to an auditory cue in order to receive a large food reward. Both environments were geometrically identical and were surrounded by identical distal cues, but differed in wall color, floor scent, and the flavor of the food reward. Comparison of position-encoding medial entorhinal cells between these environments revealed grid translation and re-mapping of non-grid position-encoding cells, consistent with prior work (Jeffrey et al., 2015). In addition, the degree to which grid cells encoded position information was selectively altered during the spatial task. Further, we

observed task-specific re-mapping of position tuning, such that position decoding was improved near the unmarked reward zone. Combined, our results indicate that MEC can shift its coding properties along task-relevant dimensions to better support accurate navigation.

Disclosures: K. Hardcastle: None. **W.N. Butler:** None. **L.M. Giocomo:** None.

Poster

604. Place Cells

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Topic: H.01. Animal Cognition and Behavior

Support: National Science Foundation Graduate Research Fellowship
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Title: Heterogeneous coding for position and context across hippocampal subregions

Authors: *M. PLITT, L. GIOCOMO
Stanford Univ., Stanford, CA

Abstract: The ability to generate novel associations between stimuli is a hallmark of learning and often depends critically on the hippocampus. One example of this process is context discrimination, in which animals must remember outcomes that are paired with a particular constellation of sensory cues, some of which may be overlapping with other outcomes. Here, we aim to gain insight into how the hippocampus supports the creation of distinct representations for similar sensory experiences by imaging hippocampal neurons as animals are trained animals to perform a difficult context discrimination task and are then presented with ambiguous context-related stimuli. In this study, mice performed a novel two alternative forced choice context discrimination task while we imaged calcium activity in CA1 or the dentate gyrus (DG) using two photon microscopy. Animals ran in one of two virtual reality environments and learned to report which hallway they were in by licking to a particular side. Once animals reached expert level performance, they were occasionally placed in an ambiguous environment that was a morph of the known contexts. Behaviorally, mice gradually increased their licking to a particular side as the evidence for that context increased. During this task, we found that the hippocampus formed a robust and low dimensional representation of both position and context. Coding for context was heterogeneous. Some neurons showed “engram”-like coding, in which cells were constitutively active in one context or the other, while others showed a partial reorganization of the place field map across contexts. Taken together, this data can shed light on whether

representations of contexts in the hippocampus are discrete entities like fixed point attractors or more continuous representations of the stimuli in the environment.

Disclosures: M. Plitt: None. **L. Giocomo:** None.

Poster

604. Place Cells

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Topic: H.01. Animal Cognition and Behavior

Support: Simons Foundation
James S McDonnell Foundation
Office of Naval Research Young Investigator Program
New York Stem Cell Foundation

Title: Distinguishing grid from non-grid cells in virtual reality

Authors: *M. G. CAMPBELL, M. PLITT, C. S. MALLORY, L. M. GIOCOMO
Stanford Univ., Stanford, CA

Abstract: Head-fixed rodent behavior is now a popular experimental paradigm because it allows an ever-expanding toolkit of recording techniques, including 2P imaging, whole cell recording, and acute silicon probe recording, to be applied in awake, behaving animals. One prominent example is head-fixed virtual reality, in which animals move through a visual, tactile, or olfactory environment by running in place on a cylinder, belt, or spherical treadmill. By enabling whole cell recording and imaging, as well as near-total control of the animal's sensory environment, this tool has greatly advanced our understanding of the neural mechanisms underlying the spatial response properties of hippocampal and entorhinal neurons during navigation. In medial entorhinal cortex (MEC), one prominent cell type is the grid cell, which fires at the vertices of a triangular lattice that spans the environment. However, the MEC also contains many other spatially-responsive neurons which are not grid cells. This leads to the question of whether grid cells can be accurately identified in linear virtual environments during head-fixation. To address this issue, we recorded hundreds of medial entorhinal neurons using tetrodes while animals explored both an open field environment (OF) and virtual linear track (VR). We labeled the cells as either grid or non-grid based on the OF data and asked whether these cells could be distinguished based on their VR response properties alone. We found that our ability to distinguish grid from non-grid cells based on the VR recordings alone was limited, but could be improved by manipulating the gain between the animal's locomotion and the movement of the visual cues, which separates cells that respond differentially to these two cues.

These results provide a benchmark for future work using virtual reality to study medial entorhinal cells during navigation.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NIDA
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New York Stem Cell Foundation

Title: Experience-dependent evolution of place cell coding during spatial learning

Authors: *Y. SUN, L. M. GIOCOMO
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Abstract: Hippocampal place cells are thought to provide a neural substrate for spatial learning and memory. However, how place cell ensembles evolve their representation of space as a function of experience remains incompletely understood. As the progression of spatial learning can occur over long time scales (weeks), this topic has been challenging to study using conventional electrophysiological techniques, due to the inherent difficulty in tracking the same cell population for many days. Here, we used a miniaturized fluorescence microscope to investigate the dynamics of spatial learning by imaging long-term calcium dynamics of CA1 place cells in freely behaving mice. For these experiments, mice were exposed to two previously unexplored environments (square vs. circular open field) every other day for 20 days and the same group of CA1 neurons were tracked and imaged repeatedly. Consistent with previous work (Ziv et al., 2013), we found that a subset of place cells remained relatively stable after the environment became familiar. However, the number of cells that showed place fields in both environments were linearly decreased over time. More interestingly, the majority of the place cells that were present on day 1 of the experiment were unstable in subsequent early sessions (days 1-7) and did not show coherent place fields in later sessions (days 9 - 20). In parallel, another group of CA1 neurons, many of which were not classified as place cells on day 1, came online and increased in stability and information in the later sessions (days 9 - 20). Thus, our data suggest a the place cell population undergoes a major reorganization in its activity pattern during spatial learning and provide new insights on the circuit dynamics that occur as environments transition from novel to familiar.

Disclosures: Y. Sun: None. **L.M. Giocomo:** None.

Poster

604. Place Cells

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Topic: H.01. Animal Cognition and Behavior

Support: NSF CRCNS #1429937
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Title: Dorsal-Ventral place cell representations in multi-scale environments

Authors: *B. HARLAND[1], M. CONTRERAS[1], P. SCLEIDOROVICH[2], A. WEITZENFELD[2], J.-M. FELLOUS[1]
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Abstract: Most of what is known about spatially-tuned cells in the brain has been learned while recording from rodents in small, highly controlled environments. Hippocampal 'place cells' generally exhibit only one 'place field' in small boxes or cylinders (typically between 40 cm and 1.5 m across), and narrow walkways. However, there is evidence that place cells possess multiple place fields in larger environments [1], perhaps indicating a fundamentally different coding mechanism in complex natural environments. We recorded wirelessly from dorsal and ventral CA1 hippocampal place cells while rats performed two different behaviors in a large open environment (530 x 350 cm) containing multiple intra- and extra-maze cues. In the majority of sessions, the rat chased a small robot baited with food [2]. The advantage of this technique is that it allows control over navigation without restricting the animal. Other sessions involved 'classical foraging' in which the rat searched for small food pellets scattered on the floor. Each session consisted of a rest/sleep period, a recording in a small enclosure (180 x 120 cm), the recording in the large environment, another recording in the small enclosure, then a final rest/sleep period. This allowed us to compare place cell coding at multiple spatial scales and to assess the stability of place cells throughout the session. We have found that dorsal place cells indeed exhibit multiple fields in the large environment. These multiple fields can greatly vary in size even within the same cell, a result similar to that shown in bats in a corridor [3] but shown here in rodents, and in an open-field environment for the first time. Ventral CA1 place cells exhibited larger place fields and increased out-of-field firing compared to their dorsal counterparts. Establishing how spatially-tuned cells operate in this larger space may be key to understanding how we form a spatial representation of complex large-scale natural environments.
References: [1] Park E, Dvorak D, Fenton A (2011) Ensemble place codes in hippocampus: CA1, CA3, and dentate gyrus place cells have multiple place fields in large environments. PLoS

One, 2011. 6(7): p. e22349. [2] Gianelli S, Harland B, Fellous J-M (2017) A rat-compatible robotic framework for behavioural neuroscience experiments. Journal of Neuroscience Methods: 294:40-50. [3] Eliav T, Las L, Ulanovsky N (2017) Representation of large-scale spaces in the hippocampus of flying bats, SfN Poster 2017. http://www.abstractsonline.com/pp8/#!/4376/presentation/22043

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Poster

604. Place Cells

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Program #/Poster #: 604.27/HHH59

Topic: H.01. Animal Cognition and Behavior

Title: An empirically driven hierarchical anti-hebbian network model for the formation of spatial cells in three-dimensional space

Authors: *K. SOMAN[1], V. CHAKRAVARTHY[1], M. M. YARTSEV[2]
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Abstract: The discovery of spatial maps in rodents has yielded valuable insights into the brain's spatial navigation systems. However, studies of spatial navigation, both empirical and computational, are highly biased towards navigation in one or two-dimensional spaces, while three-dimensional (3D) navigation is relatively under-studied, despite the fact that most forms of navigation occur in 3D environments. The discovery of 3D spatial cells in the mammalian hippocampal formation supports the existence of 3D spatial maps; yet the underlying computations that support the formation of such maps are vastly unknown. With this motivation in mind, we propose a hierarchical oscillatory anti-hebbian network model for the formation of three-dimensional spatial cells. The proposed model is a hybrid one that incorporates both oscillatory and rate coded neurons. A virtual animal is simulated to fly freely inside a cubical enclosure. The model is driven by animal's velocity components in 3D space viz. azimuth and pitch. The model has a hierarchical architecture with two parallel neural layers representing azimuth and pitch respectively. The distribution of the directional coding neurons (azimuth and pitch) in the model is made to match experimentally observed distributions. This neural representation of flight direction is passed to the downstream low-frequency oscillatory layer (0.5 Hz) that, integrates the incoming neural velocity information into the phases of the oscillators, thereby performing path integration (PI). The final anti-hebbian neural network layer is trained on the output of the oscillatory PI layer. The anti-hebbian network is a recurrent neural network whose afferent and lateral weight connections are trained using Hebbian and anti-

Hebbian rules respectively. The model, after training, accounts for the natural emergence of place, border and grid-cells in 3D. Furthermore, it provides experimentally testable prediction for the existence of two new, previously undescribed, types of 3D spatial cells that we call 'plane cells' and 'stack cells'. Interestingly, it naturally provides a mechanistic explanation for the discrepancy between the anisotropic coding of place and grid-cell firing fields observed in rodents, and the isotropic coding reported (in the case of place-cells) and predicted (in the case of grid-cells) during 3D volumetric navigation in flying bats. Lastly, it provides evidence for the importance of unsupervised learning rules in the formation of higher dimensional spatial maps.

Disclosures: K. Soman: None. **V. Chakravarthy:** None. **M.M. Yartsev:** None.

Poster

604. Place Cells

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 604.28/HHH60

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Support: Air-force grant (AFOSR)
New-York Stem cells foundation
NIH Innovator award
Packard
Searle

Title: Multiplexed continuous tracking of spatial location and navigational choice values in the posterior parietal cortex of foraging bats

Authors: *N. M. DOTSON[1], M. M. YARTSEV[2,1]
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Abstract: Real-life navigation is complex and often occurs in dynamically changing environments where an equilibrium must be struck between exploration and exploitation to guide navigational choices. Importantly, a hallmark of natural environments is a lack of certainty in both the source and reliability of reward. Yet, the neurobiological computations that support complex navigation and provide continuous monitoring of both spatial position and choice value remain largely unresolved. Here we addressed this topic utilizing wild caught Egyptian fruit bats - expert aerial navigators that are accustomed to foraging in such complex natural environments. Utilizing the development of a fully automated flight room behavioral setup in our laboratory we could effectively train these bats to perform a probabilistic navigation task. There, the animals had to choose (on each trial) between one of four navigational goals, each with a different underlying reward probability that varied on a daily basis. Importantly, the bats could only gain knowledge about the reward contingency for each target by trading-off exploration and

exploitation strategies, as no other explicit cues were available to the animal. The robustness of the behavior and controlled conditions allowed us to leverage reinforcement learning models to extract value estimates corresponding to the navigational choices on a trial-by-trial basis. To study the neural computations that might support such a complex form of spatial navigation we utilized the development of wireless electrophysiological methods in freely flying bats which we integrated with the automated flight room behavioral setup. We targeted the posterior parietal cortex region of the bat as this region has been previously shown to be involved in spatial navigation and decision-making. We find that many of the neurons in this region exhibit highly reproducible and spatially restricted firing fields in freely flying bats performing the task. Strikingly, a significant fraction of neurons exhibited value-modulated neural activity that reliably tracked the value of navigational choices on a moment-by-moment basis. Further analyses are aimed at unraveling the intriguing relationship between spatial and value coding exhibited by posterior parietal neurons to uncover their potential contribution to complex forms of spatial navigation.

Disclosures: N.M. Dotson: None. **M.M. Yartsev:** None.

Poster

604. Place Cells

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

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AFOSR
New-York Stem cells foundation
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Packard
Searle

Title: The automated flight room: Studying complex three-dimensional spatial navigation and its underlying neural codes in freely-flying bats

Authors: *D. GENZEL, M. M. YARTSEV
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Abstract: Bats, as the only mammals capable of self-propelled flight, freely navigate in three-dimensional space. They are renowned for their ability to extract information about the environment through their active echolocation system (biosonar), but visual, passive auditory and often olfactory signals are often used for navigation as well. How this multitude of sensory inputs are used for navigation we are only beginning to understand. Since the discovery of

position-coding neurons, considerable progress has been made in unraveling the neural mechanisms underlying how the brain guides navigation through complex three-dimensional environments, but much remains unknown, especially with respect to the influence of sensory inputs on ongoing neural activity as well as the mechanisms that come into play when more complex and varying constraints on navigation are present. To address these challenges, we designed a sophisticated setup in which we can record flight behavior, echo-acoustic attention and neural activity in a highly controlled manner. Specifically, the design of our system facilitates fully automated training, thus reducing the great variability of manual training, eliminating the biases due to experimentalists' presence during testing and increasing the number of trainable animals. Here we describe the implementation of this approach in the design of a task in which Egyptian fruit bats (a bat species with exceptional visual capabilities) are trained to approach in flight one of four targets to obtain a food reward, where a correct target is marked by a visual cue. By varying the intensity of the light cue, we can reduce the reliability of the sensory cue used for this navigational task and ask how this is reflected in the bat's psychometric navigation performance and ongoing neural activity. As it is becoming more evident that the retrosplenial cortex plays a major role in coding spatial information, in particular during visual-guided navigation, our goal is to investigate for the first time its underlying neural codes in a freely flying bat. The study of navigating bats coupled to cellular-resolution measurements of brain activity during free flight and under ethological yet controlled conditions, will provide important insight into how the mammalian brain supports complex forms of three-dimensional navigation.

Disclosures: D. Genzel: None. **M.M. Yartsev:** None.

Poster

604. Place Cells

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 604.30/III1

Topic: H.01. Animal Cognition and Behavior

Title: Explaining place field differences in hippocampal region CA3 and CA2. The role of spatial attractors and regulated plasticity

Authors: *T. STÖBER[1,2], A. B. LEHR[3,1], J. K. LEUTGEB[4], M. FYHN[5], T. SOLSTAD[6]
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Abstract: Since the discovery of place cells, stable spatial representations in the hippocampus are thought to form a cognitive map. In line with this theory, place cells in hippocampal region

CA3 have well-defined, spatial receptive fields that remain stable upon repeated exposure to the same environment. In contrast, place cells in neighboring region CA2 have very different properties. There, place cells tend to have multiple fields with changing peak firing rates and shifting positions, resulting in a gradual decorrelation of the spatial map. Integrating recent insights about synaptic plasticity in both regions, we developed a computational model to investigate the conditions under which place field instability may arise.
Pyramidal cells in CA3 form plastic recurrent connections thought to be involved in forming stable spatial representations. In contrast, in CA2 it is not yet clear whether excitatory plastic recurrent connections exist. But, it has been shown that proximal dendrites of pyramidal cells in CA2 are tightly enwrapped by dense extracellular matrix, limiting plasticity at afferent excitatory synapses from CA3.
In our model we hypothesize that recurrent excitatory synapses in CA2 exist, but that they are not plastic. In consequence, we assume that recurrent plasticity in CA3 allows the formation of a spatial attractor, but not so in CA2.
Using a rate-based model, we contrast emerging spatial representations of CA3 and CA2. Recurrent connections of CA3 are tuned to spatial input from entorhinal cortex (EC), CA2 recurrent connections are not. The spatial attractor in CA3 is able to transform multipeaked EC input to single place fields. Activity in CA2 reflects rather its input and tends to have multiple, irregularly spaced fields.
Despite the limited plasticity at proximal dendrites of CA2 pyramidal cells, it has been shown that afferent projections from EC onto distal dendrites of CA2 pyramidal cells are very plastic. Compared to the EC-CA3 synapse, LTP is much stronger at the EC-CA2 synapse.
In addition, neurotransmitters, such as vasopressin, oxytocin and substance P, selectively modulate the EC-CA2 synapse.
In our model, mimicking simple plasticity at the EC-CA2 synapse during rest is sufficient to reproduce field-specific changes in peak firing rates, position instabilities and a continuous decorrelation of the spatial map.
The proposed model provides an intuitive way of understanding the emergence of multiple, independently modulated place fields. Further, the model points to plasticity at the EC-CA2 synapse during rest as a potential source for the decorrelation of the spatial map over time.

Disclosures: T. Stöber: None. **A.B. Lehr:** None. **J.K. Leutgeb:** None. **M. Fyhn:** None. **T. Solstad:** None.

Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.01/III2

Topic: H.01. Animal Cognition and Behavior

Title: Enhanced associative competition by a latent inhibitor with a retention interval: Role of incubation

Authors: R. RICHARDSON[1], T. KILLMADE[2], D. KLAKOTSKAIA[1], P. MICHENER[1], *T. SCHACHTMAN[3]
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Abstract: Published studies have shown that a latent inhibitor is poor at competing for learning with another conditioned stimulus (CS) on a compound conditioning trial. Moreover, the poor conditioned response (CR) produced to a latent inhibitor (a CS given preexposure and then paired with the US) can be reversed by a retention interval placed after conditioning and prior to testing the CR (e.g., Bakner et al., 1991). In these experiments, a CS ("A") is given CS-alone preexposures prior to a pairing of the CS with the unconditioned stimulus during pretraining phases. A compound conditioning phase then occurs in which this CS was able to compete with an added novel CS ("B"). However, prior to the AB-US compound conditioning phase, a retention interval occurred lasting either one day or many days (15 or 21 days). The lengthy retention interval enhances the competitive potential of the pretrained CS. The present experiment extended these findings in showing that the effect is not due merely to an "incubation effect" (e.g., Spear & Riccio), in which associations appear to increase in strength over time; rather, conflicting associations appear to compete for retrieval.

Disclosures: R. Richardson: None. **T. Killmade:** None. **D. Klakotskaia:** None. **P. Michener:** None. **T. Schachtman:** None.

Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.02/III3

Topic: H.01. Animal Cognition and Behavior

Support: KIST Grant 2E27890

Title: Processing of associatively-activated representation requires PLCß1 of the left mPFC

Authors: H.-J. KIM, *H.-Y. KOH
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Abstract: According to associative learning theory, a conditioned stimulus (CS) evokes associatively-activated representation (AAR) of a paired unconditioned stimulus (US), and then this AAR can substitute for the actual US itself in the acquisition of new learning about US, which is called representation-mediated learning (RML). Studies with rodents showed that RML occurs only with a small number of CS-US pairings and not with extended training. It is

suggested that, with minimal CS-US pairings, CS evokes a highly realistic AAR which is not fully distinguished from the actual US so that RML can occur, and that, with extended training, AAR is replaced by a less perceptual one that is distinguishable from the actual US, so RML does not occur. Processing of AAR supporting RML sensitivity course is suggested to have implication for psychiatric conditions (synesthesia, hallucination, flashbacks in PTSD). Although the concept of AAR is used in psychology of associative learning in theoretical terms, this theory has never been addressed experimentally. In this study, (1) we observed the processing of AAR by analyzing the pattern of CS-evoked neural activation in gustatory cortex and nucleus accumbens, using cFos immunohistochemistry in wild-type mice; (2) In PLCß1 KO mice, in which RML sensitivity course is disrupted, processing of AAR was also disrupted, suggesting that PLCβ1 is required in AAR processing; (3) Local knockdown of PLCß1 in the left medial prefrontal cortex (mPFC) disrupted RML sensitivity course and processing of AAR. These results suggest that the left mPFC PLCβ1 is required in the processing of AAR.

Disclosures: H. Kim: None. **H. Koh:** None.

Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.03/III4

Topic: H.01. Animal Cognition and Behavior

Title: Characterizing a simple, automated active avoidance task for mice that leads to persistent avoidance behavior

Authors: *M. WEBER, A. EASTON
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Abstract: Persistent avoidance behavior is a hallmark of phobias, panic disorders and other neuropsychiatric (NP) disorders, while the extinction of avoidance behavior is a core principle of cognitive behavioral therapy. While rodent tasks of active avoidance (AA) may be useful for studying the biological basis of some of these behavioral patterns, it may also be useful for probing long-term memory (LTM). Here, we have characterized a two-way AA task for mice. C57BL6/J mice learned to avoid an unconditioned stimulus (UCS, a mild electric shock) by moving into the opposite compartment of a shuttle box during the presentation of (conditioned) visual and/or acoustic stimuli before the UCS is applied to the compartment in which the mouse was located at the beginning of the trial. We show that acquisition of AA is rapid in both sexes, reaching near maximal levels during the 2nd day of training. AA was impaired in aged mice consistent with earlier studies that were conducted with different stimulus conditions. The fact that these results lead to similar conclusions despite varying experimental conditions (# of trials, sex) supports the robustness of the task. When two separate tests were conducted under identical

conditions, but 5 years apart, virtually identical results were obtained, demonstrating high re-test reliability. The % of trials in which AA behavior was shown during acquisition depended on the number of "learning trials" previously encountered, showing that the behavior is amenable to training intensity. Once AA was stably established, AA memory was extremely long lasting, yielding near maximal % AA in tests 8 weeks post training. Mice still performed above their initial baseline % AA level 22 weeks post training. Following stable acquisition, AA behavior was resistant to complete extinction despite 9 consecutive days of AA training with 100 trials/day in the absence of the UCS. In sum, this AA task is simple, automated, highly reliable, and robust, and leads to a long-lasting memory that can be elicited when tested several months post training. This AA task could not only be useful for studying the biological basis of LTM or extinction, but also for testing the effects of compounds that may affect LTM or extinction.

Disclosures: M. Weber: A. Employment/Salary (full or part-time):; Genentech Inc. **A. Easton:** A. Employment/Salary (full or part-time):; Genentech Inc.

Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.04/III5

Topic: H.01. Animal Cognition and Behavior

Support: KBRI basic research program 18-BR-01-06

Title: The ability of song recognition learning does not depend on age in male and female zebra finches

Authors: *D. LEE, K. KAI, S. KOJIMA
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Abstract: Just as speech acquisition in humans, song learning in many oscine species strongly depends on age. In the zebra finch, male birds develop a good copy of their tutor's song only when they are exposed to the tutor song during a restricted period of development. In this sensory phase of song learning, birds form an auditory memory of tutor song that will be used as a template to guide song development in the subsequent sensorimotor learning phase. Although zebra finches do not learn to produce new song once the sensory learning phase is over, however, they maintain the ability to memorize songs for conspecific recognition even in adults: a number of previous studies have demonstrated that adult zebra finches learn to identify or discriminate conspecific songs. What is the relationship between song memorization for song learning and song memorization for conspecific recognition? One possibility is that juvenile birds learning song have a higher ability to memorize conspecific song compared to that of adult birds and that such enhanced ability of song memorization critically contributes to the age dependence of song

learning. To test this hypothesis, we trained juvenile and adult zebra finches to memorize a single conspecific song using a go/no-go operant-conditioning paradigm, and compared their learning speed and memory retention between the two age groups. Birds were trained to respond to a particular conspecific song (target song) to obtain a food reward, and to withhold their responses to 5 other conspecific songs (non-target songs) to avoid a mild punishment (a 20-sec time-out). Despite the strong age dependence of song learning, we found no significant differences between juvenile and adult male birds in either learning speed or memory retention. In addition, no significant differences were found between juvenile males and age-matched females in the same measures despite the lack of song learning ability in female birds. These results sharply contrast the strong dependence of song learning on age and gender, raising the possibility that song memory formed with operant conditioning is fundamentally different from song memory formed with tutoring. In support of this idea, we also found that the juvenile birds that have memorized a target song with our operant conditioning paradigm later developed adult song that does not resemble the memorized target song. We will discuss possible differences in neural mechanisms between these two forms of song recognition learning and mechanisms that regulate the sensory period of song learning.

Disclosures: D. Lee: None. **K. Kai:** None. **S. Kojima:** None.

Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.05/III6

Topic: H.01. Animal Cognition and Behavior

Support: Technical assistance Gabriela Vera and Alejandro Rangel
DGAPA-PAPIIT IN201018

Title: Effect of long term sugar consumption on insular cortex glutamate levels during new aversive learning

Authors: *D. BADILLO JUAREZ[1], M. I. MIRANDA[2]
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Abstract: Conditioned taste aversion (CTA) is an associative learning in which subjects associate a novel taste with visceral malaise, which results in a robust aversion. However, when a familiar or pre-exposed taste is associated with a visceral malaise a delay in the ability to associate the stimuli is observed; this effect is known as latent inhibition (LI) of CTA. Glutamatergic activity in the Insular Cortex (IC) plays a crucial role in memory formation and several studies have reported that during CTA (for novel saccharin) there is a significant increase

of glutamatergic activity in the insular cortex that it is related with the aversive stimulus; however, glutamate activity during LI of CTA has been little studied. Therefore, the objective of this work was to evaluate glutamate levels in the IC during CTA acquisition for novel sugar or after 21 days of permanent sugar exposure. Adult male Wistar rats were long-term exposd to permanent access to sugar solution (10%); on day 14 they were stereotaxically implanted with one microdialysis cannula directed to the right IC. At the end of the long-term sugar consumption (21 days), rats were trained in CTA during in vivo microdialysis (MD) as well as during aversive memory retrieval. Glutamate levels in the MD samples were analyzed with a HPLC coupled to fluorescence detector. During novel sugar CTA acquisition, a significant increase on IC glutamate levels where observed immediately after LiCl i.p. injection. However, during CTA acquisition, with high familiar sugar, glutamate levels did not present changes during or after LiCl injection, in rats that also presented a strong LI of CTA. This result shows that the LI of CTA induced by long-term sugar consumption had a differential glutamate release during visceral-aversive signaling. This result confirms that glutamatergic activity in the IC during CTA acquisition is required for aversive taste association and suggests that visceral information signaling could be altered after long-term periods of sugar consumption, since highly familiar taste that induces strong LI of CTA is associated with a non-effective glutamatergic cortical activity.

Disclosures: D. Badillo Juarez: None. **M.I. Miranda:** None.

Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.06/III7

Topic: H.01. Animal Cognition and Behavior

Support: NIMH
HHMI

Title: CA1 hippocampal ensemble neural activity reveals associative representations in mice acquiring a context-dependent learning task

Authors: *T. ROGERSON[1], J. MAXEY[1], P. JERCOG[2], T. H. KIM[1], S. EISMANN[1], B. AHANONU[1], B. F. GREWE[3], J. LI[1], M. J. SCHNITZER[1]
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Abstract: Substantial research has shown that hippocampus has a key role in spatial cognition, but the role of hippocampus in associative learning and memory is less well understood [Wirth *et al., Science (2003)*]. Prior studies have identified hippocampal neurons that encode conjunctions

of spatial information and sensory stimuli [*Rolls et al*., J. Neurophysiol. (2005), Komorowski *et al*., J. Neurosci. (2009)], but how these conjunctive representations develop with associative learning and are evoked in contexts with conflicting information remains largely unknown. To study the role of hippocampus in associative memory, we used a miniature head-mounted fluorescence microscope to record neural calcium dynamics in hippocampal area CA1 in freely behaving mice as they learned a task requiring mastery of a bi-conditional rule. This behavioral task involved two visuo-tactile stimuli, each of which we presented to the mice in two different U-shaped running tracks with distinctive features. In each context, only one of the two stimuli signaled the presence of a reward; thus, to receive rewards successfully in both contexts the mouse had to learn two different context–stimulus associations. Mice learned to perform this task well above chance even when rapidly alternating between the two contexts. Pharmacological inhibition of dorsal hippocampus in trained mice impaired their performance of the task, consistent with a hippocampal role in context-dependent associative memory retrieval. In trained mice, we found hippocampal cells that encoded stimulus-context associations; we hypothesize that the formation of conjunctive coding features such as these underlie the ability to respond appropriately to varied stimuli in distinct contexts. We are presently analyzing the development of these coding features during learning and their dependence on the training regimen.

Disclosures: T. Rogerson: None. **J. Maxey:** None. **P. Jercog:** None. **T.H. Kim:** None. **S. Eismann:** None. **B. Ahanonu:** None. **B.F. Grewe:** None. **J. Li:** None. **M.J. Schnitzer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MJS is a scientific co-founder of and consults for Inscopix Inc., which makes the miniature microscope used in this study.

Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

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Topic: H.01. Animal Cognition and Behavior

Support: ARC Grant DP170103952

Title: Conditions that govern false fear memories in rats

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Abstract: Memories are not often faithful records of our experiences: they often contain information about events that did not happen or did not happen the way in which they are remembered. These so-called false memories can have potentially far-reaching consequences, yet we know little about the conditions under which they are formed. We recently developed a

laboratory model to study false memories (Bae, Holmes and Westbrook, 2015), and showed that rats pre-exposed to a context, A, on day 1, then given an immediate shock upon placement into a similar context, B, on day 2, show more fear to context A than context B at test, despite the fact that rats were shocked in B and not in A. These findings have been explained in terms of mediated conditioning: rats retrieve the configural representation of context A upon placement in the similar B context; the shock in B associates with the representation of context A. Implicit in this description is that the amount of fear that accrues to each context is determined by the level discriminability between the two contexts. Thus, manipulations that prevent or impair discrimination between the pre-exposed A and shocked B contexts should increase false fear conditioning of A while minimizing true fear conditioning of B; and conversely, manipulations that permit or enhance discrimination between A and B should reduce false fear conditioning of A and simultaneously increase true fear conditioning of B. We tested these predictions in a series of experiments using the protocol developed by Bae et al (2015). We found that disrupting the formation of the configural representation of context A by shocking the A context increased false memory to A, but increasing the placement-to-shock interval in context B restored the amount of true fear memory to context B. Importantly, we showed that these two factors (i.e., an immediate shock in A and the placement-to-shock interval in B) interact in determining the strength of the false and true fear memories. These findings have important implications for how false fear memories might be formed, prevented or reduced.

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Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

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Program #/Poster #: 605.08/III9

Topic: H.01. Animal Cognition and Behavior

Support: KAKENHI 17K10270
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Title: Sustained temporal attention prevents habit formation in rats

Authors: *Z. LIN[1], H. NISHIKAWA[1], Y. IGUCHI[2], A. IWANAMI[3], Y. MINABE[1], S. TODA[1,3]
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Abstract: It is well known that operant learning with distinct reinforcement schedules results in different consequences in terms of habit formation. For example, the operant learning with random interval schedule is difficult to predict action-outcome contingency, but it is prone to be a habit, whereas the one with fixed interval (FI) schedule is easy to predict action-outcome contingency, but it is resistant to be habitual. However, what promotes or interferes with habit formation has yet to be fully elucidated. We hypothesized that the sustained attention for monitoring the interval that is required for FI, but not RI, schedule during sessions could prevent habit formation. To verify this, we first prepared three cohorts of male Sprague-Dawley rats (N=13/each) for the operant learning with FI60 (i.e., a reward is given in every 60 seconds by lever pressing) schedule for 2 weeks. As previously reported, all groups gradually adjusted their lever pressing according to the FI schedule, but their goal-directed actions did not transfer to a habit. We next allocated distinct training conditions for each cohort; for the first cohort, the operant learning with the same FI schedule was continued as before. For the second cohort, the operant learning with the same FI schedule was continued just as the first group, however, the timing of lever pressing was informed to the subjects just before it by an auditory cue, thus the subjects need not pay attention to the interval. For the last cohort, the operant learning with FI schedule was continued with auditory cues just as the second group, however, the auditory cues were provided in a yoked fashion to the ones for the second group, thus were non-contingent to the timing of reward. After 2 weeks of additional FI sessions, we found that the first group remained as goal-directed as before, meanwhile, the second and third groups developed a habit. The results of outcome devaluation also revealed that habit formation was the most robust in the second group. These results implicate that habit formation requires attention-free situation rather than action-outcome contingency, therefore continuous cognitive burden such as monitoring a certain interval by oneself prevents an action from acquiring automaticity. The reason why a habit was also developed among the last cohort is not clear, however, it is possible that the non-contingent auditory cues, which were functionally irrelevant noises, may have dispersed and/or attenuated the attention by preventing focusing on the interval monitoring.

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Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

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Topic: H.01. Animal Cognition and Behavior

Support: KAKRNHI16K14557
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Title: All-go behavioral state with resetting cue-outcome associations in ventral striatum during reversal learning

Authors: *Y. TANISUMI, Y. SAKURAI, J. HIROKAWA, H. MANABE
Grad. Sch. of Brain Sci., Doshisha Univ., Kyoto, Japan

Abstract: In changing environments, animals must adaptively select actions based on the predictive information and their motivational state to achieve their goals. Previous studies have shown that neurons in the ventral striatum(VS) fire to predictive cues associated with outcomes and play a critical role in promoting behavioral responses required to obtain outcomes. However, little is known about how VS neurons associate cues with outcomes during learning and guide behavioral choices. We therefore investigated the firing of VS neurons during go/no-go odors discrimination and reversal learning using simultaneous multiple single-unit recordings in rats. In this task, we trained rats to associate go-cue odors at the odor port with the behavior to go to the reward port to obtain water reward (”go” responses), and to associate no-go-cue odors with staying near the odor port to wait for the next trial (”no-go” responses). Firstly, we analyzed the behavioral responses during reversal learning. After reversal of odor-outcome contingencies, rats reached the 80% correct response criterion in a mean of 89 trials in 121 sessions. In addition, during an initial phase of reversal learning, rats made go responses following both go and no-go cue odors and continued this behavioral strategy in a mean of successive 43 trials. We called this behavioral state “all-go state”. Next, we focused on neural activity correlated with behavioral choices. We found that a subset of VS neurons fired selectively to go-cue odors or no-go-cue odors when rats showed cue odor-induced behaviors. There was a strong correlation between their firing rates during sampling cue odors and the selected behaviors after sampling. However, in all-go state, the selective firing during odor cue sampling was dramatically reduced and did not correlate with behavioral choices. As rats began learning to withhold responding after sampling new no-go cue odors, these neurons tended to generate their selective firing to the new cue odors. In addition, a subset of VS neurons fired to water reward regardless of changes of cue-outcome association rule. These results indicate that during the initial reversal learning phase, VS neurons were in a preliminary state to learn the new association between cue odors and outcome without affecting behavioral choices.

Disclosures: Y. Tanisumi: None. **Y. Sakurai:** None. **J. Hirokawa:** None. **H. Manabe:** None.

Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.10/III11

Topic: H.01. Animal Cognition and Behavior

Support: R01MH099505

Title: The effect of habit formation on reinforcer devaluation in rhesus macaques

Authors: *E. LAFLAMME[1], P. A. FORCELLI[2], L. MALKOVA[1]
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Abstract: Actions may be goal-directed, in pursuit of a specific result, or they may be driven by mechanistic habit in response to a familiar stimulus. The switch from goal-directed behavior to habitual responding is one of the major challenges in fighting addiction, making this type of research highly translational. Reinforcer devaluation is a task that probes goal-directed behavior, and has been used in rodents (Balleine & Dickinson, 1998) and non-human primates (Malkova *et al.*, 1997). Overtraining on stimulus-reward pairings (~360 pairs) impairs reinforcer devaluation in rodents: animals begin responding habitually (Dickinson & Balleine, 1995). However, the degree to which this is true in primates is unknown. Here, animals are repeatedly exposed to a set of pairs of objects (e.g. 40 per day) presented as discrimination problems (one rewarded and one non-rewarded). They learn implicitly over time which objects are associated with specific food reinforcers (peanut or fruit snack). Upon reaching criterion, they are offered a choice between the peanut-associated objects and fruit snack-associated objects to assess baseline preference. An experimental reduction of reward value by selective satiation (i.e., providing one food to satiety) produces a devaluation effect, i.e. a decrease in the proportion of objects associated with the sated food that are selected. We set out to determine how many exposures are necessary for a monkey to develop a habitual response, manifest as loss of the devaluation effect. In this task, three male rhesus macaques (ages 4-5) are being trained on the discrimination problems beyond criterion with some objects with high exposure per day and some with low exposure (< 50 total). Reinforcer devaluation is measured after 100, 260, and 430 exposures to designated high-exposure object pairs. Preliminary data demonstrate that 260 exposures are insufficient to establish a habit response, as the animals continue to display the same devaluation effect when choosing between high-trained objects as they do between low-trained objects. Whether this will be the case after further overtraining remains to be determined.

Disclosures: E. Laflamme: None. **P.A. Forcelli:** None. **L. Malkova:** None.

Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.11/III12

Topic: H.01. Animal Cognition and Behavior

Support: Intramural Research Program of the NIMH

Title: The spatiotemporal profile of diffusion MRI based measures of microstructural tissue changes evoked by learning novel skills

Authors: *C. THOMAS[1], M. B. MOYER[2], B. COLEMAN[2], P. BROWNING[2], F. Q. YE[2], D. K. YU[2], A. AVRAM[3], C. I. BAKER[1], E. A. MURRAY[2]
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Abstract: The ability to learn novel skills throughout our lifetime is known to be mediated by structural changes in the brain. However, the nature of the structural changes, and the spatiotemporal dynamics of such changes during the course of learning are unclear. Here, we trained naïve, adult rhesus monkeys (Macaca Mulatta; N=8) in two tasks differing in complexity. The monkeys were first trained to criterion (90%) in the "one-place" task, a visuomotor task which required the monkey to reach and touch an object on a computer screen to earn a reward. Next, the monkeys were trained in the "scenes" task, in which they learned to touch a target foreground object placed in an artificial "scene" composed of multiple geometric elements to earn a reward. The monkeys learned several unique scenes concurrently; the identity and location of the target object differed across scenes but was fixed within scenes.We acquired multishell Diffusion MRI (dMRI) images from the monkeys across two timepoints using a Bruker 4.7T MRI system. The pre-training scans were acquired before any formal training and the post-training scans were acquired when the monkeys reached criterion in the second (scenes) task or failed to reach criterion despite extensive training. Behaviorally, the monkeys showed wide individual variability in their ability to acquire the different tasks, with 2 of the 8 monkeys failing to learn the one-place task. Interestingly, monkeys that required fewer trials-to-criterion in the one-place task learned faster in the scenes task ($rho = - 0.77$ $p < 0.04$). Examination of the spatial topography of changes in dMRI measures based on a median split analysis with group (good/bad learners) and timepoint (Pre/Post) as factors in a linear mixed effects model revealed a significant main effect of group, with the good learners showing higher Fractional Anisotropy (FA) of the right internal capsule, anterior commissure and crus of the fornix. Finally, correlation analysis between the learning profile and changes in dMRI measures revealed: (a) a trend towards a positive correlation between faster learning in the scenes task and global increase in white matter (WM) volume ($p < 0.13$); (b) more trials-to-criterion in the one-place task correlated positively with an increase in FA and a decrease in Radial Diffusivity in the dorsal parieto-occipital WM. This region is considered part of the fronto-occipito fasciculus (FOF), which has been associated with reaching and grasping arm movements. Overall, the pattern of changes in the dMRI measures suggest that prolonged experience in performing the training tasks evokes changes in tissue microstructure consistent with changes in myelination.

Disclosures: C. Thomas: None. **M.B. Moyer:** None. **B. Coleman:** None. **P. Browning:** None. **F.Q. Ye:** None. **D.K. Yu:** None. **A. Avram:** None. **C.I. Baker:** None. **E.A. Murray:** None.

Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.12/III13

Topic: H.01. Animal Cognition and Behavior

Support: NIAAA (R21AA025172)
NIH (RO1 MH087542)
Robert Wood Johnson Foundation Health & Society Program

Title: Developmental experience of food insecurity reduces cognitive flexibility in a rodent model

Authors: *W. LIN[1], L.-H. TAI[1], E. GALARCE[2], L. WILBRECHT[1]
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Abstract: In the United States, approximately 17 percent of the households with children experience food insecurity, defined as uncertain or irregular access to food. Globally, hundreds of millions of people experience developmental food insecurity. Experience of food insecurity is positively linked to obesity and associated with poor academic performance, greater risk of developing psychological issues and substance use disorders. In human subjects, food insecurity is difficult to isolate from other factors associated with adversity that may also affect brain development. To understand what role food insecurity may play on the developing brain, we developed a mouse model of developmental food insecurity in which we delivered limited amounts of food on a variable schedule from P21, just after weaning, to P40, a late adolescent timepoint. Controls were mice fed ad libitum through this P21-40 period and mice that experienced a more stable food restriction. After P41, all groups were returned to ad libitum access to chow. We then tested mice in adulthood in odor based discrimination learning and cognitive flexibility. We found that adult male mice (P61-70) with developmental history of food insecurity performed similarly to controls in discrimination learning but were more perseverative than controls in reversal learning. Females showed no effect of developmental experience of food insecurity on discrimination learning or cognitive flexibility in adulthood. We conclude developmental feeding history in mice can have sex-specific effects on adult behavior. We are engaged in further research to determine the mechanisms underlying differences in cognitive flexibility in male mice.

Disclosures: W. Lin: None. **L. Tai:** None. **E. Galarce:** None. **L. Wilbrecht:** None.

Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.13/III14

Topic: H.01. Animal Cognition and Behavior

Support: This study was supported by PAPIIT IN301717 (UNAM, Mexico)

Title: Renewal attenuation by extinction in multiple contexts after amphetamine-induced place preference conditioning

Authors: *R. RUÍZ GARCÍA, L. N. CEDILLO, L. D. GUTIERREZ, S. Y. NUÑEZ, J. C. JIMENEZ, F. MIRANDA-HERRERA*
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Abstract: Drug craving plays an important role in turn to drug use or relapse. A high relapse rate suggests that drugs of abuse produce persistent changes in drug-induced behaviors. Some researchers have suggested experimental extinction as the basis for many therapies. Although extinction produces a reduction of the target behavior, several results indicate that this behavioral change is difficult to maintain, indicating that extinction procedure does not destroy the first learned behavior. One of the post extinction phenomena that support this notion is renewal. Several studies have suggested renewal effect as a model for relapse. Renewal effect has been reported if after acquisition, extinction takes place in a different contexts and testing in the acquisition context. Because of vulnerability of extinguished behavior to relapse, researchers have been looking for better treatments to prevent drug relapse. Bouton (1994) suggested extinction in several contexts rather than in the same context to enhance the generalization of extinction to other contexts. Learning in multiple contexts may reduce context specificity of conditioned responding. Specifically, renewal should be attenuated when behavior was extinguished in several contexts. The main goal of this study was to evaluate the effects of extinction in multiple contexts on the renewal effect after amphetamine-induced place preference conditioning (CPP). Forty male Wistar rats (250 g) were used. After establish the place of preference (PP), for training in the CPP procedure, subjects underwent drug- or saline-trials as follows: drug-trials, animals were administered AMPH (1.0 mg/kg, ip) and placed in the non-preference place (NP) for 30 min. Saline-trials, the animals were administered isotonic saline (1 ml/kg, ip) and placed in the PP for 30 min. Subjects received a total of 10 drug- and 10 saline-trials. Drug- and saline-trials alternated randomly, with the restriction that drug trials did not occur more than two consecutive occasions. In the extinction, subjects were administered isotonic saline as in the saline-trial, and were placed in the NP (Ext-A Group) or in multiple contexts (Ext-ABCD). With each group 8 sessions were carried out. For renewal test, three sessions were carried out (24-36-48 h after extinction session ended). In these sessions subjects

were put in the middle compartment. Results showed that CPP response decreased after the extinction process. However, renewal was observed when the subjects returned to the context where the AMPH was administrated. The renewal of the CPP can be attenuated by the extinction in multiple contexts. This study was supported by PAPIIT IN301717 (UNAM, Mexico).

Disclosures: R. Ruíz García: None. **L.N. Cedillo:** None. **L.D. Gutierrez:** None. **S.Y. Nuñez:** None. **J.C. Jimenez:** None. **F. Miranda-Herrera*:** None.

Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.14/III15

Topic: H.01. Animal Cognition and Behavior

Support: Supported by funds provided by the University of California Irvine to CDF.

Title: Characterization of lynx1 in sensory processing, learning and memory function

Authors: *Y. SHERAFAT, J. P. FOWLER, C. D. FOWLER
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Abstract: Nicotinic acetylcholine receptors (nAChRs) have been implicated in various cognitive processes, including learning, memory and sensory processing. However, little is known about the endogenous mechanisms that modulate the function of nAChRs and their impact on behavior. Here, we examined the role of an endogenous protein modulator of nAChRs, lynx1, in a knockout mouse model. We hypothesized that the absence of lynx1 would increase operant learning and sensory gating, since its presence has been proposed to dampen nAChR signaling. To test the effects of lynx1 on sensory processing, lynx1 knockout mice and their wildtype littermates were examined in the prepulse inhibition test. To examine the effects on operant learning and cognitive flexibility, lynx1 knockout and wildtype mice were trained to press a lever to receive food reward under a fixed ratio schedule of reinforcement. After acquisition and establishing baseline levels of responding, mice were then assessed in a lever reversal task. Together, these findings further define the function of lynx1 proteins in behaviors mediated by cholinergic signaling mechanisms.

Disclosures: Y. Sherafat: None. **J.P. Fowler:** None. **C.D. Fowler:** None.

Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

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Program #/Poster #: 605.15/III16

Topic: H.01. Animal Cognition and Behavior

Support: NIH NINDS R01 NS075531

Title: A cortical reinforcement prediction error computed by VIP interneurons

Authors: *Q. CHEVY, H.-J. PI, E. T. GIBSON, A. KEPECS
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Our ability to interact with our environment strongly relies on how well we can predict the outcome of our present actions. The computation leading to such predictions can be framed using a reward prediction error (RPE) model. Neuronal correlates of RPE computation have been observed in midbrain dopamine neurons but it has been unclear whether similar prediction error signals also exist in cortical circuits. Interestingly, VIP interneurons, a subtype of cortical interneuron which drive strong cortical disinhibition, are strongly recruited by reward and punishment (Pi et al. 2013). VIP interneurons are therefore in a key position to participate in reinforcement learning by controlling cortical plasticity. We now show that VIP interneurons convey not simply the presence of reinforcers but rather a prediction error about reinforcers. To characterize the response of VIP interneurons to reinforcers, mice were trained in a range of classical conditioning tasks with auditory cues. We measured the activity of VIP interneurons either using optogenetically-identified single unit recordings with tetrodes or using fiber photometry to collect calcium-transients. After learning, we found that outcome expectations modulated the reinforcer-activation of VIP interneurons. VIP interneuron responses were also modulated at cue presentation by the predictive value of the cue. VIP interneuron responses increased both for outcome that were unexpectedly worse or better than predicted, a hallmark of an unsigned reward prediction error signal (‘surprise’). Finally, we demonstrated that the modulation of VIP interneurons by expectancy is independent of the sensory modality of the predictive cues. This suggests that cortical VIP interneurons may be the recipients of inputs conveying an unsigned RPE signal to neocortex. We propose that RPE signaling by VIP interneurons could provide a cortical teaching signal, shaping local computation.

Disclosures: Q. Chevy: None. **H. Pi:** None. **E.T. Gibson:** None. **A. Kepecs:** None.

Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.16/III17

Topic: H.01. Animal Cognition and Behavior

Support: Graduate Summer Research Mentorship

Title: Development of a raven's progressive matrices to examine fluid intelligence in the pigeon, columba livia

Authors: *M. FLAIM[1], A. P. BLAISDELL[2]
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Abstract: Raven's progressive matrices (RPM) is a nonverbal intelligence test that examines fluid intelligence by asking subjects to correctly complete a stimulus matrix where transformations between stimuli in the matrix follow one or more rules. While this test has been used since 1936, and has been modified to accommodate a variety of humans (genius, children, dementia, etc.), it has never been adapted for testing non-human animals. Our study is an attempt to adapt the RPM-task for pigeons. Pigeons were trained on a task that could progressively increase in complexity through the addition of rules by which the matrix is completed. Pigeons were initially presented with a partially completed 2x2 matrix. Two shapes were presented in either the row or column of the matrix. The two shapes followed a rule, either size change along the rows or orientation change in the columns. Pigeons were initially trained on one rule at a time, using a go/no-go discrimination procedure with pecking reinforced when the rule was present (Go) but not when the rule was absent (NoGo). As discrimination performance reached criterion, pigeons were tested for transfer of the rule to novel stimuli. Following this, pigeons were then trained on a second rule. In the first version of this study, there were 6 subjects, 2 of which learned both rules to criterion. Both subjects were able to maintain the size change rule when presented with novel shapes. However, only one of these subjects was able to maintain the orientation change rule with novel shapes. This subject was able to maintain performance when both rules were presented in the same session and received full matrix presentation probe trials. In the second version of this study, 4 new subjects are learning the same rules, but along opposite dimensions. For these subjects, the size change occurs in the column and size change goes across the row. This was to determine if the rule learning was affected by the way information was presented or if the rules have different levels of difficulty. Two of the subjects have learned the size change to criterion, but none have learned the orientation change. Individual differences are expected in an intelligence test, but learning failures and modifications will be discussed.

Disclosures: M. Flaim: None. **A.P. Blaisdell:** None.

Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.17/III18

Topic: H.01. Animal Cognition and Behavior

Support: NIH grants RO1 NS029563

Title: Allyl isothiocyanate (mustard oil) mediates short-term sensitization in restrained larval zebrafish (danio rerio)

Authors: *J. ALZAGATITI, D. T. LUY[1], J. CHORNAK[2], J. RICHARDS[7], G. ZAVRADYAN[1], A. BAIBUSSINOV[1], A. RAZEE[1], F. OSADI[1], Y. MA[2], C. S. CAMPBELL[8], E. DEUTSCH[8], S. C. HERNANDEZ[8], J. CARMONA[3], A. C. ROBERTS[8], D. L. GLANZMAN[1,4,5,6]
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Abstract: Zebrafish are rapidly emerging as a model vertebrate system for neurobiological investigations of learning and memory. The larvae of zebrafish are particularly attractive for such investigations due to their transparency, which enables robust optogenetic manipulations and *in vivo* imaging of neural activity; the relative simplicity of the neural circuitry underlying some of their behaviors; and their capacity for simple forms of learning such as habituation and classical conditioning. In the present study, we examined a form of short-term sensitization induced by application of allyl isothiocyanate, a chemical irritant, in restrained larval zebrafish. We found that allyl isothiocyanate increased heart rate and tail movements for several minutes after the chemical irritant was removed from the bath. Our results demonstrate that a noxious stimulus, allyl isothiocyanate, can reliably induce sensitization in restrained larval zebrafish, and set the stage for future investigations of this simple form of learning using optical monitoring of neural activity in the intact larva.

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Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.18/III19

Topic: H.01. Animal Cognition and Behavior

Support: NIH grants RO1 NS029563

Title: Allyl isothiocyanate-induced sensitization of movement in freely swimming larval zebrafish (Danio Rerio)

Authors: *D. T. LY[1], J. ALZAGATITI[1], J. CHORNAK[2], A. KUMAR[1], U. KHAN[3], M. GARCIA[1], A. JAFARPOUR[1], R. STARK[2], A. NATARAJAN[4], J. LEWIS[2], A. ROBERTS[6], D. L. GLANZMAN[5]
[1]Integrative Biol. and Physiol., [2]Neurosci., [3]Psychology, [4]Ecology and Evolutionary Biol., [5]Integrative Biol. and Physiology; Neurobio., UCLA, Los Angeles, CA; [6]Psychology, CSU Fullerton, Fullerton, CA

Abstract: The larval form of the zebrafish (*Danio rerio*) holds significant promise as a model vertebrate system for understanding the neural mechanisms of learning and memory. Zebrafish larvae possess two particularly valuable properties: translucence, which permits robust optogenetic manipulations and in vivo optical imaging; and behaviors mediated by relatively simple neural circuits, which facilitate cellular analyses of behavior. In addition, larval zebrafish have demonstrated the capacity for simple forms of learning, including habituation and classical conditioning. In the present study, we examined the effect of a known chemical irritant, allyl isothiocyanate (mustard oil), on movement in freely swimming zebrafish larvae (5 dpf). We found that mustard oil (MO, concentration = 10 μM) induced increased locomotor activity, as well as thigmotaxis, a behavioral correlate for anxiety, in the larvae. These results demonstrate sensitization of movement in the larval zebrafish. In future work we will attempt to determine the specific changes induced by MO in the behavioral circuits that mediate swimming in the zebrafish. The results of this work may contribute to an understanding of the neural basis of anxiety disorders.

Disclosures: D.T. Ly: None. **J. Alzagatiti:** None. **J. Chornak:** None. **A. Kumar:** None. **U. Khan:** None. **M. Garcia:** None. **A. Jafarpour:** None. **R. Stark:** None. **A. Natarajan:** None. **J. Lewis:** None. **A. Roberts:** None. **D.L. Glanzman:** None.

Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.19/III20

Topic: H.01. Animal Cognition and Behavior

Support: Swartz foundation

Title: Understanding sensorimotor processing via a novel close-loop odor tracking task for head-fixed mice

Authors: *P. GUPTA[1], M. DUSSAUZE[2], U. LIVNEH[1], D. F. ALBEANU[1]
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Neurosci., Ecole Normale Superieure, Paris, France

Abstract: An emerging view of brain function is that of a simulator that generates predictions of sensory inputs via an internal model which maps motor actions to sensory outcomes (Craik 1943, Wolpert 1995). Sensory errors, the mismatches between observed and predicted sensory outcomes (Keller et al. 2012), in turn serve as feedback for updating the internal model. Experimental validation of this predictive coding framework necessitates close-loop behaviours where animals continually refine their motor actions based on the current and desired sensory percepts.
Here we present a novel behavioral paradigm, where water-restricted, head-fixed mice learn to control the lateral location of an odor source by maneuvering a light-weight lever with their front paws. Throughout the task, a fixed linear gain maps the 1-D movement of the lever onto left-right displacement of the odor source, such that a unit displacement of the paws (lever) generates a predictable, fixed displacement of the odor source. Each trial begins with the animal pulling the lever to a fixed start location which initializes the odor source location with a random offset with respect to the animal's snout. To obtain water rewards, animals are required to steer the lever such that the odor source is aligned to their snout and parked in place for >200 ms. Mice can learn this task within ~4 weeks, performing ~300-400 trials per session with >80% accuracy.
The close loop control enables us to flexibly manipulate the coupling between sensory feedback and motor action by delaying the sensory feedback or decoupling the sensory outcome (odor location) from reward availability. Using these feedback perturbations, we show that animals update their movements moment-by-moment based solely on the current odor location, and do not rely on either motor memory or other sensory cues (air flow, vision, whiskers etc).
Successful performance of this task requires at least two parallel processes: (i) directed movement of the paws (lever) based on the learned mapping between paw movement and odor displacement, i.e. an internal model, and (ii) continual assessment of the current versus expected odor location, i.e. sensory prediction errors. We are currently probing activity (via extracellular

recordings and activity suppression) in olfactory and motor cortex as well as olfactory striatum to understand the sensorimotor transformations that enable this behaviour.

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Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.20/III21

Topic: F.01. Neuroethology

Title: Deterministic and stochastic influences in oscillatory synchronization for information transfer: Complementary roles affecting global regulation

Authors: *D. C. LARRIVEE
Toronto, CANADA, Intl. Assn. Catholic Bioethicists, Chicago, IL

Abstract: Numerous studies now show that executive mechanisms evoke dynamic, oscillating activity by synchronization, which coordinates information transfer between global and regional circuits [1]. Shifting information content to generate novel information flow, conversely, requires oscillatory desynchronization prior to reassociation of new combinatorial variants, a mechanism likely to be employed in memory retrieval [2]. How this transfer is regulated is unknown but is likely to be broadly used, particularly for behavioral flexibility and to entail ubiquitous features of neural activity, including deterministic factors like phase resetting and stochastic activity that impact spike discharges of oscillating circuits. This paper explores how these mechanisms are likely to be used for regulating regional activity on the presupposition that oscillator associations are weakly coupled, a widely acknowledged model for cognitive information transfer. We show here that weak coupling constrains synchronization, leading to phaselocking values less than one, that is, to less than full synchronization [3]. Partial synchronization entails frequency modulation and phase precession through all phase angles, with cycling between periods of enhanced and weakened coupling within each phase cycle. By employing pulse trains for phase resetting, periodic weak coupling can be amplified, thereby favoring dissociation [4], with subsequent formation of new synchronous combinations. Conversely, stochastic events can modulate the phase range over which coupling strength is minimized, broadening phase variance and increasing susceptibility to transfer. Stochastic processes, moreover, can enhance transfer and responsivity by exposing coupled pairs to a wide variety of input firing patterns. That is, they can assess the information capacity of the neural system as a function of the total variation of spiking patterns that are elicited stochastically, that is, the maximum Shannon entropy. The selection of unique informational content, in turn, is achieved through a minimization of entropic variance, a mechanism likely to be employed in retrieval of unique engrams [5]. Subject to weak coupling constraints, therefore, the modulation of information content is likely to be regulated

complementarily by deterministic and stochastic events. References:1. Hellyer M, et al (2014). J Neurosci 34(2):451-461. 2.Almeida L, Idiart M, Lisman J (2007). Cold Spring Harbor Press 14:795-806.3. Lowet et al (2015). PLoS One, doi:10.1371/1-37. 4. Canavier CC (2015). Curr Opin Neurobiol 31:206-213. 5. Holzel RW, Krischer K (2013) Physics Letters A 377:2766-2770.

Disclosures:

Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.21/III22

Topic: B.03. G-Protein Coupled Receptors

Support: NIH Grant DA042779
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Title: Mu- and delta-opioid receptors differentially modulate thalamo-cortico-striatal pain circuitry

Authors: *W. BIRDSONG[1], B. C. JONGBLOETS[1], K. A. ENGELN[1], D. WANG[2], G. SCHERRER[2], T. MAO[1]
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Abstract: Affective/ motivational pain processing involves the medial thalamus, anterior cingulate (ACC) and prefrontal (PFC) cortical regions and striatum. Opioid analgesics are known to modulate this circuitry and relieve the negative emotional effects of pain. However, the identity of the opioid receptors, their cellular locations, and their physiological effects on this affective circuitry remain uncharacterized. In the present study, synaptic connections between three brain regions; thalamus, striatum, and midline cortex (ACC and PFC), were studied in mice (both sexes, 8-12 weeks of age) to investigate the ability of opioid agonists to modulate glutamatergic transmission between these regions. Using optogenetic and electrophysiological approaches, it was determined that single medium spiny neurons (MSNs) in the dorsomedial striatum received direct input from both medial thalamic and cortical afferents. It was found that opioid receptors differentially modulated thalamic and cortical glutamate release in striatum. Additionally, μ and ∂-opioid receptors were found to play distinct roles in modulating thalamo-cortical and intracortical synaptic transmission in the ACC. Using electrophysiological and immunological approaches, it was determined that delta opioid receptors could disinhibit cortical

circuitry through inhibition of local cortical interneurons. Finally, it was demonstrated that a two-armed polysynaptic circuit involving direct thalamo-striatal projections and indirect thalamo-cortico-striatal projections could converge on single striatal MSNs with delta opioid receptor activation facilitating thalamo-cortico-striatal glutamate transmission and mu opioid receptor activation inhibiting both direct and indirect thalamo-striatal glutamate transmission. These data identify opioid receptor subtype-dependent inhibition of specific pathways within the thalmao-cortico-stiatal circuitry, using multiple approaches and across multiple medial thalamic and midline cortical brain regions providing insights into how opioid analgesics modulate affective pain at the synaptic and circuit level and more broadly affect thalamo-cortico-striatal circuit dynamics.

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Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.22/III23

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH R01 NS085167
NIH R01 NS094384

Title: Neuromodulatory pathways required for targeted plasticity therapy

Authors: *D. HULSEY[1], S. SADMAAN[2], S. ABE[3], S. HAYS[2], M. KILGARD[1]
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Abstract: Targeted plasticity therapy (TPT) utilizes vagus nerve stimulation (VNS) paired with physical rehabilitation to direct plasticity and promote recovery after neurological injury. Precise timing of VNS is required to drive plasticity and functional recovery after injury in rodent models. VNS engages pro-plasticity neuromodulators, but there is no direct evidence that they mediate plasticity effects of VNS. We have shown that VNS drives rapid phasic activation of the noradrenergic locus coeruleus (LC). Parametric variation of VNS intensity, frequency, and duration shape LC responses. We believe this precisely timed noradrenergic activation reinforces task specific circuitry to drive reorganization of motor cortex output. Additionally, we show that VNS-directed plasticity requires cortical innervation of norepinephrine, acetylcholine, and serotonin. Pairing VNS with a lever-press task for one week reliably causes expansion of proximal forelimb representations. Depletion of each neuromodulatory input with targeted immunotoxins prevents VNS promoted reorganization. These results make substantial

contributions to elucidating the mechanisms, resoundingly confirming the neuromodulatory basis for TPT and VNS-directed plasticity. Further experiments assessing the involvement of the dopaminergic system are planned. Pharmacological interventions altering the release and reuptake levels of these key neuromodulators may also influence plasticity outcomes. These experiments can help guide clinical considerations in terms of patient selection for TPT based on pharmacological profiles.

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Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 605.23/III24

Topic: H.01. Animal Cognition and Behavior

Support: ERC advanced grant 2015
grant from the Bettencourt Schueller Foundation

Title: Cross-modal association learning in humans and monkeys

Authors: *T. VAN KERKOERLE[1,2], L. E. PAPE[2], M. EKRAMNIA[2], J. TASSERIE[2], M. DUPONT[2], B. JARRAYA[2], S. DEHAENE[2], G. DEHAENE-LAMBERTZ[2]
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Abstract: Compared to other animals, humans have been suggested to have a unique capacity for symbolic representations, as exemplified in their ability for language and mathematics. However, direct evidence for this hypothesis remains sparse. One element of symbolic representation is that an association is bidirectional, a symbol can be presented either before or after the associated object for them to be paired. Behavioral studies have indicated that non-human primates do not spontaneously reverse learned associations. Here, we directly compared humans with macaque monkeys in their ability to reverse a cross-modal association, and if so, which cortical areas were involved. Different views of four objects (for example a chair or a purple prolate ellipsoid) were associated with speech sounds (such as 'mush', or 'bugnunu'). The sound was always presented before the image for 2 of the 4 objects (sound-image order), or after the image for the other 2 (image-sound order). Three days of training with 2-4 minutes long videos presenting the 4 pairs were followed by one day of scanning (3T Siemens Tim Trio), both in human adults (31 subjects) and in monkeys (32 sessions in 2 macaque monkeys). To test learning, incongruent pairs were presented during the scanner sessions either keeping the same pairing order than during learning (canonical pairs) or reversing it (reversed pairs). Crucially,

when the pairing order was reversed, the same number of congruent and incongruent pairs were presented to prevent any associative leaning in that direction. Furthermore, as the training comprised both orders (sound-image and image-sound pairs), it should prevent surprise effect for the mere reversal of the order and also facilitate generalization in monkeys. In humans, a network of areas, including the inferior frontal gyrus and the superior temporal gyrus reacted to incongruent pairs independently of the direction of the pairing (canonical or reversed), indicating that humans store the association in a reversible manner, at a symbolic level. In contrast, monkeys only showed an effect of incongruency in the canonical (i.e. learned) direction, without generalization to the reversed pairs. The network was limited in monkeys to the early sensory areas and the inferior frontal gyrus. These results demonstrate a fundamental difference between humans and monkeys, using the exact same paradigm in both species. Humans, but not macaque monkeys, can spontaneously access to a symbolic format, going beyond simple associative learning. This study confirms animal difficulties for symbolic representations as indicated in previous studies.

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Poster

605. Animal Cognition and Behavior: Associative, Nonassociative, and Skill Learning

Location: SDCC Halls B-H

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Program #/Poster #: 605.24/III25

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH081153
Kavli Foundation

Title: Reward associations do not explain performance on transitive inference tasks in monkeys

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Abstract: The observation that monkeys appear to make transitive inferences about the stimuli in ordered lists has been taken as evidence of their ability to form mental representations of those lists and to manipulate their contents. However, alternative explanations have been proposed, which argue instead that transitive inference performance can be explained entirely by "model-free" mechanisms (i.e. by expected or experienced reward value). To test the contribution of reward value to monkeys' behavior in TI paradigms, we performed two experiments in which we manipulated the amount of reward associated with each item in an ordered list. In these experiments, monkeys were presented with pairs of items drawn from the list, and rewards were delivered if subjects selected the item with the earlier list rank. In one condition, stimuli that

were correct more often were paired with lower rewards, whereas those correct less often were paired with larger rewards (creating a "reversed gradient" of reward magnitude). This created a scenario in which the reward magnitude associated with the correct item in any pair of stimuli was smaller overall than that associated with the incorrect item. When subjects were presented with a reversed reward gradient, correct responding was reduced, but nevertheless remained above chance. Over time, monkeys eventually learned to make correct rule-based choices despite countervailing incentives to select incorrect stimuli. In the second experiment, monkeys were trained with a subset of pairs and tested with the remaining pairs. Despite experiencing reversed reward magnitudes during training, they were still able to perform above chance when tested on novel pairs, a strong indication of the transfer of ordinal knowledge. In another condition, the opposite gradient was used, such that larger rewards were paired with stimuli that were correct more often (a "concordant gradient" of reward magnitude); this did not result in disrupted performance. These results demonstrate that monkeys' performance in TI paradigms is not driven solely by expected reward, but that they are able to make appropriate inferences in spite of competing reward associations.

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Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 606.01/III26

Topic: H.02. Human Cognition and Behavior

Title: Theta phase-coupled gamma power in human memory structures strongly correlates with memory performance

Authors: *Y. SALIMPOUR[1], W. S. ANDERSON[2]
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Abstract: The cross-frequency coupling (CFC) between neural oscillations at different frequency bands is a currently investigated neuronal mechanism for complex information processing in the central nervous system. Phase-amplitude coupling (PAC) is a commonly implemented form of CFC. PAC reflects the coupling of the phase of oscillations in a specific lower frequency band to the amplitude of oscillations in another higher frequency band. In a healthy brain, PAC occurs in mesial temporal structures, and changes in PAC have been associated with neurological disorders such as Parkinson's disease and Alzheimer disease (AD). The purpose of this study is to investigate the neurophysiological function of CFC across memory-related structures in the human brain during multi-item working memory tasks. the electrocorticographic (ECoG) activity from the intracranial grid, strip, and depth electrodes was

analyzed in fifteen epilepsy subjects while they performed image sequence encoding and recall tasks. Subjects were involved in remembering (encoding phase) and also reporting (recall phase) the temporal order of sequentially presented images and ECoG was recorded while subjects performed the task. After preprocessing the recorded signals, the frequency spectrum and also the phase-amplitude coupling between the phase of the theta band rhythms (4-9 Hz) and the amplitude of the gamma band oscillations (50-250 Hz) on each trial were estimated. CFC analysis of ECoG signals recorded from the parahippocampal gyrus of epilepsy patients is demonstrated by the strong correlation between levels of PAC and memory performance. Additionally, the portion of gamma activity coupled to the phase of the theta oscillations shows a higher correlation with memory performance in comparison to total gamma band power. These results point toward the possible role of cross-frequency coupling (specifically theta phase-coupled gamma power) in memory related activities in the human brain and demonstrate its crucial function in memory formation and retrieval.

Disclosures: Y. Salimpour: None. **W.S. Anderson:** None.

Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 606.02/III27

Topic: H.02. Human Cognition and Behavior

Title: The primacy of processing speed: Distributed neural activity during digit-symbol performance discriminates individual differences in working memory

Authors: *Y. ZHAO[1], M. MOTES[1], N. HUBBARD[2], M. TURNER[1], B. RYPMA[1]
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Abstract: The neural and cognitive bases of individual differences in working memory (WM) performance remain unknown. One hypothesis is that processing speed underlies WM performance differences. In this view, WM performance differences arise from differences in the speed of fundamental operations. Such speed would afford faster individuals additional mental resources for succeeding WM and higher-level processes (e.g., rehearsal), to benefit their performance. Consistent correlations between processing speed and WM capacity make it difficult to adjudicate the primacy of processing speed or WM from behavioral data. Neuroimaging data provide additional leverage on this question by providing information about the extent to which variability accompanying one task (e.g., a processing speed task) mediates the performance on another task (e.g., a WM task). Similar to the behavioral studies, fMRI studies reveal similar neural mechanism for the two cognitive processes. However, activation pattern characteristics across the brain could be more informative about the fundamental substrate of WM. In this study, we tested the hypothesis that individual differences in processing

speed underlie WM utilizing a multivariate barycentric discriminant analysis (BADA) method, that permits us to predict individuals' WM performance from the patterns of multi-regional fMRI data. 25 healthy younger adults (Mage = 24.3 years, 15 females) completed a digit symbol verification task (DSVT) and a Sternberg WM task (SWMT) during fMRI scanning. In DSVT trials, participants saw an array of nine digit-symbol pairs and a digit-symbol probe pair below it. They indicating whether the probe-pair was present in the key or not by button-press. In the SWMT, participants encoded 2, 4, or 6 letters, maintained them over a delay, and then decided whether a probe letter was present in the encoded set. Average β values from eight frontal and parietal regions were used in the BADA analysis. There was a strong correlation between composite DSVT scores and composite SWMT Cowan's K ($r = .67$, $p < .001$). Consistent with behavioral findings, BADA analysis indicated that, with .95 bootstrap confidence interval, the DSVT task-related fMRI pattern differences could successfully separate the SWMT poor performers from the moderate and the best performers. SWMT could not discriminate those performance differences on SWMT. Our findings support the hypothesis that individual differences in processing speed and the neural substrate underlying processing-speed tasks account for WM performance differences.

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Poster

606. Human Cognition and Behavior: Working Memory II

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Topic: H.02. Human Cognition and Behavior

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NIMH Silvio O. Conte Center (21560-685)
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Title: Event segmentation reveals working memory forgetting rate

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Abstract: We perceive the world as series of segmented sequence of events. People generally agree with segmenting when salient events occur but there is inter-individual variation in the number of perceived segments. According to a prominent theory of event perception (Event

Segmentation Theory; Zacks et al 2007), the working memory system plays a key role in tracking and segmenting a sequence of events; however, data linking individuals' event segmentation to their working memory system is missing. Here, we tested if individuals' event segmentation rates predict their working memory capacities and forgetting rates. Forgetting rate reflects how fast working memory system forgets an encountered event. Healthy adults (n=36) segmented three movies that had different storylines (movie 1 had an overarching story with recurrent events, movie 2 had no overarching story but with recurrent events, and movie 3 had a linear overarching story) and performed a source memory test about the movies. These individuals also participated in an image-action association learning task that was used to extract the individual's working memory capacity and forgetting rate (Collins et al, 2017). Model-free and model-based analyses of the association learning task both showed that memory decay rate is linked to event segmentation. We found an inverted U-shape relationship between the number of reported segments in the movies and working memory forgetting rates in that people who perceived either a very low or a very high number of events had a higher forgetting rate. The working memory forgetting rate did not correlate with source memory performance. The results were robust to the storylines. Working memory forgetting rate is a less studied parameter of working memory system because of the high computational effort for extracting the parameter. The results suggest using individuals' event segmentation performance to infer working memory forgetting rate.

References:
Collins AGE, et al. Interactions Among Working Memory, Reinforcement Learning, and Effort in Value-Based Choice: A New Paradigm and Selective Deficits in Schizophrenia. Biol. Psychiatry (2017), doi:10.1016/j.biopsych.2017.05.017.
Zacks JM, et al. Event perception: A mind/brain perspective. Psychol. Bull. 2007;133:273–293

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Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

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Program #/Poster #: 606.04/III29

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01EY028746

Title: Recovery of working memories after delay-period interference

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Abstract: Distractors presented during the delay period of a working memory task can have a negative impact on performance. However, some memory items can be protected from this interference if a retro-cue informs subjects that only a subset of items will be tested (Makovski & Pertzov, 2015). This retro-cue is theorized to bring one of multiple memory items into the "focus of attention" (FoA) within working memory (Oberauer, 2002). Discussions about the protective effects on items inside the FoA have only been made relative to items that are no longer task-relevant, and therefore not only outside the FoA, but also outside of working memory altogether. Here we sought to understand whether it is the FoA specifically that provides interference protection to working memories, or if this protection is provided to all items in working memory regardless of their attentional state. Participants performed a double retro-cue paradigm (Oberauer, 2005) in which after the encoding of two memoranda (face and scene), a first retro-cue indicated which item would be probed after the first delay, and a second retro-cue indicated which item would be tested after the second. On half of trials, participants maintained the same memorandum throughout both delays (repeat trials), and on the other half participants switched to the previously uncued memorandum for the second probe (switch trials). Crucially, half of trials included interference during the first delay period on which participants made a sub-category judgement for an otherwise task-irrelevant picture of an animal. This paradigm allows us to, during the second delay period, investigate the "recovery" of a memorandum that was either inside the FoA (repeat trials) or outside the FoA (switch trials) during interference. Behavioral memory performance on the second memory probe was degraded by interference more on switch trials than on repeat trials, indicating that memoranda outside the FoA are impacted more by delay interference than those inside the FoA. Preliminary fMRI results (tracking category-level pattern instatement via MVPA) show that during the second delay, classifier evidence was greater for the cued item compared to the uncued item after interference, but only on repeat trials. On switch trials, when participants were asked to recover an item from outside the FoA after interference, classifier evidence was not differentiable between cued and uncued items. Both behavioral and fMRI results indicate that delay interference has a down-stream effect on the recovery of memory representations, and that this effect preferentially impacts memoranda outside the FoA.

Disclosures: R. Mallett: None. **J.A. Lewis-Peacock:** None.

Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

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Program #/Poster #: 606.05/III30

Topic: H.02. Human Cognition and Behavior

Support: Sloan Research Fellowship
Whitehall Foundation (2017-12-73)

NSF 1736028

Title: Predicting memory formation using theta oscillations and temporal-frontal oscillatory coupling

Authors: *T. TRAN[1], B. VOYTEK[2]
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Abstract: Successful memory formation is associated with dynamic changes in temporofrontal cortical activity. These changes are apparent when examining 4 - 8 Hz theta oscillations and population-level spiking activity as well as when measuring the degree of oscillatory coupling between them. However, it is unclear which changes are most predictive of memory encoding as well as how consistently these changes predict encoding across individuals. To investigate this, we analyzed previously collected electrocorticographic (ECoG) recordings from 17 patients undergoing treatment for drug-resistant epilepsy (Solomon et al., 2017). These patients performed a free recall task in which they were serially and visually presented multiple lists of words, from which they were asked to memorize as many words as possible. From these recordings, we measured temporal and frontal cortical theta amplitude, high-frequency activity (HFA), phase-amplitude coupling (PAC), and aperiodic (1/f) slope, as well as interregional, temporofrontal PAC. We used these features to train individualized logistic regression models to correctly classify whether a word would be recalled, on a within-subjects basis, using neural activity during the presentation of subsequently remembered versus forgotten words. With these models, we achieved above-chance classification performance in 13/17 patients. To determine which features most accounted for prediction of memory formation, we used algorithmic feature selection and found that similar classification performance was achievable with a smaller subset of features. Moreover, comparison of model coefficients indicated that this feature subset was relatively consistent across patients. Using this approach, we identify reliable predictive patterns of neural activity that ultimately suggest crucial neural mechanisms underlying memory formation.

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Poster

606. Human Cognition and Behavior: Working Memory II

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Topic: H.02. Human Cognition and Behavior

Support: Hotchkiss Brain Institute/Pfizer Canada Research Award
Canadian Institutes of Health Research New Investigator Award

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Title: Maintenance and manipulation components of working memory and associated structural brain regions in bipolar disorder

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Abstract: BACKGROUND Cognitive deficits are thought to play an important role in the functional impairments seen in patients with bipolar disorder (BD). A domain of cognition that has been found to be impaired even during euthymic periods is working memory (WM). Based on Alan Baddeley's model, WM can be divided into separate *maintenance* and *manipulation* components. Despite the separation of WM into these two components, no study to date has investigated both components separately in BD and associated them with underlying structural brain regions.
OBJECTIVE To investigate differences in maintenance and manipulation components of WM and prefrontal and parietal cortex regions between controls and patients with bipolar disorder.
METHODS Forty-nine participants (24 controls) between 18-60 completed a visuospatial WM task and underwent neuroimaging. For the maintenance condition, participants determined if the position of the initial objects were the same as the newly presented objects, following a delay. The manipulation condition required the mental flip of objects along a horizontal line. Participants determined if the flipped positions of the previous objects matched the position of the new objects. Both accuracy and reaction time (RT) were measured. Structural neuroimaging data of regions generated by using FreeSurfer will be analyzed.
RESULTS 2 group x 2 WM condition ANOVAs examined accuracy and RT data. For accuracy, a significant main effect of WM condition was found across groups ($F(1,47) = 10.249$, $p = 0.002$), with the manipulation condition being more difficult; this did not significantly interact with group. However, the main effect of group approached significance ($F(1,47) = 3.69$, $p = 0.061$), with controls being more accurate than patients. A similar ANOVA for RT found a significant main effect of WM condition ($F(1,47) = 62.35$, $p < 0.001$), with the manipulation component having longer response times. There was no main effect of group ($F(1,47) = 0.142$, $p = 0.708$) or interaction between condition and group ($F(1,47) = 1.69$, $p = 0.199$). These results will be presented alongside findings from the structural neuroimaging data.
DISCUSSION Although both groups found manipulation to be more difficult, only the main effect of group approached significance for accuracy (with controls having higher accuracy). However, these results will be augmented by structural integrity indexes (i.e., gray matter volume, cortical thickness, and surface area) within the prefrontal and parietal cortex. Structural integrity measures may provide alternative information regarding the underpinning of WM dysfunction and brain-behaviour associations in bipolar disorder.

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Poster

606. Human Cognition and Behavior: Working Memory II

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Program #/Poster #: 606.07/III32

Topic: H.02. Human Cognition and Behavior

Support: NIH R01MH110831
NIH U01NS103792
McKnight Foundation
NSF 1554105

Title: Evidence for domain-specific working memory buffers from human single-neuron recordings

Authors: *J. KAMINSKI[1], A. BRZEZICKA[1], J. M. CHUNG[2], C. M. REED[2], A. N. MAMELAK[1], U. RUTISHAUSER[1,2,3]
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Abstract: There are two main views on the nature of working memory (WM) buffers. One proposes that information is stored in a central capacity-limited modality-free buffer; the other suggests that domain-specific buffers exist for different modalities. In the Medial Temporal Lobe (MTL) of humans, cells represent multimodal concepts and they also exhibit persistent activity during WM maintenance. To explore the nature of WM storage in MTL, we performed single-neuron recordings in Medial Temporal Lobe in neurosurgical patients as they performed a working memory task. We asked subjects to maintain in WM images. These images were preselected for each of them individually using a screening task to ensure image-specific neuronal activation with image presentation. In addition to keeping in mind these images, subjects performed a verbal or a spatial distractor task in the middle of the maintenance period. Here, we investigated the properties of neurons that showed visual selectivity for one of the images. These neurons remained persistently active and tuned during maintenance. We found that this image selective persistent activity was significantly disrupted by the spatial but not verbal distractor task. We also found that persistent activity recovered after completion of the spatial task. This result shows two aspects of the specific nature of the neuronal representation of WM information in the MTL. First, the observation that only the spatial task disrupted the persistent activity demonstrates existence of domain-specific buffers independently from those used in other tasks operating in different modality. Second, the observation that persistent activity recovers after the spatial distractor could be an evidence of a distributed system holding multiple copies of the same information in different brain areas. These copies might be used to recover information in the MTL.

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Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 606.08/III33

Topic: H.02. Human Cognition and Behavior

Title: Sex differences in electroencephalographic activity in default mode related with processof attention and memory

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Abstract: ABSTRACT
Cognition results from the synchronic interaction of bioelectric activity of the glio-neuronal assemblies that construct the brain circuits. However, to identify these circuits it is essential to describe absolute power (PA) profiles of the spectrum with its topography in default mode network (DMN) and its modifications in simple learning processes. The purpose of the present study is to simultaneously analyze four frequencies of the EEG (δ, θ, α and β) in DMN, habituation to the repeated photostimulation (FR) and visual-motor association (asso-vm) describing possible differences between genders and correlating the PA of the DMN with performance in neuropsychological evaluations. A total of 40 undergraduate students (20 ± 2 years) were divided into a group of women (GM) and a group of men (GH). The EEG was recorded in DMN and before (pre-FR) and during FR of 20 series from 2 s to 5 Hz (condition of habituation). A similar FR was performed with the indication that upon sensing it, a switch (aso-vm condition) would be pressed with the dominant hand. The analysis of EEGc revealed differences between genders in the power spectrum: GH showed higher PA of δ with respect to GM, while in the other bands (θ, α and β) the opposite phenomenon occurred. In the habituation, synchronization activity was predominant (understood as greater inhibitory activity), while during the asso-vm greater desynchronization was observed (greater activation). Finally, a correlation of the PA of θ in DMN with the total memory score was found. These duly identified data could be established as quantitative electroencephalography biomarkers for the support of neurological diagnoses and follow-up of cognitive neuro-rehabilitation interventions.
Keywords: Glioneuronal assemblies; absolute power; topographic profiles; simple learning processes; habituation; association; Correlation between electrical activity and cognitive processes.

Disclosures: Y.M. Serrato: None.

Poster

606. Human Cognition and Behavior: Working Memory II

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Program #/Poster #: 606.09/III34

Topic: H.02. Human Cognition and Behavior

Title: EEG Neurofeedback training for memory enhancement and rehabilitation

Authors: *G. B. PATRUDU
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Abstract: Several studies show that EEG Neurofeedback training of selective bands has a positive effect on memory and several other cognitive functions. This review summarizes the studies investigating the effect of EEG neurofeedback training of different rhythms like Upper Alpha , Sensory Motor Rhythm , Gamma , Beta and Theta bands on various aspects of memory. EEG Neurofeedback training has been shown to be beneficial to both healthy subjects and patients with cognitive impairment. A study by Alekseeva etal (2017) showed that Alpha based Neurofeedback training enhanced memory in stroke patients. Other studies on Alpha Neurofeedback training had shown specific improvements in Episodic memory (Wei etal, 2017 , Hsench etal 2016), Working memory (Wei etal, 2017; Hsench etal, 2016; Kober etal , 2015; Escolano etal, 2011) , Mental rotation abilities(Escolano etal, 2011; Hanslmayr etal,2005) and Strategic and controlled recollection (Guez etal, 2015) . Sensory motor rhythm NFT improved Visuospatial short term memory (Gomez-Pilar etal, 2016; Kober etal, 2015), Automatic item specific and Familiarity based processes in memory (Guez etal, 2015) and Semantic working memory (Vernon etal, 2003). Both Upper alpha NFT and Sensory motor rhythm NFT nonspecifically improved Short and Long term memory(Kober etal, 2015). Frontal mid line theta up training improved performance on Working memory along with other cognitive functions in aging adults(Wang & Hsieh, 2013).Similarly, up regulating the theta to alpha power ratio in the anterior parietal region improved Working memory performance(Xiong etal, 2014).In a study by Keiser etal (2010), Gamma band activity targeted NFT improved Recollection and Beta band targeted NFT improved Familiarity memory.These findings show that EEG neurofeedback training has a potential to play a promising role in enhancement and rehabilitation of specific aspects of memory

Disclosures: G.B. Patrudu: None.

Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 606.10/III35

Topic: H.02. Human Cognition and Behavior

Title: Perceptually-matched images that are meaningful are remembered better and result in increased CDA in visual working memory

Authors: *I. E. ASP, V. S. STÖRMER, T. F. BRADY
Psychology, Univ. of California San Diego, La Jolla, CA

Abstract: People are able to hold more items in visual working memory (VWM) when asked to remember meaningful stimuli (e.g., umbrella) than abstract stimuli (e.g., blue square) (e.g., Brady et al. 2016). However, in previous work, real-world objects and abstract stimuli were not controlled for perceptual equivalency. It is therefore possible that differences in VWM capacity were driven by perceptual properties (e.g., visual information load) rather than representational meaning. Here we address this concern by using perceptually-matched stimuli and manipulating only their meaningfulness. We used two-tone images (Mooney faces) that can be perceived as meaningful faces when upright, but meaningless blobs when inverted or shuffled. In particular, we measured VWM capacity for faces vs. non-face stimuli and recognized vs. unrecognized faces while simultaneously measuring the contralateral delay activity (CDA) using EEG. The CDA is believed to be a neural marker sensitive to the number of items being actively held in mind (e.g., its amplitude increases as the number of items held in VWM increases). By combining behavior and CDA we can assess the effect of meaningfulness on VWM capacity.In Experiment 1, we found that participants had higher VWM capacity for trials with more faces present compared to perceptually-matched non-faces ($t(11)=3.23$, $p=0.008$), and on trials where participants recognized more of the faces compared to trials with the same stimuli where they recognized fewer faces ($t(11)=3.99$, $p=0.002$). In Experiment 2, we found that in addition, CDA amplitudes were larger when the memory sets consisted of more faces than when they consisted of fewer faces ($t(18)=2.94$, $p=0.001$). We also found that the CDA tended to be larger on trials where participants recognized more of the faces compared to trials where they recognized fewer faces ($t(14)=2.11$, $p=0.053$), suggesting that VWM capacity depends on whether an object is perceived (subjectively) as meaningful by an individual or not. Together these results indicate that meaningfulness plays an important role in enabling more items to be held in VWM, independent of perceptual properties. Broadly, this suggests that VWM capacity is not fixed but critically depends on what type of information is being remembered.

Disclosures: I.E. Asp: None. **V.S. Störmer:** None. **T.F. Brady:** None.

Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 606.11/III36

Topic: H.02. Human Cognition and Behavior

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Cellex Foundation
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Title: Glutamatergic modulation of working memory precision and serial biases

Authors: *H. STEIN[1], D. LOZANO-SOLDEVILLA[1], J. DALMAU[2,3], A. COMPTE[1]
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Abstract: Continuity of mnemonic contents in time contributes to integrating information into coherent memory representations. Recently, attractive response biases towards previously memorized locations in visuospatial delayed response tasks have been reported as evidence for continuous integration of memory contents between trials. These serial attractive biases emerge specifically during working memory (WM) delay. Assuming a beneficial role of attractive biases for the coherence of memory representations, psychiatric and neurological disorders could be characterized by atypical serial memory biases, along with impairments in memory maintenance and precision. We tested a unique population of patients recovering from anti-NMDAR encephalitis to study possible synaptic mechanisms of memory maintenance and continuous memory integration. These patients still have a decreased NMDAR mediated neurotransmission and reportedly suffer from long-term and WM deficits. We collected behavioral and electroencephalography (EEG) data from anti-NMDAR encephalitis patients and healthy control subjects performing a visuospatial delayed response task. While healthy controls' responses were significantly biased towards previous memoranda, serial attractive biases were absent in patients with reduced glutamatergic synaptic transmission. Moreover, encephalitis patients reported memorized spatial positions with lower precision than healthy controls. Both serial biases and WM precision normalized with recovery from the synaptopathy. In EEG data, we analyzed task-related changes in alpha-band power during WM delay and prior to stimulus onset. Both during WM encoding and delay, encephalitis patients showed reduced decodability of the stimulus, compared to healthy controls. Similarly, past stimulus locations could be decoded just before the onset of the new stimulus in healthy controls, but not in encephalitis patients. Persisting target-

specific neural activity during delay and in the inter-trial interval might play a role in explaining behavioral differences between anti-NMDAR encephalitis patients and controls. Taken together, our findings suggest a fundamental role of the NMDAR in the within- and between-trial maintenance of short-term memory traces, potentially leading to deficits in the continuous integration of memory contents in NMDAR synaptopathies.

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Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 606.12/III37

Topic: H.02. Human Cognition and Behavior

Support: 2-R01-DC-009209-11
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R01-MH107235
R01-MH107703
R01MH109520
1R01NS099348
R21-M MH-106799

Title: Structural support for brain state transitions that contribute to working memory

Authors: *E. J. CORNBLATH[1,2], R. CIRIC[3], G. L. BAUM[3], K. RUPAREL[3], T. M. MOORE[3], R. C. GUR[3], R. E. GUR[3], D. R. ROALF[3], T. D. SATTERTHWAITE[3], D. S. BASSETT[2]
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Abstract: In the healthy brain, large-scale white matter architecture and local neuronal membrane properties facilitate seamless transitions between cognitive states. However, the manner in which white matter supports the brain's recurrent spatial patterns of activity, or states, remains unknown. Here, we ask whether structural connectivity predicts trajectories of brain states in the resting state, and whether those predictions are conserved as participants engage in a cognitively demanding task. Using a large (n = 690) community-based sample of healthy youths from the Philadelphia Neurodevelopmental Cohort, we identify common brain states by applying unsupervised clustering to functional neuroimaging data acquired during the resting state and during the performance of an n-back working memory task to classify each time point as a discrete state. Highly active regions in the cluster centroids closely mirror resting state functional networks, with larger dwell times in visual and frontoparietal states during task and default mode network (DMN) states during rest. Furthermore, state transition probabilities differ between rest

and n-back and change over the course of normative neurodevelopment. Using diffusion-weighted imaging acquired from the same subjects, we show that increasing structural connectivity between highly active regions in each state positively correlates with the probability of transitioning between the respective states. These trends are similar for resting state and n-back task data, persist when accounting for spatial distance, and are robust to the choice of cluster number. State probabilities and state transition probabilities also predict working memory performance: increased DMN dwell times and transitions into DMN states at rest positively predict working memory performance. These results challenge the notion that the default mode network is a task negative system, suggesting that frequent, simultaneous activation of the entire DMN at rest and during the n-back task represents brain activity favorable for working memory. Overall, these findings shed new light on the relationship between brain structure and brain activity, as well as the role of regional coactivation in cognition.

Disclosures: E.J. Cornblath: None. **R. Ciric:** None. **G.L. Baum:** None. **K. Ruparel:** None. **T.M. Moore:** None. **R.C. Gur:** None. **R.E. Gur:** None. **D.R. Roalf:** None. **T.D. Satterthwaite:** None. **D.S. Bassett:** None.

Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 606.13/III38

Topic: H.02. Human Cognition and Behavior

Support: Johns Hopkins Lyme Disease Research Center Innovation Fund

Title: An fMRI study of cognition in early post-treatment Lyme disease

Authors: *C. L. MARVEL[1], J. A. CREIGHTON[1], O. P. MORGAN[1], M. B. SLAPIK[1], E. A. MIHM[2], A. W. REBMAN[2], C. B. NOVAK[2], J. N. AUCOTT[2]
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Abstract: Background: Lyme Disease (LD) is caused by the bacterium Borrelia burgdorferi, which is transmitted by infected deer ticks. Symptoms of LD typically include a variety of physical symptoms and cognitive complaints. Specifically, cognitive complaints include difficulty with concentration, executive function, and working memory, despite antibiotic treatment. Contrasting patient reports, however, neuropsychological tests describe normal cognitive function in early post-treatment LD patients. This can be frustrating news for patients who feel their cognitive symptoms are not being adequately addressed in the clinic. We hypothesized that people with LD are able to function at a cognitively normal level, but do so at a physiological cost, which could be measured as hyperactive brain activity. That is, their brains need to work harder than before LD to achieve normal performance. Methods: We administered

a functional MRI (fMRI) paradigm of working memory to 7 patients and 10 healthy controls at three weeks post LD diagnosis and again at six months (N=7 both groups). The working memory paradigm followed a Sternberg design which presented 1 or 2 letters at encoding and consisted of two rehearsal conditions: 1) passive storage of those letters, or 2) manipulation of those letters, such that the subject counted two alphabetical letters forward of each and held those new letters in mind. When a probe was presented, subjects indicated by button press whether the probe matched the original letters (storage) or the newly created letters (manipulation). Event-related fMRI analyses focused on the rehearsal phase that involved storage/manipulation processes only. Contrasts were conducted on the manipulation minus control conditions. Results: Accuracy and response time did not differ between groups or time points. At initial testing, fMRI data between groups showed hyperactivity in the LD group within a fronto-cerebellar pathway relevant to working memory. At 6 months, within-group comparisons between time points showed that the LD group activated regions similar to their own baseline (fronto-cerebellar). However, controls shifted activity to other regions (including inferior frontal, anterior insula, inferior parietal). Regions of interest created from the LD's fronto-cerebellar regions revealed that greater fMRI activity in the frontal lobe correlated with higher test accuracy, but also with fewer days since diagnosis, suggesting that frontal hyperactivity is compensatory yet systems are recovering with time. Summary: This study supports the notion that Lyme Disease impacts cognition and the brain.

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Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 606.14/III39

Topic: H.02. Human Cognition and Behavior

Title: A comprehensive paradigm to test neurocognitive and neuromotor effects of shift work

Authors: *M. O. CONRAD[1], N. A. DIB[2], C. EDWARDS[2], E. CARPER[2], A. HARRINGTON[2], A. STEWART[2], A. MIDDLEMAN[2], J. FELLOW[2], M. T. MAHAR[2], H. S. BAWEJA[2]
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Abstract: Extended workdays (more than10 hours) are increasingly prevalent in healthcare, manufacturing and the public service sectors. Published studies regarding shift length rely on subjective data rather than physical measurements. To our knowledge, no paradigm exists capable of rapidly conducting an overall physical and mental work assessment for work.
The purpose of this study was to (1) develop an efficient battery of tests quantifying the physical

and mental effects of extended work shifts on employees, and (2) validate the test protocol and data analysis system through a pilot study of physical therapy students with two distinct yet rigorous work schedules.
A battery of tests was developed to assess both motor and cognitive aspects of work. Tests included 1) Neuropsychological testing: Groton Maze Learning Test (GML), Two Back Memory Test (TWOB), Continuous Paired Associate Learning Task (CPL) (CogstateTM, CogState Ltd., Melbourne, AU)), and 2) Motor performance testing: maximal pinch & grip strength for both hands, balance (Better Balance Test, Balance Tracking Systems, San Diego, CA), and dexterity (Purdue Pegboard, Lafayette Instruments, Lafayette, IN). A pilot study was conducted to validate the protocol.
Nineteen participants were divided into 2 groups consisting of a 4-day work week (10 total; 5 females, mean age 26.2 years ± 3.68 years) or a 5-day work week (9 total; 7 females, mean age: 24.4 ± 0.73). All subjects participated in two, one-hour test sessions at the start and end of the work week. All participants were tested on the entire battery of tests in each session.
Results indicate a significant effect for both groups on work week in the cognitive domains of associate learning (CPAL; $F_{1,17} = 4.73$; P=0.044) and executive function (GML; GML $F_{1,17} = 29.71$; P=0.00). Within groups there is not a significant effect on motor performance. There were additional significant between group differences for CPAL ($F_{1,17} = 4.73$; P=0.044). Initial interpretations suggest a learning effect for some of the measures.
The study presented a comprehensive battery of tests that objectively and efficiently quantified multiple domains of physical and mental function. Young adults experienced an effect of work week on cognitive, but not motor function. Future studies will apply experimental paradigm to test effect of work week on healthcare profession, manufacturing applications and less active older adults.

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Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 606.15/III40

Topic: H.02. Human Cognition and Behavior

Title: Multiple visual working memory items can guide attention and facilitate perceptual processing

Authors: *J. R. WILLIAMS, T. F. BRADY, V. S. STÖRMER
Psychology, Univ. of California San Diego, La Jolla, CA

Abstract: Previous research has shown that contents of visual working memory (VWM) can guide attention to features that match those actively held in mind. This memory-based attentional guidance has been shown for a single item, but whether similar guidance occurs for multiple items in VWM is under debate. Furthermore, it is unclear whether VWM contents can facilitate perceptual processing in tasks that do not require a narrow focusing of attention.
Here we demonstrate that VWM can guide attention and facilitate visual processing of features that match the memory content, even for two items. Participants were instructed to remember one or two colors while performing another task. In Experiment 1, on 80% of the trials, instead of reporting the memory color, participants performed an unrelated visual search task in which the target either appeared in a circle that matched the color held in VWM or not (as in Soto, 2005). Participants were faster in finding the target when it matched the memory color relative to when it did not for both set sizes even though the memory color was uninformative in the search task ($t(19)=-2.5, p<0.05$), consistent with automatic memory-based guidance. In Experiment 2, instead of a visual search task, we used a perceptual dot estimation task in which participants had to determine which one of two briefly presented dot arrays showed an overrepresentation of one color (as in Fang, Becker, & Liu, 2017 VSS). We found that the number of dots required to accurately identify the target array was significantly lower when the target color matched a memory color, suggesting that VWM contents facilitate visual processing. Importantly, this pattern was present for single and multiple memory items ($t(28)=-2.6, p<0.01$).
Overall, this suggests that two items held in VWM can affect perceptual tasks and attentional guidance in a relatively automatic fashion.

Disclosures: J.R. Williams: None. **T.F. Brady:** None. **V.S. Störmer:** None.

Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

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Program #/Poster #: 606.16/III41

Topic: H.02. Human Cognition and Behavior

Support: RTG Grant 2175

Title: Speeded visual working memory performance during standing and exercise: New insights from event-related EEG lateralizations

Authors: *T. TÖLLNER[1,2], G. DODWELL[2], H. J. MÜLLER[1,3]
[1]Ludwig-Maximilians-University Munich, Muenchen, Germany; [2]Grad. Sch. of Systemic Neurosciences, Munich, Germany; [3]Birkbeck Col., London, United Kingdom

Abstract: While a substantial body of research has investigated the effects of aerobic exercise on cognitive performance, few have monitored exercise-concurrent cognitive processes via

electroencephalography (EEG), and fewer still using an event-related lateralization (ERL) approach. As such, little is known regarding how the temporal dynamics of cognitive processing are influenced during aerobic activity. Here, we aimed at elucidating what influence aerobic exercise and upright posture might have on the temporal dynamics of a concurrent visual working memory (VWM) task.
To this end, participants performed a retro-cue task during both rest (sitting vs. standing) and acute aerobic exercise (cycling vs. walking), using a stationary bicycle and a treadmill, respectively. In the analyses, we combined mental chronometry with three specific EEG markers that can be directly linked to functionally different stages of the VWM processing pipeline. Behaviorally, we found reaction times (RTs) being speeded during exercise, while both RTs and error rates were decreased during upright posture. At the electrophysiological level, we observed CDA waves - indicating the access of WM representations - to be delayed for upright as compared to seated conditions, with no influence of exercise. However, the sLRP waves - indicating motor-response selection - mirrored the RT pattern, showing earlier onsets in active and upright conditions.
This pattern of effects demonstrates that acute aerobic exercise and upright body posture can have faciliatory effects on VWM performance. Within an optimal range of cardiovascular load, aerobic exercise can significantly improve processing speed, while upright posture can enhance both processing speed and response accuracy. Interestingly, VWM performance was found to be lowest in resting, seated conditions - the physiological state in which nearly all other neuro-cognitive research is conducted.The present study is unique in these findings, as to the best of our knowledge no prior research has attempted to disentangle the temporal dynamics of exercise concurrent VWM performance using a staged ERL approach. As such, this study provides an ample theoretical and methodological basis to inform future investigations of visual cognition during exercise.

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Poster

606. Human Cognition and Behavior: Working Memory II

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Program #/Poster #: 606.17/III42

Topic: H.02. Human Cognition and Behavior

Title: Shift in representation explains error in precision working memory tasks

Authors: *M. WOLFF[1,2], J. JOCHIM[2], T. BUSCHMAN[3], E. AKYÜREK[1], M. G. STOKES[2]
[1]Univ. of Groningen, Groningen, Netherlands; [2]Univ. of Oxford, Oxford, United Kingdom; [3]Princeton Neurosci. Inst. & Dept of Psychology, Princeton Univ., Princeton, NJ

Abstract: Recent research suggests that imprecise recalls in spatial working memory (WM) tasks are not only attributable to a noisy neural representation of the remembered location, but also to drift of the location code over time (Wimmer et al., 2014; Schneegans & Bays, 2018). We investigated whether similar processes are involved in non-spatial WM. Human participants completed a free-recall WM task while EEG was recorded. Two randomly orientated gratings were presented simultaneously at the beginning of each trial and a retro-cue indicated which of the two items was relevant shortly after. Participants were instructed to reproduce the orientation of the cued item at the end of the trial. "Impulse" stimuli were presented at two separate time-points during the maintenance period. The neural impulse response is WM content-specific (Wolff et al., 2017), and was used here to track the evolution of the neural representations of WM content during the delay. We found that orientation recalls that were either clockwise or counter-clockwise relative to the actual orientation were accompanied by corresponding shifts in the neural representations of the cued item as revealed by the "impulse" stimuli. The effect was stronger later in the delay, suggesting that the neural code of the item in WM drifts towards the final response, replicating and extending previous findings.
Schneegans, S., & Bays, P. M. (2018). Drift in neural population activity causes working memory to deteriorate over time. *Journal of Neuroscience*, 3440-17.
Wimmer, K., Nykamp, D. Q., Constantinidis, C., & Compte, A. (2014). Bump attractor dynamics in prefrontal cortex explains behavioral precision in spatial working memory. *Nature neuroscience*, *17*(3), 431.
Wolff, M. J., Jochim, J., Akyürek, E. G., & Stokes, M. G. (2017). Dynamic hidden states underlying working-memory-guided behavior. *Nature neuroscience*, *20*(6), 864.

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Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

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Topic: H.02. Human Cognition and Behavior

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NSF DMS NRT Grant 1514743
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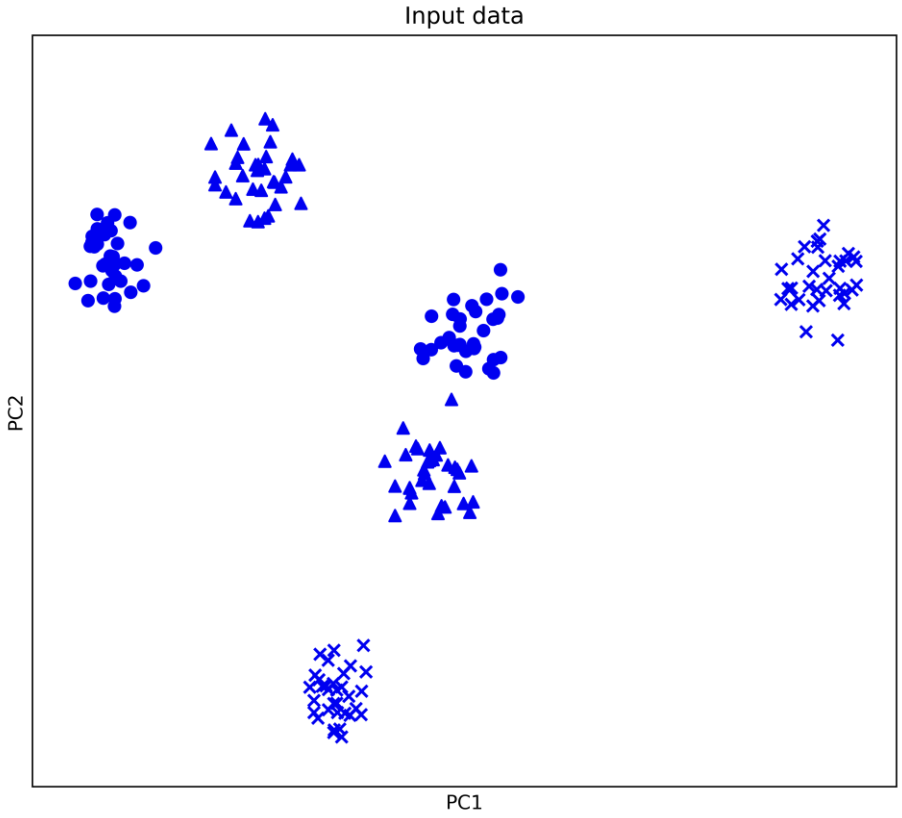
Title: A look into the dimensionality of recurrent neural networks

Authors: *M. S. FARRELL[1], E. SHEA-BROWN[2], S. RECANATESI[3], G. LAJOIE[4]
[2]Applied Mathematics, [3]Physiol. and Biophysics, [1]Univ. of Washington, Seattle, WA;
[4]Mathematics and Statistics, Univ. de Montréal, Montreal, QC, Canada

Abstract: The functional role of neural circuits in the brain can be modeled as a transformation of inputs into some desired outputs. In this work, a recurrent neural network is trained to classify spatially clustered inputs into classes using working memory. This task serves as a testbed to ask questions about the nature of neural representations as inputs are transformed into outputs defined by class assignment. We use a metric of dimensionality to quantify this transformation and see how it behaves as a function of the input and output dimension. We explore possible ways that this metric can be used to gain a deeper insight into the workings of the network and used to influence the training process for the purpose of finding new classes of solutions.



Shapes denote classes (here there are three classes and six clusters)

At the end of the working memory delay period, the trained network compresses its representation from six clusters into three.

Disclosures: M.S. Farrell: None. **E. Shea-Brown:** None. **S. Recanatesi:** None. **G. Lajoie:** None.

Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 606.19/III44

Topic: H.02. Human Cognition and Behavior

Title: Both facilitation and impairment: Similarity affects interference during visual working memory

Authors: *L. YANG[1,2,3,4], T. XIA[1,5], L. MO[2,3,4], C. SEGER[1,3]
[1]Dept. of Psychology, Colorado State Univ., Fort Collins, CO; [2]Ctr. for Studies of Psychological Application,South China Normal Univ., Guangzhou, China; [3]Sch. of Psychology, South China

Normal Univ., Guangzhou, China; [4]Guangdong Key Lab. of Mental Hlth. and Cognitive Science, South China Normal Univ., Guangzhou, China; [5]Art and Design Sch., Guangdong Univ. of Technol., Guangzhou, China

Abstract: Previous research from our laboratory has demonstrated similarity effects in visual working memory for multiple items. We identified a "U-shape" relationship: working memory for a set of moderate similarity items is worse than for either high or low similarity items. Here we extended this research to investigate how the similarity of interfering information relative to working memory content impacts visual working memory. We hypothesized that interference effects would also show a U-shape relationship, with greatest interference by moderate similarity items. To test our hypothesis, we manipulated degree of similarity of visual and semantic properties of items across high, moderate and low similarity levels. We collected BOLD fMRI images while participants performed a continuous free recall task. In this task, a study stimulus was presented first. After a mask there was a delay period, during which four interfering images were presented sequentially. The interfering stimuli varied in degree of similarity to the study items. Finally, a probe stimulus was presented and participants were required to respond whether it was the same as the study stimulus. To ensure that interfering items were attended to, participants also performed a one-back recognition task during the delay phase. We identified regions of interest in occipitotemporal, frontoparietal executive control, dorsal attentional, and salience networks that were active during the study, delay, and probe phases of the task. We then used model-based fMRI and multivariate pattern analysis to characterize how activity in each region was affected by the degree of similarity of the interfering stimuli to the study items.

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Poster

606. Human Cognition and Behavior: Working Memory II

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Program #/Poster #: 606.20/III45

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01 MH063901

Title: Attentional effects on working memory representations: Comparing information-detection techniques and metrics

Authors: *J. MILLER[1], J. M. SCIMECA[1], N. S. ROSE[2], M. DESPOSITO[3]
[1]Helen Wills Neurosci. Inst., UC Berkeley, Berkeley, CA; [2]Dept. of Psychology, Univ. of Notre Dame, Notre Dame, IN; [3]Helen Wills Neurosci. Inst., Univ. of California Berkeley, Berkeley, CA

Abstract: Deploying attention within working memory (WM) helps determine which WM representations should guide behavior. When retrospective cues (retrocues) are used to guide attention during WM maintenance, neural evidence reveals different representational states: attended items are generally decodable from brain activity measures (fMRI or EEG), whereas unattended items are not generally decodable (even if later task-relevant). However, multivariate pattern analysis (MVPA) decoding techniques have yet to fully characterize the nature of attended and unattended WM representations, and mixed results render the neural substrates for "latent" WM representations unclear. For example, a recent study used a novel pattern analysis technique to successfully decode unattended WM representations in the intraparietal sulcus (Christophel et al., 2018). It remains unknown how much variability in detecting WM representations stems from true differences in attentional effects (e.g., change in representational format or cortical localization when outside the focus of attention) versus the informational approach (e.g., training different MVPA approaches on different tasks and task phases).
Here we investigated the nature of WM representations inside and outside the focus of attention using fMRI and several MVPA approaches. Human participants completed a multi-item WM task with two retrocues and two recognition probes (Rose et al., 2016). The first retrocue indicated an attended memory item (AMI) that would be tested at the first probe. The next retrocue could direct attention to the same item (repeat trials) or the previously unattended memory item (UMI; switch trials). We analyzed the fMRI timeseries with two primary MVPA methods: (1) decoding with a linear classifier, and (2) an inverted encoding model with an explicit feature space based on the stimulus categories (words, faces, and motion). Consistent with previous results, training either method on delay-period activity from an independent single-item WM task reveals high evidence for the AMI and chance-level evidence for the UMI in the retrocue task. Training on data from the retrocue task, however, revealed different evidence patterns for remembered items; e.g. training on the final delay of switch (vs. repeat) trials produced lower evidence during the initial multi-item delay but similarly high evidence after the first retrocue. These results suggest that information that was previously unattended is maintained in a modified representational format even when brought back into the focus of attention, implying a complex coding scheme for WM representations at different levels of attentional priority.

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Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 606.21/III46

Topic: H.02. Human Cognition and Behavior

Support: NSF EPSCoR 1632738

Diamond Foundation

Title: Trends in interictal epileptiform activity are correlated with free recall performance

Authors: *S. MEISENHELTER[1], B. C. JOBST[2]
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Abstract: We examined the relationship between the rate of interictal epileptiform activity (IEA) over long time periods and performance in a free recall working memory task.
Our previous work has demonstrated that IEA can momentarily impair cognitive function, but the cumulative effects of IEA over long periods of time have remained unstudied. A recent study has shown that epilepsy patients commonly have multi-dien rhythms in IEA rate and that these rates can govern seizure likelihood, hinting that IEA rate is associated with slow processes that govern brain activity.
We conducted this study primarily in subjects who are receiving epilepsy treatment using the NeuroPace RNS System, an implantable neurostimulator that can deliver therapeutic stimulation in response to clinician-specified patterns in electrocorticography. Using this system, we can obtain continuous records of how many times per hour the neurostimulator detected IEA, as well as electrocorticography (ECoG) recorded from the subjects' seizure onset zones during testing sessions.
We found that when subjects had elevated rates of IEA relative to their baseline rate, they had reduced performance on cognitive tasks. We also examined ECoG recorded during cognitive testing to determine whether there are changes in brain activity during periods of heightened IEA rates.

Disclosures: S. Meisenhelter: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NeuroPace, Inc. provided some equipment for this study. **B.C. Jobst:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NeuroPace, Inc. provided some equipment for this study.

Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 606.22/III47

Topic: H.02. Human Cognition and Behavior

Support: NIMH Grant MH059299

Title: Task switching and network inflexibility in obsessive compulsive disorder

Authors: *A. Z. CHOWDURY[1], L. PIVETTA[1], P. EASTER[1], P. ARNOLD[2], G. HANNA[3], D. R. ROSENBERG[1], V. A. DIWADKAR[1]
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Abstract: Background
Obsessive compulsive disorder (OCD) is characterized by dysfunctional activation and brain network profiles (Diwadkar et al, 2015). How network profiles adapt to *changes* in cognitive load is not well understood, yet such dynamic adaptation is related to functional reserve in brain networks. We investigated *similarities* in network profiles *within* OCD and healthy controls (HC) as subjects transitioned between attention- and memorial-based processing. Processing was induced using a standard n-back paradigm with two levels (Attention: 0-Back and Memory: 1-Back). Network profiles were investigated using undirected functional connectivity (uFC) based on bivariate correlations (Silverstein et al, 2016).
Methods
Data were collected from 105 participants (40 OCD, 3T Siemens Verio). During the task, letters were projected in sequence and subjects signaled their response (to targets). fMRI data were processed using typical methods (SPM12). Ten co-activated peaks (**Ins**ula, **F**rontal **P**ole, **I**nferior **F**rontal **T**riangularis-1,2, **M**iddle **F**rontal **G**yrus-1,2, **S**upplementary **M**otor **A**rea, **I**nferior **f**rontal **O**percularis, **P**osterior **S**upramarginal **G**yrus and **I**nferior **P**arietal) were identified from conjunction analysis (HC ∩ OCD). Correlation coefficients from time series of the co-activated peaks were computed and normalized to the bivariate distribution curve (Fisher's Z). For each of the 45 sub-network pairs *Z* coefficients from each of the 0- and 1-Back conditions were submitted to second level correlational analyses *within* each group (OCD, HC). The resultant Pearson correlation coefficient (*r*) served as a metric of similarity between sub-network behavior in the 0- and 1-Back conditions. A higher *r* represents greater similarity and by inference, lower flexibility of network function across conditions. Corresponding *r*s (OCD vs HC) were compared for differences in significance (Wuensch, 2002).
Results
OCD evinced significantly *greater* correlations for the following network pairs, **SMA↔IFT**-1 ($p<0.05$), **SMA↔PSG** ($p<0.02$), **SMA↔IP** ($p<0.02$) and **MFG↔IP** ($p<0.02$). HC had significantly greater correlations for **IFT**-1↔**IP** ($p<0.05$).
Discussion
Our data imply that OCD subjects are characterized by a relative lack of dynamic flexibility during task switching on frontal-parietal (FPN) and frontal-motor sub-networks. This inflexibility may reflect a network-based representation of exaggerated mechanisms of frontal-cingulate control during basic processing (Diwadkar et al., 2015; Friedman et al., 2017). Successful task-switching depends on network *dynamics*; A focus on network inflexibility may enhance the search for OCD bio-markers.

Disclosures: L. Pivetta: None. **P. Easter:** None. **P. Arnold:** None. **G. Hanna:** None. **D.R. Rosenberg:** None. **V.A. Diwadkar:** None.

Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 606.23/III48

Topic: H.02. Human Cognition and Behavior

Support: PRODEP-SEP Grant UABC-PTC-486

Title: Working memory EEG power spectrum and academic achievement

Authors: *M. L. GARCIA-GOMAR, B. JIMÉNEZ-HIGUERA, A. J. NEGRETE-CORTÉS, P. FERNÁNDEZ-RUÍZ
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Abstract: Working memory (WM) is defined as an important system for the maintenance of information during the execution of complex tasks. It has been described that WM is associated with information seeking knowledge. Alpha and Theta frequency bands are associated with WM. It has been described that in general students with high academic achievement have higher power in all frequency bands during WM tasks. Objective: To study the behavioral and electrophysiological differences associated with a working memory task between university students of high vs low academic achievement. Methods: 40 university students participate in the study. WM was assessed by a Sternberg verbal working memory task. We register brain electric activity while the students were responding the WM task. Results: The students included in the study were 29 women and 11 men, with an average of 18.8±0.89 years old. EEG Power spectrum was analyzed during three different conditions: Baseline, Attention and WM. We did not find significant differences in the behavioral performance in the WM task. However results indicate significant differences in EEG power spectrum between high vs low academic achievement groups. During almost all the conditions, low academic achievement group showed higher delta and theta absolute power. However, high achievement group show higher alpha absolute power during almost all the conditions. Regarding topography during working memory condition, low academic achievement group involved almost all brain regions while high academic achievement group involved only left frontal and posterior brain regions. Conclusions: There are EEG power spectrum differences that can underlie differences in academic achievement as it has previously been demonstrated. High academic achievement group show the brain activity associated with WM, higher alpha power over frontal and posterior regions. WM is a cognitive process that is very important in real life function as in the academic field.

Disclosures: M.L. Garcia-gomar: None. **B. Jiménez-Higuera:** None. **A.J. Negrete-Cortés:** None. **P. Fernández-Ruíz:** None.

Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 606.24/III49

Topic: H.02. Human Cognition and Behavior

Support: U01 AG050618

Title: 5Hz repetitive transcranial magnetic stimulation to enhance working memory and neural factors underlying the rTMS induced behavioral plasticity in old and young adults

Authors: *L. BEYNEL[1], S. W. DAVIS[1], C. CROWELL[1], S. HILBIG[1], W. LIM[1], H. PALMER[1], A. BRITO[1], A. V. PETERCHEV[1], B. LUBER[2], S. H. LISANBY[2], R. E. CABEZA[1], L. G. APPELBAUM[1]
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Abstract: Working memory (WM) is a critical cognitive function relying heavily on the prefrontal cortex (PFC), and widely affected with aging. In this study, we tested the capacity of repetitive transcranial magnetic stimulation (rTMS) to enhance WM in a group of young and elderly participants. To advance our knowledge of what neural factors underlie rTMS-induced behavioral plasticity, brain activations in the targeted PFC were tested. This study involved 6 days of participation. Participants were trained on a Delayed-Response Alphabetization Task in which an array containing 3 to 9 letters was presented, followed by a 5-second delay period during which subjects were asked to keep the stimulus in mind and reorganize the letters into alphabetical order. At the end of the delay, a probe letter with a number above it appeared on the screen. Participants were asked to report if the probe letter was in the original set and matched (Valid), or not (Invalid), the number when re-organized into alphabetical order, or if the letter was not in the original set (New). During the second visit, the same task was performed in the fMRI scanner and individualized statistical map predicting the parametric increase in BOLD activity associated with increasing set size was identified. On days 3 through 6, 25 pulses of 5Hz rTMS at resting motor threshold were delivered to the identified PFC target, on each trial, either with active or electrical sham rTMS. rTMS-induced accuracy changes were assessed, as well as correlations between those changes and the parametric PFC activations associated with increasing set sizes. Active rTMS significantly improved accuracy relative to sham only in the hardest condition (largest set size in Invalid trials). Besides, the magnitude of improvement with active rTMS showed an age-dependent pattern of correlation with the parametric PFC activation. For the young, subjects showing the lowest increase in PFC activation with difficulty increase are the ones benefitting the most from rTMS, while a positive but not significant correlation was found for the elderly. This study showed that online 5Hz-rTMS can enhance working memory abilities, but only in the most challenging condition. Interestingly, the magnitude of this rTMS-

induced improvement was found to express significantly different patterns for old adults and young adults, indicating that baseline fMRI activation in the PFC may play a mediating role in rTMS effects. These findings provide important implications towards the use of non-invasive neuromodulation to enhance cognitive function and provide specific prescriptive recommendations that may lead to optimal efficacy.

Disclosures: L. Beynel: None. **S.W. Davis:** None. **C. Crowell:** None. **S. Hilbig:** None. **W. Lim:** None. **H. Palmer:** None. **A. Brito:** None. **A.V. Peterchev:** None. **B. Luber:** None. **S.H. Lisanby:** None. **R.E. Cabeza:** None. **L.G. Appelbaum:** None.

Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 606.25/III50

Topic: H.02. Human Cognition and Behavior

Title: Identifying Chronic Cognitive and Electrophysiological deficits in individuals with and without a history of Concussion

Authors: *A. TAPPER[1], E. NIECHWIEJ-SZWEDO[2], R. STAINES[2]
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Abstract: Current clinical tests for concussions have been ineffective at detecting chronic cognitive effects. In contrast, dual-task paradigms that stress cognitive resources appear to be helpful in identifying underlying cognitive deficits in individuals with a history of concussion who are symptom free. We hypothesize that persisting cognitive impairments (i.e., poorer performance) will be present in individuals with a history of concussion compared to those without when using a visual-auditory dual-task paradigm. The visual-auditory dual-task involved two tasks including, a visuospatial working memory task (i.e. computerized Corsi block Test) - subjects needed to encode and recall a sequence of blocks, and an auditory Go-NoGo task - subjects responded as quickly as possible to a target tone and withheld a response to a standard tone. Both tasks were performed individually and simultaneously. Eleven recreational athletes (6 reported a medically diagnosed concussion, 5 without) participated in the study. Three dual-task measures (Corsi cost, auditory cost and total cost) were used to compare single versus dual-task performance between groups. Additionally, event-related potentials (ERPs) were time-locked to the auditory task in both conditions (single, dual) to assess brain functioning. Two ERP measures (P50, N100) of early auditory gating were analyzed by comparing the amplitude of target tones to standard tones. Preliminary findings show that individuals with a history of concussion perform significantly worse on the Corsi cost ($p<.05$) and total cost ($p<.05$) measures compared to those without. No significant differences are shown in P50 or N100 gating mechanisms; however, there is a trend showing that the P50 gating ERP is smaller in individuals with a history

of concussion. Furthermore, a larger P50 difference between target and standard tones may be linked to the total cost measure. In conclusion, concussions may produce subtle long-term deficits in executive functions that can be detected using a dual-task paradigm and these deficits may be linked to early gating mechanisms.

Disclosures: A. Tapper: None. **E. Niechwiej-Szwedo:** None. **R. Staines:** None.

Poster

606. Human Cognition and Behavior: Working Memory II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 606.26/III51

Topic: H.02. Human Cognition and Behavior

Support: R01 NS106822

Title: A scalp EEG signature of delay period activity in verbal working memory

Authors: *A. D. FRIEDMAN, P. K. BISARYA, V. MURALHIDARAN, A. R. ARON
UC San Diego, La Jolla, CA

Abstract: Research on working memory maintenance and distractibility has been hampered by difficulty in identifying sustained neural activity signatures during the delay period. Here we ran human participants in a scalp EEG experiment of verbal working memory. On each trial, participants encoded a sequence of verbally-presented phonemes, held them across a delay period, and then, when prompted, tried to say them in the correct order. We manipulated load so that, within each participant, there were four vs. two phonemes. As expected, accuracy was significantly lower for high vs. low load ($Z=1.9$, $p<0.05$). We analyzed the scalp EEG data using Generalized Eigenvector Decomposition. This looks for a subspace of weights which optimally separates signals from two conditions (here, high vs. low load, time-locked to the start of the delay period). In each participant a component was identified in the range of 10 to 15Hz. For this component there was a sustained power change (over 2 seconds of delay period) for high vs. low load. Importantly, in high load this was reduced when errors were made. Further analysis will test if and how this sustained signature relates to a left sensorimotor component for speech. Overall, the results show that a maintenance signature for verbal WM can be derived from scalp EEG.

Disclosures: A.D. Friedman: None. **P.K. Bisarya:** None. **V. Muralhidaran:** None. **A.R. Aron:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

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Program #/Poster #: 607.01/III52

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant 1R56AG049793-01A1
NRSA Grant #T32AG000175

Title: Spontaneous resolution of competing memories is impaired with age

Authors: *B. CORBETT[1], S. M. POLYN[2], M. R. DULAS[3], A. L. DUARTE[4]
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Abstract: Proactive interference can impair our memory in daily tasks such as retrieving a recently updated email password or the updated dosage of a medication. Previous research has found that older adults are more susceptible to proactive interference, which may contribute to their episodic memory impairments. The current fMRI study investigated if age-related deficits in PFC-mediated cognitive control processes underlie age-related differences in the resolution of proactive interference in an associative memory task. Further, multivariate pattern analysis was applied to examine if competing memories (lures) were reliably reactivated during attempts to recover recent ones (targets) in both age groups and if the relative amount of target vs. lure reactivation differs as a function of mnemonic interference and age. Young and older adults were tasked with remembering which associate (face or scene) objects were paired with most recently during study, under conditions of high, low or no proactive interference. Following scanning, we tested participants' memory for varying levels of episodic detail about the pairings (i.e. face category vs. gender vs. specific face). Behavioral results show that as proactive interference increased, associative memory performance worsened similarly across groups. Across age, memory performance was worse for the specific target associate than the target category. Imaging results revealed that lures were reliably reactivated during attempts to retrieve the target. Importantly, stronger reactivation of the lure was associated with less accurate retrieval of the target. Additionally, imaging results suggest the ability to spontaneously resolve interference at encoding may be impaired in older adults. Collectively, these results shed light on the neural mechanisms behind overcoming interference in associative memory and how this differs with age.

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Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

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Program #/Poster #: 607.02/III53

Topic: H.02. Human Cognition and Behavior

Support: NIAAG047979
UL1TR002319

Title: Cognitive and subjective side effect ratings following exposure to oxycodone in middle age and older adults with healthy and unhealthy alcohol consumption patterns

Authors: *M. CHERRIER[1], C. KRAY[2], X. TAO[3], J. LI[4], R. WANG[4], W. YEUNG[5], G. W. TERMAN[6], T. SIMPSON[5], A. SAXON[5], D. SHEN[7]
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Abstract: Chronic unhealthy levels of alcohol use, may predispose adults to use illicit substances and/or modify their response to prescribed medications, such as pain medications. There is increasing recognition of an association between chronic pain conditions and heavy alcohol use. This relationship may also impact the associated side effects experienced by adults when taking a pain medication. We examined the cognitive and side effect response of middle age and older adults who met criteria for healthy and unhealthy alcohol drinking patterns after exposure to a single 10mg dose of oxycodone. We anticipated that regular unhealthy alcohol consumption would result in a different cognitive and side effect response of cognition and side effects.
Methods: Participants underwent an initial phone screen, followed by an in person screening visit that included informed consent. Eligible participants underwent a day long study, that included baseline administration of cognitive tests and side effect questions that were repeated at three intervals (90 minutes, 5 and 8 hours) following administration of 10mg of oxycodone. The cognitive test battery included measures of memory, working memory and a computerized test of sustained attention and working memory (digit symbol substitution test). Side effect assessment included an opioid adjective list.
Results: Ninety four adults completed the initial screening visit, and thirty six adults completed all study measures. Fourteen met criteria for NIAAA criteria for unhealthy alcohol consumption and 22 met criteria for healthy alcohol consumption. Participants in the middle age group had a mean age of 51 (11.2) years and older adults had a mean age of 72 (4.2) years. Between group (unhealthy vs healthy drinkers) comparison of performance on a computerized version of DSST revealed improvements in total score over all trials (baseline, and 90 minutes, 5 and 8 hrs post

dose) F(4, 32) = 25.8 p<.01 with the exception of the older, heavy alcohol consumption group which did not improve. Subjective rating of side effects was rated as more severe in the older unhealthy group compared to middle age and healthy.
Conclusion: These findings indicate that older adults, particularly those with unhealthy alcohol consumption behaviors, may experience more adverse cognitive impact from pain medication and may also subjectively rate the experience of the medication as more adverse compared to middle age participants. It is possible that alcohol consumption patterns may impact cognitive and subjective side effects of pain medications in older adults.

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Poster

607. Human Cognition and Behavior: Cognitive Aging II

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Program #/Poster #: 607.03/III54

Topic: H.02. Human Cognition and Behavior

Support: NRSA Grant T32AG000175
NSF Grant BCS-1125683

Title: Aging affects integration of multiple episodic details

Authors: *T. JAMES[1], M. N. RAJAH[2], A. L. DUARTE[3]
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Abstract: The anterior prefrontal cortex (aPFC) is believed to play a critical role in integrating the outputs of lower-order processes, such as evaluations of item or inter-item properties. While the high-order integration functions attributed to aPFC have been shown to support complex reasoning, the region's role in episodic memory is less well understood. Emerging data suggest high-order PFC functions may be particularly susceptible to the effects of age and may contribute to older adults' associative memory impairments. It is currently unknown how aging interferes with the aPFC operations necessary for integrating multiple relations for episodic encoding and retrieval. We investigated this issue in the current fMRI study. Young and older adults were presented with an occupation and an object and were asked to judge how likely the two were to interact, either in general or within the context of a given scene. When provided with a scene, participants needed to consider and integrate the distinct relations between the three items to reach a decision - a task dependent on aPFC functions. Multivariate behavioral partial least squares (B-PLS) was used to identify patterns of brain activity during associative encoding

that were associated with age and/or subsequent pair and context memory accuracy. The PLS analysis identified two significant effects. The first effect indicated that activity in the most anterior PFC areas supported both pair and context memory in young adults. The second effect showed a pattern of activity that was differentially correlated with pair and context memory in older adults: more posterior PFC areas supported context memory while bilateral precuneus supported memory for the pairs. These effects were further supported by the behavioral data. For young adults, pair and context memory were significantly correlated; older adults did not show this association and demonstrated poorer performance overall. Failure to recruit the most anterior aspects of the PFC could indicate older adults had difficulty engaging the necessary operations to encode the scene-occupation-object associations as an integrated whole.

Disclosures: T. James: None. **M.N. Rajah:** None. **A.L. Duarte:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 607.04/III55

Topic: H.02. Human Cognition and Behavior

Support: CIHR Grant MOP-148940

Title: Aging is linked to a dissociation of future prospection from other autobiographical memory abilities

Authors: *C. FAN[1], H. ABDI[2], A. ESLAMI[3], B. LEVINE[1]
[1]Rotman Res. Institute, Baycrest, North York, ON, Canada; [2]Univ. of Texas at Dallas, Dallas, TX; [3]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: It is well known that autobiographical memory (AM), which involves retrieving information relevant to one's own life, is impacted with age. The Survey of Autobiographical Memory (SAM) is a validated self-report questionnaire that measures individual differences in four categories of AM—episodic, semantic, spatial, and future prospection—but the factor structure of the SAM has not been examined in different age groups. Here, we analyze data collected online from two samples to investigate whether the latent dimensional structure of AM abilities, as measured by the SAM, changes across the adult lifespan. All participants completed the SAM as part of a larger battery of online measures. Sample 1 comprised over 4000 subjects aged 18-85 who independently came across our laboratory's research on AM, and completed our online survey to learn more about their own memory. Sample 2 comprised over 1300 healthy older adults aged 50-93, recruited online through CARP (the Canadian Association for Retired Persons). We first examined the factor structure of the SAM in Sample 1 using PCA, and replicated previous research: Dimension 1 separated those with high from low self-reported

memory across all four memory categories, and Dimension 2 separated individuals with high versus low self-reported spatial memory relative to other memory types. However, when we repeated this analysis in Sample 2, comprised solely of older adults, we found that Dimension 2 separated individuals with high versus low future prospection—rather than spatial memory—from other memory types. To examine this age-related dissociation of future prospection from other AM abilities, we binned participants in Sample 1 based on age, ran a PCA on the SAM in each bin, and computed a matrix of RV coefficients between all age bins. The factor map of the age bins indicated that the dimensional structure of the SAM remained relatively constant from the ages of 18 to 60, after which the future component appeared to dissociate. A body of existing research has linked the processes underlying episodic AM to those supporting future prospection, but our results suggest that aging may be associated with subtle shifts in memory functioning such that with age, episodic recollection decouples from future prospection. These findings open the door for future work to examine whether this dissociation can be observed on behavioural and neural levels, and may ultimately be used to inform strategies for identifying individuals at risk for age-related cognitive decline.

Disclosures: C. Fan: None. **H. Abdi:** None. **A. Eslami:** None. **B. Levine:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

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Program #/Poster #: 607.05/III56

Topic: H.02. Human Cognition and Behavior

Support: Dana Foundation

Title: Memory benefits in older adults following real-world environmental enrichment training

Authors: *B. KOLARIK, S. RUTLEDGE, S. STARK, C. STARK
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Abstract: Memory deficits are common in healthy aging. However, in rodents, exposure to a stimulating environment (environmental enrichment) can alleviate some age-related memory deficits. Similarly, exposure to 3-D video games improves memory performance in young adults. We sought to test if environmental enrichment has the same effect on older adults using a real-world exploration task. Participants searched a local park for landmarks using a cell phone app which presented pictures of landmarks and instructed them to find that place in the park (8 landmarks/day for 20 days). We investigated whether exploration training would ameliorate mnemonic deficits as evidenced by improved performance on memory tests from pre- to post-training. We compared participant's performance on a series of neuropsychological tests as well as the Mnemonic Similarity Task, used to assess pattern separation ability. To assess

performance during the park intervention, we tracked the number of hints requested on the first and last day in each park as well as normalized excess path to each target. Participants requested fewer hints and took more efficient routes to the targets on the last day in each park than on the first day, indicating that they were learning information about the target locations during the intervention. On neuropsychological tests, participants showed no change in digit span, but did show a significant increase in RAVLT Total scores suggesting that our intervention is targeting episodic memory function. The Mnemonic Similarity Test can be used to assess both recognition memory and lure discrimination (pattern separation). Consistent with previous findings, recognition memory scores did not change, while lure discrimination showed significant improvement following the intervention. In addition, we will report on structural imaging evaluating any neural alterations following this intervention. These results suggest that a real-world behavioral intervention can help ameliorate memory deficits, specifically those related to pattern separation, associated with normal healthy aging.

Disclosures: B. Kolarik: None. **S. Rutledge:** None. **S. Stark:** None. **C. Stark:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 607.06/III57

Topic: H.02. Human Cognition and Behavior

Support: ADNI3 combination of private and federal agencies including NIH

Title: Diffusion mri metrics of brain aging in the adni3 study: Relation to clinical impairment and scanning protocol

Authors: *A. ZAVALIANGOS-PETROPULU[1], T. M. NIR[1], S. I. THOMOPOULOS[1], N. JAHANSHAD[1], R. I. REID[2], M. A. BERNSTEIN[3], B. BOROWSKI[3], C. R. JACK[3], M. W. WEINER[4], P. M. THOMPSON[1]
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Abstract: The Alzheimer's Disease (AD) Neuroimaging Initiative acquired diffusion-weighted MRI (dMRI) from a third of its participants during its second phase (ADNI2), at sites with 3T GE scanners, to evaluate the utility of white matter (WM) microstructure biomarkers of aging and AD. Recently, ADNI3 extended dMRI acquisitions to include GE, Siemens and Philips scanners, resulting in 7 protocols. ADNI3 protocols have smaller voxels and variable numbers of gradient directions compared to ADNI2. To better understand consistency of effects across

ADNI3 protocols, we assessed the ability of each protocol to detect associations with cognitive impairment. As ADNI3 data collection is ongoing, we analyzed all baseline dMRI and clinical data available as of March 2018 in the ADNI database (https://ida.loni.usc.edu/). We analyzed data from 278 (age=75.4±8.0 yrs; M/F=125:153) ADNI3 participants scanned with 5 dMRI protocols, and computed 5 dMRI indices in 24 WM ROIs - mean, radial, and axial diffusivity (MD/RD/AxD), and fractional anisotropy (FA) derived from the tensor model and from the Tensor Distribution Function (TDF). All ROI indices were tested for associations with 3 cognitive test scores (MMSE, ADAS, and CDR-sob) with multivariate linear regression, controlling for age, sex, and age*sex, with protocol and site nested as random variables. FDR was used to correct for multiple comparisons. In healthy controls (ADNI2: N=85; ADNI3: N=184), we used an ANCOVA and post-hoc pairwise tests to evaluate the stability of dMRI indices across protocols. Significant associations were detected between dMRI indices and cognitive scores throughout the WM. AxD (specifically in the Cingulum Hippocampal Bundle (CGH)) consistently showed the strongest effect sizes across cognitive tests; effects weakened with distance from the temporal lobes. As expected, protocols were significantly different, with the ADNI2 protocol differing the most, and FA measures the least consistent across protocols. Ultimately, robust clinical associations were consistently detected despite protocol differences.

a.

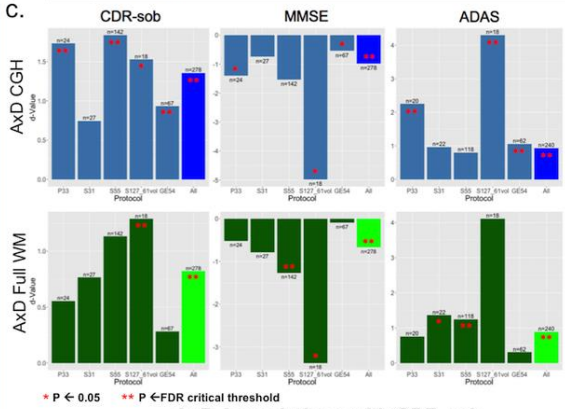
	Key	Scanner	DWI directions	b0	CN	MCI	AD	MMSE	CDR-sob	ADAS
ADNI3	GE54	GE	48	6	43	19	5	28.05±4.8	0.92±1.8	12.14±6.9
	P33	Philips	32	1	17	4	3	28.75±2.0	1.31±2.8	13.32±6.8
	S31	Siemens	30	1	15	8	4	28.26±2.3	0.91±1.4	11.74±4.7
	S55	Siemens	48	7	95	39	8	27.93±3.4	0.89±2.0	11.78±5.6
	S127_61vol	Siemens	48	13	14	4	0	28.61±1.8	0.36±0.8	10.64±2.8
			TOTAL:	184	74	20		28.83±1.7	0.61±1.1	10.17±2.8
ADNI2	GE46	GE	41	5	85	NA	NA	27.9±2.7	0.08±0.18	5.15±2.8
Key	DWI: b=1000 s/mm²; b0: b=0 s/mm²; CN: Cognitively Normal; MCI: Mild Cognitive Impairment; AD: Alzheimer's Disease; MMSE: Mini Mental Status Exam; CDR-sob: Clinical Dementia Rating Sum of Boxes; ADAS: Alzheimer's Disease Assessment Scale									

b.

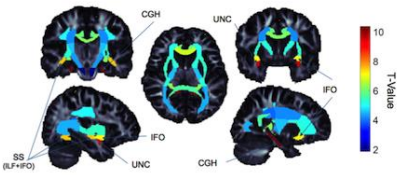
Number of Significant ROIs (out of 24)

	MMSE	CDR-SOB	ADAS
AxD	23	23	23
MD	23	24	23
RD	24	24	23
FA	21	20	16
TDF	24	24	23

c.



d.



e.

ANCOVA Across Protocols in Controls

ROI	P-Value FA	AxD	MD	RD	TDF
ACR	6.93E-05*	2.68E-05*	3.97E-05*	8.55E-05*	5.36E-04*
ALIC	2.91E-10*	5.31E-04*	1.83E-04*	3.64E-06*	1.96E-04*
BCC	1.00E-10*	4.34E-04*	2.93E-05*	8.40E-07*	1.43E-06*
CC	5.58E-09*	2.36E-05*	1.23E-03*	2.18E-04*	6.02E-04*
CGC	2.81E-11*	1.59E-06*	2.39E-08*	7.74E-11*	3.10E-06*
CGH	1.72E-09*	2.21E-01	8.06E-03*	1.80E-04*	1.55E-05*
CP	6.18E-10*	8.47E-06*	1.29E-07*	5.19E-08*	4.32E-08*
CST	6.29E-07*	1.10E-06*	4.25E-07*	2.75E-07*	9.90E-06*
EC	3.52E-09*	1.28E-03*	1.57E-04*	2.14E-05*	4.88E-03*
Full WM	1.32E-11*	3.29E-07*	2.75E-05*	2.49E-05*	1.45E-05*
Fx	2.46E-04*	7.95E-06*	7.44E-05*	2.74E-04*	7.74E-03*
GCC	5.51E-06*	6.04E-05*	2.80E-04*	3.20E-04*	1.86E-03*
IFO	1.45E-08*	1.77E-03*	1.10E-05*	3.31E-07*	2.25E-06*
PCR	1.65E-08*	3.40E-08*	1.09E-05*	1.04E-03*	1.65E-04*
PLIC	6.78E-08*	7.65E-04*	2.35E-04*	1.91E-05*	2.03E-03*
PTR	3.71E-05*	3.81E-07*	1.77E-05*	1.56E-04*	1.03E-05*
RLIC	2.69E-11*	4.15E-06*	1.11E-03*	2.09E-05*	3.18E-07*
SCC	1.26E-07*	3.00E-06*	9.88E-06*	4.83E-05*	4.28E-06*
SCR	4.49E-08*	6.31E-05*	3.52E-04*	2.98E-04*	3.17E-05*
SFO	1.19E-05*	9.08E-04*	3.89E-03*	6.05E-03*	3.97E-03*
SLF	7.93E-10*	1.26E-05*	7.00E-05*	8.26E-07*	7.07E-07*
SS	2.83E-09*	1.28E-05*	5.86E-03*	6.44E-02	9.21E-03*
TAP	4.56E-03*	2.87E-07*	2.75E-06*	1.37E-05*	2.21E-05*
UNC	8.51E-07*	4.16E-01	8.65E-03*	1.08E-03*	1.32E-02*

* Significant after FDR Multiple comparisons correction for 24 ROIs/tests

f.

Post Hoc Pairwise Tests

	# Sig ROI GE54	GE46	P33	S127	S31	S55	Measure Total:
FA	58	79	32	42	60	47	318
MD	31	41	8	36	14	40	170
RD	35	52	9	38	25	35	194
AxD	14	22	4	21	2	21	84
TDF-FA	36	44	7	36	39	26	188
Protocol-Total:	174	238	60	173	140	169	

Figure 1. a) In this analysis, we evaluated 5 of 7 ADNI3 protocols that had at least 10 subjects at the time of download. b) For each dMRI index, the number of ROIs (out of 24) significantly associated with each cognitive score reveals widespread associations throughout the WM. c) We plotted the effect size (d-Value) within protocols and across protocols for associations with AxD in the overall WM and CGH, which were consistently among the largest 10 effect sizes across all dMRI indices. d) AxD associations with CDR-sob visualized on the brain show the strongest effects in the CGH. Effects weakened with distance from the temporal lobes. e) For each ROI and dMRI index, an ANCOVA tested for significant differences between protocols. FA was the least stable dMRI index across protocols. f) For each protocol, we report the number of ROIs in which a significant difference was found in post hoc pairwise tests between protocols. ADNI2/GE46 is the most frequently different protocol across dMRI measures and ROIs. This may be due to the larger voxel size in ADNI2 (2.7x2.7x2.7 mm) as opposed to ADNI3 (2x2x2 mm).

Disclosures: T.M. Nir: None. **S.I. Thomopoulos:** None. **N. Jahanshad:** None. **R.I. Reid:** None. **M.A. Bernstein:** None. **B. Borowski:** None. **C.R. Jack:** None. **M.W. Weiner:** None. **P.M. Thompson:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 607.07/III58

Topic: H.02. Human Cognition and Behavior

Support: CONACYT 828873

Title: Generating cognitive reserve activities and the relationship with cognitive performance in healthy elderly

Authors: *C. GARCÍA-CAMACHO[1], T. VILLASEÑOR-CABRERA[1,2], M. JIMÉNEZ-MALDONADO[1,2], J. RUIZ-SANDOVAL[1,3], F. JAUREGUI[1]
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Abstract: Cognitive reserve refers to a number of factors that protect the brain against damage or cognitive decline. This reserve accumulates through the life course. The activities that we do through life are not always the same and exhibit changes in nature and frequency. Identifying frequency and life stage in which protecting activities are made could help to establish healthy profiles that we may recommend to prevent dementia and other pathologies.
Aims: In this study, we retrospectively identified cognitive reserve generating activities that exhibit significant relationship with a better cognitive functioning in old age.
Methods: Descriptive, transversal and retrospective assessment. A sample of healthy subjects between 60 and 70 years old were included. People with a history of substance abuse, cognitive impairment, psychiatric disorders or moderate to severe brain injury were excluded. Cognitive functioning was assessed with the Montreal Cognitive Assessment (MoCA), Digit Span and Vocabulary subtests of the Wechsler Intelligence Scale for Adults (WAIS-IV). The cognitive reserve was achieved through the Rami Cuestionario de Reserva Cognitiva (CRC) and protective activities frequency was obtained through Escala de Reserva Cognitiva y Envejecimiento (ERC). The project submitted and approved by the ethics committee of the "Hospital Civil de Guadalajara Fray Antonio Alcalde" registration number 217/17.
Results: We found that 66.7% of our subjects were woman, with a mean age of 63.8 years old (SD=3.0), the mean of education was 12 years (DE= 5.3), related to the occupation the 42.4% were retired, 42.4% still work and 15.2% does not work. The range of cognitive reserve was calculated in four categories; 3.0% had a lower rank, 15.2% medium-low range, 48.5% medium-high range and 33.3% a higher rank. In the cognitive examination the mean score in MoCA test

was 25.1 (DE= 1.6), Digit Span 8.6 (SD= 2.1) and Vocabulary 10.0 (SD= 1.8). Pearson correlation showed an association between Digit span and the young-age activities (r= .355, p= 0.04) and middle-age activities (r= .432, p= 0.01) even do MoCA and late-life activities showed an association (r= .393, p=0.02).*Conclusion*: These results lead us to think that there is a strong associationamong the protective activities that take place during adulthood, which agrees with different authors reported.

Disclosures: C. García-Camacho: None. **T. Villaseñor-Cabrera:** None. **M. Jiménez-Maldonado:** None. **J. Ruiz-Sandoval:** None. **F. Jauregui:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 607.08/III59

Topic: H.02. Human Cognition and Behavior

Title: Identifying brain and cognitive deficits in older adults at-risk for diabetes

Authors: *J. A. FURLANO, L. S. NAGAMATSU
Western Univ., London, ON, Canada

Abstract: Globally, dementia affects approximately 47 million people, and is a major cause of disability among older adults. Type 2 diabetes (T2D) is a known risk factor for dementia; older adults with T2D have been shown to experience cerebral atrophy and cognitive decline. Consequently, older adults at-risk for developing T2D (based on body mass and blood glucose levels) are at higher risk for cognitive decline. Pre-diabetic older adults have been shown to experience some cognitive decline, however further research is needed to determine the specific cognitive domains affected and the degree to which this decline occurs. Moreover, structural and functional brain changes that may occur with these deficits is currently unknown in this population. Therefore, the aim of this study was to assess cognitive performance and brain health (using advanced neuroimaging) in older adults at-risk for diabetes. We conducted a cross-sectional analysis of older adults (aged 60-80) at-risk for diabetes (BMI > 25 or blood glucose of 6.1-7.0 mmol/L) and healthy aged-matched controls, examining 1) memory performance and executive functioning, using a battery of standardized neuropsychological tests, 2) functional brain activation, as measured by fMRI BOLD signal during an associative memory task, and 3) structural measures, such as volume of the hippocampus as well as other brain regions implicated in memory. Based on our cross-sectional analysis, older adults at-risk for diabetes show impaired associative memory performance and executive functioning, as well as altered brain structure and function that may contribute to the observed deficits. We conclude that older adults at-risk for diabetes experience cognitive decline and decreased brain health, and have implemented a 6-month resistance training intervention strategy to prevent and delay the onset of such decline.

Disclosures: J.A. Furlano: None. **L.S. Nagamatsu:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 607.09/III60

Topic: H.02. Human Cognition and Behavior

Support: University of California, Santa Barbara
Brain and Behavior Research Foundation

Title: Reproductive aging shapes top-down, goal-directed modulation of visual processing

Authors: *L. A. PRITSCHET, E. G. JACOBS
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Abstract: The neurophysiological changes that accompany age-related declines in working memory, selective attention and inhibitory control are well-established (Cabeza, Nyberg & Park, 2016). These studies generally target adults over the age of 65, but now more attention is being paid to neural and cognitive changes that unfold in the preceding decade, as adults enter midlife and women transition through menopause. The menopausal transition is marked by a decline in ovarian hormone production and is a time when many women report changes in memory and attention (e.g. “menopause fog”). Rodent and nonhuman primate studies have established the critical role sex hormones play in modulating prefrontal cortical function. Parallel evidence from human neuroimaging studies further implicates sex hormones in the regulation of memory/attention circuitry (Jacobs et al., 2018). In this study, we examine the impact of reproductive aging on top-down modulation of goal-directed behavior. Healthy midlife women and men (N=30; ages 45-60) performed a visual selective attention task during fMRI scanning. Menstrual cycle histories and serological assessments were used to determine women’s pre/peri/post-menopausal stage. The task paradigm presents face and scene stimuli. “Bottom-up” visual information is identical across conditions, only task goals differ. Subjects are instructed to selectively attend to relevant stimuli (e.g. Faces) and ignore irrelevant stimuli (e.g. Scenes) while completing a match-to-sample task. A dual attention condition requires subjects to attend to both stimulus classes. Neural activity during each ‘attend’ condition is compared to a control condition (image categorization). Superior frontal and parietal cortices are key components of top-down modulation and attentional control. Preliminary results reveal that menopausal status shapes task-evoked activity in the frontoparietal network, specifically intraparietal sulcus (IPS)/superior parietal lobule (SPL). In the dual attention “Both” condition when cognitive load is highest, postmenopausal women showed exaggerated IPS/SPL activity relative to premenopausal women, despite no significant difference in task performance. The IPS/SPL is a key node in the frontoparietal network underlying top-down control of visual processing.

Previous studies established the impact of chronological aging on top-down modulation (Gazzaley et al., 2005). Our preliminary findings suggest that neuroendocrine changes during the middle years of life ("reproductive aging") shape attentional control mechanisms by altering activity in the frontoparietal network and should be a factor in cognitive aging.

Disclosures: L.A. Pritschet: None. **E.G. Jacobs:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 607.10/III61

Topic: H.02. Human Cognition and Behavior

Support: NIA AG032361

Title: BDNF polymorphisms are associated with local gyrification and default mode network integrity in healthy middle-aged adults

Authors: *J. K. BLUJUS[1], L. E. KORTHAUER[2], I. DRISCOLL[1]
[1]Psychology, [2]Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Brain-derived neurotrophic factor (*BDNF*) is a neurotrophin that promotes neuronal survival and differentiation and has been implicated in higher-order cognitive abilities such as learning and memory (Budni et al., 2015). *BDNF* is expressed throughout the brain but particularly in the prefrontal cortex and hippocampus (Peezawas et al., 2004), two regions important for learning and memory and vulnerable to Alzheimer's disease (AD). A Val to Met substitution at codon 66 in *BDNF* (rs6265) has been associated with impaired memory, compromised brain integrity, and increased risk of AD (Boots et al., 2017; Hajek et al., 2012), however, little work has been done to understand the relationship between a less common variant in *BDNF* (rs11030096) and brain integrity. The purpose of the current study was to characterize the relationship between polymorphisms in *BDNF* (rs6265, rs11030096), local gyrification index (LGI), and functional connectivity in the default mode network (DMN), measures of structural and functional brain integrity that are disrupted in AD. Our investigation focused on healthy, middle-aged adults to identify alterations in brain integrity that may occur years prior to the onset of cognitive impairment ascribed to pathological aging. Cognitively normal, middle-aged adults (age 40-60, N=150) underwent a multi-modal neuroimaging paradigm including T1-weighted structural imaging and resting-state functional MRI. Freesurfer v5.3.0 was used to analyze LGI and independent components analysis and general linear modeling were employed to identify and assess connectivity within the DMN. Results revealed that minor allele carriers (AA/AG) of *BDNF* (rs6265) exhibited lower LGI in the right superior frontal gyrus compared to homozygous major allele carriers (GG), however, no differences were evident in DMN

connectivity. The *BDNF* (rs11030096) analysis showed that minor allele carriers (CC/CT) exhibited lower LGI in the right posterior cingulate cortex, right superior parietal lobule, left paracentral gyrus and left fusiform gyrus compared to homozygous major allele carriers (TT). Additionally, minor allele carriers (CC/CT) had decreased DMN connectivity in the precuneus and superior temporal gyrus than homozygous major allele carriers (TT). These findings reveal a relationship between the *BDNF (*rs11030096, rs6265) polymorphisms, LGI, and DMN connectivity in frontal and temporal regions where BDNF is highly expressed and further demonstrates that changes in brain integrity in regions vulnerable to AD are evident in minor allele carriers as early as middle age, decades prior to the onset of cognitive decline.

Disclosures: J.K. Blujus: None. **L.E. Korthauer:** None. **I. Driscoll:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 607.11/III62

Topic: H.02. Human Cognition and Behavior

Support: German Federal Ministry of Education and Research (BMBF) Grant, Project: "FANS - Pedestrian Assistance System for Older Road Users".

Title: Age-dependent differences in cued peripheral visual perception and motor performance during dual-task natural walking

Authors: *J. PROTZAK[1], K. GRAMANN[2]
[1]Dept. of Psychology and Ergonomics, Junior Res. Group FANS, TU Berlin, Berlin, Germany; [2]Biol. Psychology and Neuroergonomics, TU Berlin, Inst. of Psychology and Ergonomics, Berlin, Germany

Abstract: With increasing age, a stable and secure gait may change from an automated task into an activity that requires increased concentration and executive control. Resources necessary to compensate for sensory and motoric declines are no longer available for parallel executed tasks like scanning the traffic environment. Thus, safety critical situations can emerge from insufficiently processed or unperceived environmental cues like approaching cars or obstacles. However, brain dynamics underlying efficient movements and performance in simultaneously executed tasks and their development throughout adulthood are not yet understood in detail. Using a Mobile Brain/Body Imaging (MoBI; Makeig et al., 2009; Gramann et al., 2011; 2014) approach, we established a set-up that enables the recording of brain dynamics underlying peripheral visual perception during natural locomotion. Furthermore, the potential of vibro-tactile warning cues to improve visual perception was evaluated as well as the impact of cues on gait and posture. Short visual targets were dynamically presented in the peripheral visual field of

the participant while standing or walking up and down a hallway of ten-meter length. In half of all test blocks, vibro-tactile cues were delivered to the upper arm preceding the target stimulus. Data recordings included button-press responses to visual targets, continuous EEG-recordings using a mobile 64-channel set-up, and motion capture data from a camera-based system. Data sets from 15 younger (<35 years, 9 female) and 17 older (>65years, 7 female) adults were analyzed. Dual-task effects on performance measures (response times, errors, misses), balance and gait parameter (walking speed, sway), amplitudes and latencies of the early (P1) and later (P300) stimulus-locked event-related potentials (ERP) served as dependent measures. Both groups responded faster to visual stimuli but showed more incorrect responses during walking. Warnings reduced response times, number of missed targets and errors but also led to decreased gait velocity. Older participants made generally more errors than younger but differences between age groups in number of missed targets while walking were eliminated through warnings. Modulations in P1 and P300 amplitudes and latencies indicate age-related as well as motor task-related differences in resource allocation processes. Moreover, directly comparing established EEG-setups (seated participants) with recordings of mobile participants in highly ecological valid settings reflect age-related impacts of motor activity on the processing of visual targets.

Disclosures: J. Protzak: None. **K. Gramann:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 607.12/III63

Topic: H.02. Human Cognition and Behavior

Support: NMSS Grant No RG150704951
NIH Grant R01AG029523

Title: BOLD hemodynamic response function changes significantly with healthy aging: A population-based study

Authors: *M. D. ZUPPICHINI[1], K. WEST[2], M. P. TURNER[4], D. SIVAKOLUNDU[5], D. ABDELKARIM[6], Y. ZHAO[3], J. SPENCE[4], B. P. RYPMA[7]
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Abstract: Functional magnetic resonance imaging (fMRI) has been used to infer age-differences in neural activity from the hemodynamic response function (HRF) that characterizes the blood-

oxygen-level-dependent (BOLD) signal over time. The HRF results from complex interactions between neurons, glia, and vascular structures, comprising the neural-vascular coupling system. This system is finely-tuned in healthy individuals for efficient brain function. We hypothesize that age-related changes to any component of this system could alter relationships between HRF parameters and neural activity.
We analyzed a large dataset from the Cambridge Center for Aging and Neuroscience (CamCAN) study. 74 younger (18-30 years of age; 25.4±3.6, 33 males) and 173 older (54-74 years of age; 63.7±6.0, 100 males) adults viewed two checkerboards flanking a central fixation point (34ms) and simultaneously heard a 300ms binaural tone. HRFs were estimated using FMRIB's Linear Optimal Basis Sets (FLOBS) to minimize shape assumptions. We assessed age-differences in HRF parameters using one-way ANCOVAs in which each parameter was a dependent factor, age group was the independent factor, and ROI volume was a covariate. In a visual cortex ROI, there was decreased peak amplitude ($p<.001$), longer time to peak ($p=.003$), decreased trough amplitude ($p<.001$), longer time to trough ($p=.012$), and shallower rise ($p<.001$) and fall slopes ($p<.001$) in healthy older compared to younger adults. In an auditory cortex ROI, there were longer time to peak ($p<.001$), decreased trough amplitude ($p=.001$), longer time to trough ($p=.004$), and shallower rise ($p=.039$) and fall slopes ($p=.007$) in older compared to younger. There were no significant interactions for age and volume for any parameter.
Age-changes in the shape and timing of the HRF support the hypothesis of age-related changes in neural-vascular coupling. HRF age-differences arise from complex physiologic factors that complicate interpretation of BOLD as an index of age-related neural change. New imaging methods, like calibrated fMRI, permit direct assessment of age-differences in the physiologic factors underlying BOLD signal. More precise interpretations of HRF age-differences can be formulated once these physiologic factors are disentangled and measured separately.

Disclosures: M.D. Zuppichini: None. **K. West:** None. **M.P. Turner:** None. **D. Sivakolundu:** None. **D. Abdelkarim:** None. **Y. Zhao:** None. **J. Spence:** None. **B.P. Rypma:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 607.13/III64

Topic: H.02. Human Cognition and Behavior

Support: NIA Training Grant 5T32AG000175

Title: Decoding context memory representations during retrieval across the adult lifespan

Authors: *P. S. POWELL[1], J. STRUNK[1], T. JAMES[1], S. M. POLYN[2], A. L. DUARTE[1]
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Abstract: Age-related context memory declines are due, in part, to a tendency to bind too much irrelevant contextual information during encoding (hyper-binding). However, the degree to which older adults are able to engage in selective retrieval of relevant contextual information, while suppressing retrieval of irrelevant information, is unknown. In the current study, we used multivariate pattern analysis (MVPA) to explore the degree to which task relevant and irrelevant context memory representations are reactivated during retrieval and the impact of this reactivation on memory performance.
Participants were 51 adults between 18 and 80 years old.
During encoding, participants studied pictures of objects in the presence of two contextual features: a color and a scene, and their attention was directed to the object's relationship with one of those contexts (target context) and were told not to pay attention to the other context (distractor context). At retrieval, participants judged whether the context features were similar to, or different from, the context feature shown during encoding. EEG was recorded as participants made context memory decisions for both attended and unattended features.
During encoding, we used pattern classification analyses to assess whether classifier performance and evidence varied as a function of selective attention (i.e., target vs. distractor). During retrieval, we examined classifier evidence for target and distractor features to determine whether the strength of neural representations for the target and distractor context features related context memory performance and hyper-binding.
Results showed that classifier evidence was greater for target contexts relative to distractor contexts, however evidence for the distractor was greater in older adults compare to middle-aged and younger adults. Across all subjects, the strength of the distractor representation was related to a higher probability of correctly recognizing both the target and distractor as well as greater hyper-binding. Finally, the relationship between distractor classifier evidence and hyper-binding significantly varied across age with older showing a stronger relationship than middle aged and younger adults.
These results provide direct evidence that age-related episodic memory impairments are related to the spontaneous retrieval of distracting information that interferes with the ability to recover sought after episodic details. The lifespan approach in this study reveals a linear effect of age on this increased susceptibility to mnemonic interference.

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Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 607.14/III65

Topic: H.02. Human Cognition and Behavior

Title: Music training as aneuro-cognitiveprotector for brain aging: Cognitive and neuropsychological profiles in professional musicians

Authors: *C. E. SCHNEIDER[1], Y. JIANG[3], J. WATKINS[2]
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Abstract: The proportion of older adults living with cognitive impairments is increasing rapidly. This shift will increase mortality rates, reduce perceived quality of life, and cause economic burden. Currently evidence of highly effective and noninvasive interventions that prevent or slow the onset of cognitive impairment are limited. Music playing has been shown to improve brain and cognitive functions by engaging networks of brain areas, simultaneously involving cortical mechanisms associated with executive, high-level cognitive and motor functions, and multiple sensory systems. Literature suggests strong correlations between cognition and music ability. Studies in the past have not concretely operationalized music training. Here we test the general hypothesis that music training improves neural mechanisms associated with core cognitive functions (e.g. working-memory and attention). This study was designed to control level of music involvement and genre by examining professional, classically trained orchestral musicians, establishing cognitive and neuropsychological profiles in an effort to better understand the potential for music training to protect older adults from cognitive decline. Twenty-nine professional musicians were recruited who completed five neuropsychological exams. The scalp electrophysiological signals from 14 channels were recorded wirelessly while musicians performed a modified delayed match-to-sample task, imagination of music playing, and resting states. Musicians completed neuropsychological screening (MoCA) and a music and life span questionnaire. Musicians tested above normative ranges in cognitive ability. Musicians' scores were compared with normative scores of participants at similar ages in previous studies using the same measures; current musicians performed significantly faster and more accurately on neuropsychological measures. Regression and ANCOVA showed strong positive correlations between theta oscillation in bilateral frontal sites (F3, F4) and both number of years of private music lessons and number of hours of music practice. Current new findings reveal that professional musician's cognitive scores and neural activity are associated with superior cognitive ability via enhancement of neural mechanisms of current target material and inhibition of distractions. Music training is a promising noninvasive method to control cognitive challenge, which merits further research to determine how it can be used as a beneficial cognitive training method for aging individuals.

Disclosures: C.E. Schneider: None. **Y. Jiang:** None. **J. Watkins:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

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Program #/Poster #: 607.15/III66

Topic: H.02. Human Cognition and Behavior

Support: Deutsche Forschungsgemeinschaft Grant BU 2670/7-1

Title: Age-related impairment of semantic integration into long-term memory is related to theta-alpha and low beta oscillations

Authors: *P. A. PACKARD[1], T. STEIGER[1], L. FUENTEMILLA[2], N. BUNZECK[1]
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Abstract: Long-term memory encoding is impaired in healthy aging but the underlying mechanisms in humans remain unclear. Here, we tested whether this relates to failures in associating information with previous memories to build multi-item representations. To gain insight into age-related differences in such neural mechanisms during online encoding, we employed the temporal precision of electroencephalography to examine how semantic integration during encoding is affected by healthy aging. As expected, we found that congruent matches improved subsequent recognition memory in younger adults (i.e. congruency effect) but this effect was reduced in the elderly. At the neural level, congruence caused changes in neural activity within ~1500 ms after stimulus presentation, and there were widespread differences in ERPs and alpha-beta oscillations (8-30 Hz), which are known to support semantic processing. Importantly, these ERP differences predicted increases in memory performance, especially for congruent items. Finally, age-related differences in memory were accompanied by an early positive ERP and a later decrease in theta-alpha and low beta power (5-13 Hz), during encoding, which were greater in the younger group. Our findings provide evidence that age-related memory impairments can be explained by deficits in online semantic integration, depending on theta-alpha and low beta oscillations.

Disclosures: P.A. Packard: None. **T. Steiger:** None. **L. Fuentemilla:** None. **N. Bunzeck:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 607.16/III67

Topic: H.02. Human Cognition and Behavior

Title: Age-related decreases in the retrieval practice effect directly relate to changes in alpha-beta oscillations

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Abstract: The retrieval (or testing) of information leads to better memory performance as compared to re-encoding. This phenomenon is known as 'testing effect' or 'retrieval practice effect' and has been described in several studies using various stimulus material. The underlying neural mechanisms, however, remain unclear. To address this issue, we used a previously established paradigm in healthy young (N = 27) and elderly (N = 28) participants while their brain activity was being recorded using electroencephalography (EEG). Subjects viewed pre-familiarized scene images intermixed with new scenes and classified them as indoor vs outdoor (encoding task) or old vs new (retrieval task). Subsequently, subjects performed a final recognition memory task. As expected, both young and elderly showed the testing effect but it was less pronounced in the elderly. At the neural level, the retrieval task was, as compared to the encoding task, accompanied by power decreases in the alpha (9-13 Hz) and beta bands (13-20 Hz), and this difference was more pronounced in the elderly. In line with this observation, those elderly who displayed a more pronounced testing effect exhibited a neural pattern that was more similar to the younger subjects. Our findings provide further evidence that the testing effect decreases across the life span, and they suggest that changes in alpha-beta oscillations play a direct role.

Disclosures: C. Guran: None. **N.A. Herweg:** None. **N. Bunzeck:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

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Topic: H.02. Human Cognition and Behavior

Support: CONACYT 262010 to VP
L.A.R.C. received a scholar fellowship from DGAPA-UNAM

Title: Activation of kynurenine pathway correlates with cognitive decline in non-demented elderly men

Authors: *L. A. RAMOS[1,3], P. CARRILLO-MORA[5], B. GARCÍA[5], D. GONZÁLEZ-ESQUIVEL[3], D. RAMÍREZ-ORTEGA[3], B. PINEDA[4], C. RIOS[3], G. ROLDÁN-ROLDÁN[2], V. PÉREZ-DE LA CRUZ[3]
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Abstract: Aging is a multi-etiological and degenerative process that is characterized by progressive deterioration in cognitive, physiological and metabolic processes; these changes are related to numerous age-related disorders. Cellular alterations during aging process involve oxidative stress, inflammation, mitochondrial and metabolic dysfunction, cognitive and immune response decline. Recently, it was found that changes in the tryptophan metabolites proportion during aging in women. Tryptophan (Trp) is essential for synthesis of proteins and is mostly metabolized by kynurenine pathway (KP), conducing to several metabolites with neuroactive and redox properties. Specifically, kynurenic acid (KYNA), -an endogenous antagonist of α7nACh and NMDA receptors- and quinolinic acid (QUIN), an agonist of NMDA receptors, have been related with neurodegenerative diseases. Due to KP metabolites have been related with aging and some ageing-related diseases, we investigated whether the metabolites of kynurenine pathway could be related with cognitive decline in old men. The age range of participants was 19 to 93 years old. A brief neuropsychological standardized tests series (NEUROPSI), was performed in patient over 50 years old. The NEUROPSI tests serie includes assessment of orientation, attention, memory, language, visuoperceptual abilities, motor skills, and executive functions. Serum levels of Trp, kynurenine, KYNA and 3-HK were determined in eighty old men. Results showed a negative correlation between age and Trp levels and a positive correlation between age and KYNA/Trp and 3-HK/Trp ratios. The cognitive impairment showed a significant positive association with age and with KP activation and a significant negative correlation with Trp levels. This results suggest that KP activation increases with age and it is strongly associated with the level of cognitive performance in non-demented elderly men.

Disclosures: L.A. Ramos: None. **P. Carrillo-Mora:** None. **B. García:** None. **D. González-Esquivel:** None. **D. Ramírez-Ortega:** None. **B. Pineda:** None. **C. Rios:** None. **G. Roldán-Roldán:** None. **V. Pérez-de la Cruz:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 607.18/JJJ1

Topic: H.02. Human Cognition and Behavior

Title: The positive effects of a 4-week cognitive training are not modulated by contextual novelty

Authors: *D. BIEL, T. STEIGER, T. VOLKMANN, N. JOCHEMS, N. BUNZECK
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Abstract: Introduction: An optimal cognitive training not only improves specific skills but also transfers to other cognitive domains. Although a few studies have reported such 'transfer effects', the underlying mechanisms remain unclear. **Objectives**: Here, we tested the hypothesis that contextual novelty, which is known to drive plasticity, learning and memory, may enhance the transfer effects of a 4-week cognitive training. Moreover, we aimed to investigate the underlying neural mechanisms with a focus on iron, myelination, grey matter volume and white matter tracts. **Materials & methods**: 50 healthy elderly subjects (50-80 years) participated in a 4-week training including 12 training sessions of a 2-back working memory task on a tablet computer at home. During the trainings, one group watched short sequences of novel nature movies (novelty group, n=25), while a second group performed the same training intermixed with five repeating movie sequences (familiarity group, n=25). Neuropsychological assessment of various cognitive abilities as well as structural MRI (including R2*, T1, MT) and DTI was conducted before and after the training period. **Results**: All participants showed increased accuracy and faster response times in the 2-back task over time. Moreover, performance in fluid intelligence (LPS 50+), processing speed (D2 and complex trail making) and verbal and numeric memory (VLMT and digit span backwards) significantly improved within both groups. However, the training did not lead to significant changes in a digit span forward test, simple trail making and crystalline intelligence (MWT). Finally, there were no significant differences between the novelty and familiarity group in any of the acquired neuropsychological tests. At the neural level, in both experimental groups the training led to weak changes in grey matter volume within the left insula and medial temporal lobe as revealed by VBM. **Conclusion**: Our results demonstrate that a 4-week working memory training not only improves working memory skills but also leads to transfer effects within the domains of fluid intelligence, processing speed as well as verbal and numeric memory. However, at the behavioral level, contextual novelty did not further modulate the observed transfer effects.

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Poster

607. Human Cognition and Behavior: Cognitive Aging II

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 607.19/JJJ2

Topic: H.02. Human Cognition and Behavior

Support: CIHR MOP11501

Title: Characterizing age-related changes in brain connectivity using sparse graphs

Authors: *S. HRYBOUSKI[1], I. CRIBBEN[4], J. MCGONIGLE[5], R. CARTER[2], F. OLSEN[3], P. SERES[3], N. V. MALYKHIN[3]
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Abstract: Introduction: The predominant theory of brain aging suggests that the association cortices are most affected by the aging process, while primary sensory and motor systems of the brain are moderately preserved in healthy older individuals. In the present study, we applied graphical models to resting-state functional Magnetic Resonance Imaging (fMRI) data to investigate whether connectivity profiles of the association cortices are most vulnerable to healthy aging.
Methods: A total of 105 healthy volunteers (18-85 years old) were recruited for the study. Participants were excluded if they had an unstable medical illness, history of psychiatric or neurological disorders and the use of medications that might affect brain structure or function. fMRI images were collected on a 4.7T scanner using T2*-sensitive EPI sequence [TR = 3 s; TE = 19 ms; flip angle = 90°; voxel size = 3×3×3 mm^3]. SPM, FSL, ANTS, and GIFT software packages were used for image preprocessing and network decomposition. In total, 22 independent components (ICs), representing brain networks, were identified. These ICs were grouped into visual, somatomotor, auditory, attentional, executive, and default systems. Sparse graphs for young (18-39 years), middle (40-59 years), and old (60-85 years) age groups were estimated from network time courses using the SCAD estimating method with the cross-validation selection method. From the resulting graphs, we computed graph summary metrics such as edge density, graph diameter, and measures of centrality.
Results: For intra-system connectivity, we observed: (1) no age-related differences in edge density, diameter, and centralization in any of the networks; (2) age-related reduction of node closeness and node betweenness in the default system; and (3) age-related reduction in centrality for the motor and visual systems. All other age effects were identified in the between-system connectivity, where all systems, except for auditory, default, and executive displayed greater between-system edge density in older adults. Furthermore, we observed age-related increases in

between-system centrality for the motor, visual, and attentional systems. Most of these differences were statistically significant only when comparing young and old cohorts directly. **Conclusion:** Age-related differences in between-system connectivity were most pronounced in the somatomotor and visual networks, while the default system showed a reduction in node closeness and node betweenness in intra-system connectivity. Together, these results characterize substantial age-related changes in brain organization at the systems level, extending beyond the association cortices.

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Poster

607. Human Cognition and Behavior: Cognitive Aging II

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Program #/Poster #: 607.20/JJJ3

Topic: H.02. Human Cognition and Behavior

Support: NIA Grants R01AG039103, RF1AG039103

Title: The relationships between regional cortical thickness, intra-scan motion and associative recognition memory performance as a function of age

Authors: *M. A. DE CHASTELAINE, B. E. DONLEY, K. KENNEDY, M. D. RUGG
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Abstract: We previously described the relationships between age, head motion (during functional scanning), mean cortical thickness and associative recognition performance in groups of young (18-30 yrs), middle-aged (45-55 yrs) and older (63-77 yrs) adults, totaling 133 individuals. To expand upon these relationships, here we utilized the same sample to conduct whole-brain analyses to determine which, if any, of these relationships might be regionally selective. Using Freesurfer's (v.5.3) semi-automated analysis pipeline, we obtained vertex-wise thickness estimates along the cortical surface to examine regional variations in the outcome of two main regression analyses. The first analysis, using an initial vertex-wise uncorrected threshold of $p < .001$ (10mm FWHM smoothing kernel), evidenced a linear relationship between thickness and memory performance, after correcting for motion, across large areas of the cortical surface in both the young and older groups. Consistent with our previous analyses using mean thickness measures, this relationship was found to be negative in the young but positive in the older sample. After applying a cluster-wise correction using the Monte Carlo simulation, however, these relationships appeared to be particularly robust in the parahippocampal cortex and posterior cingulate, indicating some level of regional specificity. In a second regression

analysis, we observed a weakening in what we had previously observed to be a strong negative relationship between age and cortical thickness, when including motion as a covariate of interest, particularly in lateral frontal cortical regions. Consistent with this observation, the relationship between the amount of head motion and cortical thickness, after controlling for age, was also most apparent in lateral prefrontal regions. These findings highlight the value of taking into account intra-scan head motion when investigating age-related differences in regional cortical thickness and cognitive performance. This research was funded by NIA Grants R01AG039103, RF1AG039103.

Disclosures: M.A. De Chastelaine: None. **B.E. Donley:** None. **K. Kennedy:** None. **M.D. Rugg:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 607.21/JJJ4

Topic: H.02. Human Cognition and Behavior

Title: Longitudinal frontostriatal functional connectivity and gray matter changes related to motor and cognitive symptoms in early-stage Parkinson's disease

Authors: *S. KANN[1], C. CHANG[2], R. WALES[1], H.-C. LEUNG[1]
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Abstract: Motor and cognitive symptoms are heterogeneous in both severity and progression across individuals with Parkinson's Disease (PD). However, the neural substrates underlying these differences in functional decline across time remain largely unknown. Several studies have demonstrated that PD patients with more severe akinetic/rigid (AR) motor symptoms also display greater impairment on executive function (EF) tasks, while severity in both domains is implicated in faster decline. The current study therefore examined longitudinal changes in gray matter volume and functional connectivity in relation to more severe EF and AR symptoms utilizing the Parkinson's Progressive Marker Initiative (PPMI) (http://www.ppmi-info.org) database. We conducted resting state functional connectivity (rsFC) analyses in early stage PD patients in order to examine frontostriatal circuits. These analyses were conducted at two timepoints a year apart (N=51, 56), and controlled for age, gender, medication status and scanner location. At both timepoints, better EF was associated with greater rsFC between striatum and dorsomedial and dorsolateral prefrontal cortex (dmPFC, dlPFC) across subjects, while at the first timepoint milder AR was associated with stronger connectivity between striatum and ventromedial PFC (vmPFC) across individuals. Additionally, we examined the rate of change in gray matter volume in 86 PD subjects across 4 timepoints (baseline to 48 months) controlling for

age at baseline, total intracranial volume, and gender. Baseline AR symptom severity predicted a greater rate of gray matter atrophy within the motor cortex, while similar effects were not found for tremor symptoms, general cognitive scores, or EF measures. These findings suggest distinct patterns of fronto-striatal functional connectivity, as well as rates of atrophy within frontal cortex, related to motor and cognitive symptom variability within PD.

Disclosures: C. Chang: None. **R. Wales:** None. **H. Leung:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

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Program #/Poster #: 607.22/JJJ5

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01 AG030311

Title: Enhanced associative memory and mid-cingulate activity during memory retrieval in 'superagers'

Authors: *J. M. ANDREANO[1], A. TOUROUTOGLOU[1], H. POPAL[1], B. C. DICKERSON[1], L. F. BARRETT[2]
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Abstract: Aging is frequently accompanied by cognitive decline, including loss of memory abilities. However, recent studies have identified a remarkable group of elderly people, known as 'superagers', who maintain cognitive abilities equivalent to middle aged and even young people on some measures (Sun et al., 2016; Harrison et al., 2012). 'Superaging' has been associated with preserved cortical thickness (Sun et al., 2016) and functional connectivity (Zhang et al., 2018) in multiple regions, notably the mid-cingulate cortex, a functional 'hub' region. However, no study to date has compared task-related activity between superagers and typical elderly adults during a cognitive task. In this study, 17 elderly adults classified as superagers, and 39 typical older adults performed a paired-associate recognition memory task during fMRI. Behavioral performance and retrieval activity were compared between the two groups. As predicted, the results indicated superior paired associate memory performance in superagers ($p < .05$), suggesting that previously observed advantages in verbal recall extend to associative recognition. Additionally, region of interest analysis indicated a significant difference in activity associated with successful retrieval between superagers and typical older adults, with a substantially greater signal difference between hits and correct rejections in superagers in a cluster of mid-cingulate cortex closely matching the area of preserved cortical thickness observed in previous studies ($p < .05$). Across all subjects, hit vs. correct rejection signal in this cluster predicted recognition memory performance, suggesting that the preserved structure and function of this region

contribute to superior cognitive abilities in superaging. Implications for successful aging are discussed.

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Poster

607. Human Cognition and Behavior: Cognitive Aging II

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01AG024972

Title: Age-related declines in neural-vascular coupling: Regional variability, effects of task demand, and relationship to cognitive performance

Authors: *B. P. RYPMA[1], M. P. TURNER[1], K. WEST[1], D. SIVAKOLUNDU[2], Y. ZHAO[1], D. ABDELKARIM[1], B. P. THOMAS[3], H. LU[4]
[1]Behavioral & Brain Sci., [2]Biol. Sci., Univ. of Texas At Dallas, Dallas, TX; [3]Advanced Imaging Res. Ctr., Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [4]Radiology, Johns Hopkins Univ., Baltimore, MD

Abstract: Blood-oxygen-level-dependent signal (BOLD) as measured with functional magnetic resonance imaging (fMRI) arises principally from two physiologic factors underlying BOLD: cerebral blood flow (CBF), which quantifies the rate of oxygen delivery to metabolically active neural tissue as measured by cerebral metabolic rate of oxygen ($CMRO_2$). The coupling of these two factors ($CBF/CMRO_2$) reflects the integrity of the neural-vascular coupling system that underlies efficient cognitive function. Sixteen healthy younger (mean age = 23.6, SD = 3.4, 10 F) and eighteen healthy older (mean age = 58.9, SD = 4.6, 11 F) right-handed adults that had been screened for any potential cardiological, respiratory, pulmonary, or vascular conditions performed block-designed visual and motor tasks while undergoing calibrated fMRI scanning. During the visual task, participants responded via bilateral button-press whenever a fixation cross at center-screen changed in luminance. During stimulation blocks, flickering checkerboards were presented at varying frequencies (2 Hz, 4 Hz, and 8 Hz). During stimulation blocks of the motor task, participants pressed buttons bilaterally in rhythm with an auditory cue (1 Hz, 2 Hz, and 3 Hz). To estimate maximum possible BOLD, participants completed a hypercapnia challenge, in which they breathed room air for 4 minutes and then an isometabolic gas containing 5% CO_2 21% O_2, and 74% N_2 for 6 minutes while being scanned at rest. During all functional scans, BOLD and CBF were collected in separate echoes using a novel pCASL-based pulse sequence (parameters TE1/TE2=11/30 ms, TR = 4 s, 22 6-mm axial slices, no gap, in-plane

resolution = 3.4 × 3.4 mm²). In visual cortex, during visual stimulation, younger adults exhibited monotonic increases in BOLD, CBF, and CBF/$CMRO_2$ with increasing task demand. BOLD, CBF, and CBF/$CMRO_2$ for older adults plateaued as task demand increased. In motor cortex, CBF/$CMRO_2$ increased for younger but remained low for older adults. In both regions, CBF/$CMRO_2$ was lower in older than in younger adults. The lower CBF/$CMRO_2$ ratio observed in older adults supports the hypothesis that age-related changes to the neural-vascular coupling system underlie age-related declines in cognitive efficiency.

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Poster

607. Human Cognition and Behavior: Cognitive Aging II

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Program #/Poster #: 607.24/JJJ7

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01AG047972

Title: Age-related decline in arterio-venous compliance and relationships to cognitive performance

Authors: *D. H. ABDELKARIM[1], M. P. TURNER[1], D. SIVAKOLUNDU[2], Y. ZHAO[1], K. WEST[1], B. P. THOMAS[3], H. LU[4], B. P. RYPMA[1]
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Abstract: The neural mechanisms of age-related cognitive decline remain unknown. We hypothesize that this decline is related to compromised integrity of the neural-vascular coupling system that mediates local blood flow increases in response to increased neural activity. This system involves well-coordinated signaling between neurons, glia, and cerebral vasculature, and is known to be compromised in older adults. One way to characterize cerebrovascular decline is through measurement of cerebrovascular reactivity (CVR) of veins (CVR_V) and arteries (CVR_A). CVR describes the compliance of blood vessels in the brain in response to vasoactive stimuli. We predicted that in young, healthy brains, arterio-venous compliance (AVC), the degree to which arterial compliance changes precipitate proportional increases in arterial compliance following stimulation, will be high. In older brains, we also predicted this synchronization will be lost, and that this loss will be related to cognitive decline in aging.In this study, dual-echo functional magnetic resonance imaging (fMRI) was used to obtain blood-oxygen level dependent signal (BOLD), a measure of venous oxygenation, and cerebral blood flow (CBF), a measure of

arterial flow rate, near-simultaneously. Younger (ages 18-34) and older (ages 55-70) adult participants were scanned while they breathed room air (normocapnia) for four minutes followed by six minutes of inhalation of 5% CO2 solution (hypercapnia) to induce cerebral vessel dilation. After scanning, participants completed cognitive assessments, including the Digit-Symbol Substitution Task and Box Completion task. CVR was assessed by comparing signal change between normocapnia and hypercapnia and calculating the amount of dilation per unit of CO_2 blood concentration increase. CVR_A was calculated using whole-brain CBF signal, that measures arterial flow rate, and CVR_V was calculated using whole-brain BOLD, a measure of venous oxygenation. AVC was calculated as CVR_A/CVR_V. We found that AVC was higher in younger than in older participants, suggesting lower venous compliance in response to arterial dilation in older adults. CVR was also related to performance on cognitive tasks in young adults but not in older adults.The strong relationship between CVR_V and CVR_A in young adults indicates that arterial and venous blood flow vary together in the intact system. The loss of this relationship in older adults is probably due to age-related vascular pathology. The age-related decline in CVR-performance relationships supports the hypothesis that compromised neural-vascular coupling underlies age-related cognitive decline.

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Poster

607. Human Cognition and Behavior: Cognitive Aging II

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Program #/Poster #: 607.25/JJJ8

Topic: H.02. Human Cognition and Behavior

Support: NIA R01 AG034613

Title: Cortical thickness as a predictor of memory success across the lifespan and in the 90+

Authors: E. DOMINGUEZ, M. CORRADO, C. KAWAS, S. STARK, *C. E. STARK
Univ. of California Irvine, Irvine, CA

Abstract: Previous research has found a specialized group of individuals 80 and above, also known as SuperAgers, that exhibit superior episodic memory performance comparable to the performance of their much younger counterparts. Previous reports have shown that specific regions of the cerebral cortex are significantly thicker in SuperAgers when compared to both younger and similarly aged groups (Rogalski et al., 2012, Sun et al. 2016). In the present study, we aimed to examine the relationship between cortical thickness and memory performance across the lifespan using two existing cohorts. In one, a cohort of 20-89 year-olds, RAVLT scores were used to define SuperAgers as individuals aged 70-89 that scored within the range of

younger adults on the RAVLT delay (≥10) and within one standard deviation of normal scores on Trails B. In all participants, RAVLT was used to examine its overall relationship with cortical thickness. Our second cohort consisted of individuals aged 90 and above (provided by the 90+ Study), where SuperAgers were defined by scores greater than or equal to the mean of younger adults on the CVLT delayed recall (≥8 on short-form) and performance within 1 standard deviation of the age-based norms on Trails B. Using T1-weighted structural MRI images, cortical thickness was calculated using both ANTS and FreeSurfer pipelines and segmented according to the standard DKT atlas to examine previously implicated cortical regions. In our 70-89 SuperAger dataset, we saw no differences in cortical thickness between normal and SuperAgers in these regions, despite a correlation in the entire 20-89 year old cohort between RAVLT and anterior cingulate thickness. However, in the 90+ cohort, we found greater cortical thickness in 90+ SuperAgers in the posterior cingulate and entorhinal cortex when compared to the normal agers. These data contribute to a growing literature addressing the factors that underlie superior memory and cognitive performance in the elderly, which will be informative for further understanding and defining resilience and brain maintenance later in life.

Disclosures: E. Dominguez: None. **M. Corrado:** None. **C. Kawas:** None. **S. Stark:** None. **C.E. Stark:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 607.26/JJJ9

Topic: H.02. Human Cognition and Behavior

Title: Metformin associated with mild cognitive impairment in an older adult diabetes mellitus type 2 patient. A case report

Authors: *K. LIRA-DE LEON[1], A. ALCAZAR-RAMOS[1], M. MERAZ-RIOS[2], G. SOTO-OJEDA[3], M. OCAÑA-SANCHEZ[4], M. HERNANDEZ-LOZANO[3]
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Abstract: Life expectancy has increased substantially in recent decades. Thus diseases related to older people (aged 65 years or older) have become a serious public health problem worldwide. Specifically the non-communicable diseases such as: cardiovascular disease, diabetes mellitus (DM), cancer and dementia are the most affecting for this group. In addition, DM is associated with a faster rate of cognitive decline in those with mild cognitive impairment (MCI) and is considered a risk factor for developing Alzheimer disease (AD). The term DM describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with

disturbances in carbohydrate, fat and protein metabolism, resulting from defects in insulin secretion, insulin action, or both. MCI has been used to describe an isolated memory deficit in older adults in the context of otherwise normal cognitive functioning and can be the first step in the progression to AD or cognitive functional impairment. Approximately 90% of patients with diabetes have type 2 diabetes and Metformin is a first-line treatment, which increasing glucose uptake in muscle while reducing liver gluconeogenesis. The current study examined the association between the use of Metformin and the risk of developing MCI. Clinical case: We report a 78-year-old male patient that according with the Yesavage scale has depression. Their Pharmacological treatment includes: Metformin/glibenclamide, Sennosides, pentoxifylline and omeprazole. Within the analysis, mild cognitive impairment was detected (score = 19) according to the Montreal cognitive assessment (MoCA) test. Our observation agrees with previously reported for patients who are taking metformin and developed cognitive impairment.

Disclosures: K. Lira-De Leon: None. **A. Alcazar-Ramos:** None. **M. Meraz-Rios:** None. **G. Soto-Ojeda:** None. **M. Ocaña-Sanchez:** None. **M. Hernandez-Lozano:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 607.27/JJJ10

Topic: H.02. Human Cognition and Behavior

Title: Can a deep learning neural network improve ability of the MOCA (Montreal cognitive assessment) score to predict amyloid pet positives? Sensitivity and specificity analyses from a memory clinic

Authors: *A. K. NAIR[1], S. P. NAIR[2]
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Abstract: Background: Deep learning techniques could improve sensitivity and specificity of Montreal cognitive assessment (MOCA) score to predict amyloid positive scans to make it more clinically meaningful.
Objectives: To use a deep learning neural network model to analyze sensitivity and specificity of MOCA scores for predicting positive amyloid PET scans.
Methods: Memory clinic patients from July 2010 to Dec 2017 with available MOCA score and binary amyloid-PET result were analyzed retrospectively. Univariate and multivariate analyses of age, race, gender and education with MOCA score and amyloid-PET status was performed. Sensitivity and specificity of MOCA score to predict amyloid positivity was calculated. Receiver Operating Conditions (ROC) analysis measured area under the curve (AUC) and selected the ideal cut point. A neural network model was tested to improve item level sensitivity and specificity.

Results: From July 2010 to Dec 2017, 99 patients had available MOCA scores and Amyloid-PET imaging. Mean±SD of age, education, MOCA score of subjects were 71.31±9 years, 13.33±2.52 years and 20.09±4.84 respectively. 49 females (49.5%), 93 Caucasians (94%) and 56 amyloid-PET positive (56.57%) patients were included. Lower MOCA scores significantly correlated to amyloid positivity in univariate ($\chi 2=6.39$, df=1, $p<0.05$) and multivariate logistic regression analyses after adjusting for age, gender, education, and race (OR= 0.89, 95% CI= 0.80-0.99, $p<0.05$). The established clinical cut point of MOCA<26, had 98.2% sensitivity and 9.3% specificity for predicting Amyloid-PET positivity. AUC was 0.65 (95% CI 0.54 - 0.76). Prior to applying the neural network algorithm, the ideal MOCA cut point score was 20, with 67% sensitivity and 64% specificity. A Neural network model was trained to identify items and improve accuracy for clinically meaningful use.
Conclusions: A real-world use of a neural network algorithm improved clinical utility of MOCA score to predict amyloid positives. The commonly used clinical cut point for diagnosis was not adequately specific for amyloid positivity. Additional studies are needed to detect the pre-clinical stage of dementia.

Disclosures: A.K. Nair: 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 Clinical Trial (Site PI) for Avanir, Lilly, Genentech, Otsuka, Acadia, Axovant, Avid, Piramal, MNI, Allergan, Novartis, Pfizer, VTV. **S.P. Nair:** None.

Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 607.28/JJJ11

Topic: H.02. Human Cognition and Behavior

Support: University of Louisville 21st Century Initiative

Title: Feature fusion based cad system for a detailed diagnosis of mild cognitive impairment diagnosis using smri

Authors: *X. QIU[1], F. GAMAL[2], M. ELMOGY[2], M. GHAZAL[3], H. SOLIMAN[2], A. ATWAN[2], R. KEYNTON[2], G. N. BARNES[4], A. EL-BAZ[2]
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Abstract: Alzheimer's disease (AD) is an irreversible neurodegenerative disorder that faces the central nervous system and that mainly goes through three stages. Diagnosing the disease in its early stage is the main obstacle in front of the researchers due to number of factors. These factors

include mainly the variability of the disease effect among the patients. Among the diagnosing tests, brain biomarkers show effective role in this context. This paper utilizes structure Magnetic Resonance Imaging (sMRI) to mainly present a local computer-aided diagnosis (CAD) system for serving the personalized diagnosis of AD and monitoring the progression of the disease. A dataset of 146 sMRI scans, 60 normal controls (NC), and 86 mild cognitive impairment (MCI), obtained from Alzheimer's Disease Neuroimaging Initiative (ADNI) database, are used to evaluate the proposed system that goes into five steps: (1) Preprocessing to standardize the scans to the labeling atlas's space, and to extract the brain's cortex to serve the following analysis, (2) Feature extraction through: (I) cortex re-construction using marching cube algorithm, (II) shape features extraction (i.e., mean and Gaussian curvatures, sharpness, curvedness, and volume), (3) Brain labeling using automatic anatomical labeling (AAL) atlas to serve the local diagnosis goal, (4) Feature fusion using canonical correlation analysis (CCA) based technique to produce more informative features, and (5) Two diagnosis levels: (I) local diagnosis, using probabilistic support vector machine (pSVM) to visualize the disease's severity in each of the cortical regions, and (2) a global diagnosis, using standard SVM, to present a final diagnosis of the subject. System's performance evaluation is performed from three perspectives: (a) the evaluation of different SVM-based kernels, (b) the comparison with state-of-the-art classifiers, and (c) the validation of the results with related work. First, testing SVM-based kernels (i.e., radial basis function, linear, and polynomial) shows superior results of the linear with 86.3%, 88.33%, and 84.88% of the accuracy, specificity, and sensitivity, respectively. Second, the comparison with a number of the state-of-the-art classifiers shows higher results of our system where the accuracies of the tested classifiers are 71.91%, 75.34%, and 60.95% for decision tree, ensemble classifier, and k-nearest neighbor, respectively. Finally, the system's validation with the related work shows promising results in the differentiation task between NC and MCI groups.

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Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 607.29/JJJ12

Topic: H.02. Human Cognition and Behavior

Title: Human motion discrimination, confidence in it, and metacognitive sensitivity decrease with age, while selective visual attention effects on these visual perception performance measures do not

Authors: *L. ZIZLSPERGER[1], H. EITLER-KLENK[2], T. HAARMEIER[3]
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Abstract: Visual perception has been described as a process of probabilistic inference featuring metacognitive evaluations of choice confidence, the degree to which one believes that a choice is likely to be correct. Here we examine the influence of selective visual attention on human perceptual sensitivity, the subjects' confidence in it, and metacognitive sensitivity across the lifespan. 50 healthy subjects between 20 and 69 years of age performed a precue-postcue paradigm discriminating between four directions of global motion in one of two random dot kinematograms (RDK) simultaneously presented in each hemifield. A central arrow correctly indicated which of two RDKs to attend in 80% of the trials. Motion coherence levels were varied in a 4-alternative forced-choice design and subjects indicated the confidence in their decision via post-decision wagering. To quantify how precise subjects assessed their objective performance we examined their metacognitive sensitivity via a non-parametric signal detection theoretic approach. We show that objective visual motion discrimination performance and subjective confidence in the perceptual decision significantly decrease with age (perceptual sensitivity: $p = 0{,}006$; $F(4,43) = 4{,}195$ and confidence: $p = 0{,}024$; $F(4,43) = 3{,}128$). Metacognitive sensitivity was observed to decrease with age, as well ($p = 0{,}001$; $F(4,43) = 5{,}674$). Analyzing valid and invalid cueing conditions, we found significant increases in perceptual sensitivity, choice confidence and metacognitive sensitivity for attended targets, showing effects of visual spatial attention on objective and subjective performance measures. Covertly shifting attention to a target in the visual field significantly improved these three performance measures. But it was only for metacognitive sensitivity that we observed improvements with attention to differ between the age groups ($p = 0{,}030$; $F(4,43) = 2{,}963$), with the biggest increase in the oldest group (60 to 69yo). Based on these findings we conclude: (i) Visual motion discrimination performance, the confidence in this perceptual decision and metacognitive sensitivity decline with age. (ii) Improvements in cognitive and metacognitive performance due to selective attention, in contrast, do not abate with natural aging. (iii) This points to a compensatory effect of this top-down contextual neural modulation on objective and subjective measures of human visual perception that appears to be particularly essential for maintaining metacognitive sensitivity in advanced age.

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Poster

607. Human Cognition and Behavior: Cognitive Aging II

Location: SDCC Halls B-H

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Program #/Poster #: 607.30/JJJ13

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01AG029523
NMSS Grant No RG150704951

Title: Age-differences in canonicality of the hemodynamic response function and relationships to cognitive performance: A population-based study

Authors: *V. PRABHAKARAN[1], M. P. TURNER[2], K. WEST[3], M. D. ZUPPICHINI[7], D. SIVAKOLUNDU[4], Y. ZHAO[5], D. H. ABDELKARIM[8], B. P. RYPMA[6]
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Abstract: Functional magnetic resonance imaging (fMRI) has been used to infer age-differences in neural activity from the hemodynamic response function (HRF) that characterizes the blood-oxygen-level-dependent (BOLD) signal over time. The HRF results from complex interactions between neurons, glia, and vascular structures, comprising the neural-vascular coupling system. This system is finely-tuned in healthy individuals for efficient brain function, and may underlie performance differences observed between younger and older adults. We hypothesize that age-related changes to any component of this system could alter relationships between the shape of the HRF and cognitive performance. We analyzed a large dataset from the Cambridge Center for Aging and Neuroscience (CamCAN) study. 74 younger (18-30 years of age; 25.4±3.6, 33 males) and 173 older (54-74 years of age; 63.7±6.0, 100 males) adults viewed two checkerboards flanking a central fixation point (34 ms) and simultaneously heard a 300ms binaural tone. HRFs were estimated using FMRIB's Linear Optimal Basis Sets (FLOBS) to minimize shape assumptions. FLOBS-generated HRFs were compared to canonical HRFs using several different measures, including Pearson's correlation coefficient and Kolmogorov-Smirnov goodness-of-fit test statistics. These metrics were combined to create a composite score indicating how closely the FLOBS-generated HRF resembled the canonical HRF, the Quantitative Canonicality Index (QCI). Results showed that for older adults, the QCI significantly predicted reaction time on the task performed in the scanner ($r = -0.21$, $p < 0.004$). Age reductions in the conformity of HRF shape (as measured by QCI) support the hypothesis of age-related changes in neural-vascular coupling. They also suggest the importance of an intact neural-vascular coupling system to fast, efficient cognitive performance. New imaging methods, like calibrated fMRI, permit direct assessment of age-differences in the physiologic factors underlying BOLD signal. More precise interpretations of HRF shape-cognitive performance relationships can be formulated once these physiologic factors are disentangled and measured separately.

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Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 608.01/JJJ14

Topic: H.02. Human Cognition and Behavior

Support: NIH DA023248
NIH AA021449

Title: Neural compensation for proactive inhibitory control in healthy aging

Authors: *S. HU[1], M. JOB[2], S. K. JENKS[1], C.-S. R. LI[3]
[1]Dept. of Psychology, [2]Dept. of Psychology, Dept. of Biol. Sci., SUNY Oswego, Oswego, NY; [3]Dept Psychiatry, Yale Univ., New Haven, CT

Abstract: Aging is associated with impaired cognitive functions including inhibitory control. Previous research has reported reduced stopping efficiency during reactive inhibition, along with decreased brain activations, in older adults. Whether older adults are also compromised in proactive or anticipatory inhibition remains unclear. The current study aimed to investigate age-related behavioral and neural changes in proactive inhibitory control. One-hundred-and-forty-nine adults (83 women) between the age of 18 and 72 (31.6 ± 11.9) years participated in the study. All participants underwent fMRI performing a stop signal task (SST) in which frequent "go" signals instructed a button press and occasional "stop" signals demanded withdrawal of the response. Proactive inhibition was operationalized by the sequential effect, the correlation between the probability of stop signal occurrence, or P(Stop), and go trial reaction time (RT). P(Stop) was estimated trial by trial with a Bayesian belief model (Hu et al., 2015). Two generalized linear models were built each on trial and go signal onsets to model fMRI signals. P(Stop)s and RTs were entered as parametric modulators each in the first and second model. Behaviorally the magnitude of sequential effect was not correlated with age, suggesting spared proactive inhibitory control in older adults. On the other hand, age was associated with increased activation during P(Stop) and prolonged RT. Specifically, the left lateral prefrontal cortex (PFC), paracentral lobule, superior and inferior parietal lobule, and cerebellum showed increased activation with age during stop signal anticipation, and the right middle occipital gyrus (MOG) showed age-related increase in activation during prolonged RT. Further, Granger Causality analysis showed that the PFC Granger caused MOG, with the PFC-MOG connectivity significantly correlated with P(Stop) in older but not in younger adults, suggesting that the PFC and MOG activations and PFC-MOG connectivity may compensate for sequential effect during aging. These results revealed distinct neural processes underlying proactive inhibitory control in aging and highlighted neural plasticity in the aging brain.

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Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

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Program #/Poster #: 608.02/JJJ15

Topic: H.02. Human Cognition and Behavior

Support: CONICET
FONCyT-PICT 2012-0412
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INECO Foundation

Title: The neural basis of metacognition in lesion models

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Abstract: Metacognition, the knowledge about own mental abilities, is strongly linked to self-control and self-awareness. While neuroscientific study on this domain has accrued in recent years, only few studies have compared metacognitive performance across brain pathologies and none has applied the lesion model approach combined with neuroimaging (MRI) analysis. To bridge this gap, we evaluated metacognition in patients with focal frontal-insular lesions (FIS) and dementias -behavioral variant frontotemporal dementia (bvFTD) and Alzheimer's disease (AD)-, who present damage in key metacognitive areas. Participants performed a visual perception task and provided two types of metacognitive report: *confidence* (judgment of trust about the performance) and *wagering* (betting on their accuracy in the perceptual task). Then, damaged areas were analyzed via structural MRI to identify an association with impaired metacognitive outcomes.Results showed that, relative to controls, FIS and bvFTD patients did not present differences in confidence, whereas AD patients proved significantly overconfident. In contrast, wagering performance was affected in all patient groups. MRI analysis evidenced that lesions in orbitofrontal regions were involved in overconfidence, and damage in dorsolateral regions was associated with excessive wagering. Therefore, this study allowed a differentiation between metacognitive performance (confidence vs. wagering) and pathologies (orbitofrontal lesions vs. dorsolateral lesions). The impairment of confidence and wagering in AD patients evidenced a lack of self-awareness in both types of metacognitive measures. Remarkably, in the frontal pathologies (FIS and bvFTD), confidence was preserved, but wagering was excessive, showing a failure to use metacognitive information to bet adequately. Finally, overconfidence was associated with orbitofrontal damage, while impaired wagering was related with dorsolateral

lesions. These results and the application of the lesion model approach across contrastive pathologies contributed to a better understanding of the brain functions, and specifically, of the metacognitive processes.

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Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 608.03/JJJ16

Topic: H.02. Human Cognition and Behavior

Support: MOST Grant 106-2410-H-194-038

Title: Age differences in brain mechanisms underlying processing of a face and its components: An fMRI study

Authors: *G. C.-W. SHYI[1,2,3], P. CHENG[4,2], Y.-C. CHEN[2], A. KUO[1,2], T. HUANG[1,2], M.-C. LIAO[2]
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Abstract: A variety of behavioral tasks have been devised to examine component, configural, and holistic aspects of face processing. Using simultaneous presentation of face pairs, our previous study in age differences has demonstrated that an inversion effect of the component task was evident with younger but not with older adults, despite the fact that regression models suggested younger and older adults relied upon the same aspects of holistic and non-holistic processing revealed by the component task for encoding and later retrieving memory of faces (Cheng, Shyi, & Cheng, 2016, *CJP*). Here we aimed to investigate the brain mechanisms underlying the performance of component task in order to better understand whether and how younger and older adults may differ in the processing of a face and its components. 17 young adults (mean age of 22.6 yrs and *SD* of 1.80) and 9 older adults (mean age of 67.5 yrs and *SD* of 6.35) were asked to judge whether or not two simultaneously presented faces, displayed either upright or inverted, were identical. When faces were not identical, they differed in terms of the eyes or mouths. Their brain were scanned using fMRI while performing the task. The behavioral results showed the inversion effect among younger adults but not among the older adults, replicating our previous findings, and both groups did equally well in the scrambled-face control condition. Brain imaging results on the other hand revealed more regions were activated among

younger adults than among older adults for processing upright and inverted faces in contrast to processing scrambled faces. Furthermore, for younger adults, the contrast between upright and inverted faces revealed a broad array of brain regions involving frontal, prefrontal, and temporal areas that have been widely identified for their roles in processing faces. In a sharp contrast, older adults appeared to have engaged primarily non-face processing regions, such as caudate body and retrosplenial cortex, in processing the components of a face. These differences in the pattern of brain activations may help to elucidate the neural mechanisms in support of the presence of inversion effect among younger adults as well as its absence among the older adults.

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Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

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Program #/Poster #: 608.04/JJJ17

Topic: H.02. Human Cognition and Behavior

Support: Medical Research Council
BRACE Bristol
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Title: The neuromodulatory effects of L-DOPA across human verbal memory processes

Authors: *H. K. ISOTALUS[1], J. P. GROGAN[2], N. IRIGORAS IZAGIRRE[2], A. HOWAT[2], L. KNIGHT[3], R. A. KAUPPINEN[2], E. J. COULTHARD[4,1]
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Abstract: One framework for understanding long term memory is to divide it into three core phases of processing; encoding (transcribing information into a neural representation), consolidating (organising and strengthening) and retrieving (accessing). We have previously shown that increasing dopamine availability in the brains of patients with Parkinson's disease enhances consolidation and retrieval but impairs encoding. Understanding when dopamine influences memory is pivotal to optimally target future therapeutics in memory impairment. Here, we tested if exogenous dopamine administration improves either retrieval or encoding of verbal episodic information in healthy elderly. In this placebo-controlled double-blind randomised crossover trial, 33 healthy elderly (65+ years) adults performed a verbal recognition memory task. Volunteers first learnt a word list on Day 1 without medication. On Day 2, to examine the effect of dopamine on retrieval, they were dosed with 150mg L-DOPA or placebo before their memory was tested. To target encoding, they then learnt a novel word list for which

they were tested on immediately, and 1, 3, and 5 days later, with unique targets at each test. There was no difference in verbal recognition accuracy or signal detection measures between L-DOPA and placebo when administered prior to retrieval or encoding. Furthermore, Bayesian analysis of these data provided moderate support against L-DOPA affecting retrieval (BF_{01}=4.243) or encoding (BF_{01}=4.300) in elderly adults. However, post-hoc analyses revealed that L-DOPA during encoding enhanced retrieval 3-days later for those with high trait depression or anxiety (r =.425 p=.030; r =.467, p =.016, respectively). Our findings suggest that exogenous dopamine does not enhance encoding or retrieval in healthy ageing, and that earlier results may be explained by dopamine boosting consolidation. However, elderly with high trait depression or anxiety may benefit from L-DOPA during encoding or early consolidation, although these findings need replicating in independent datasets, which we aim to do in our future work. Our ongoing work investigates the efficacy of L-DOPA administration in targeting consolidation during sleep in both healthy ageing and in amnesic disease.

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Poster

608. Human Cognition and Behavior: Cognitive Aging III

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Topic: H.02. Human Cognition and Behavior

Support: DFG (Deutsche Forschungsgemeinschaft) in the Cluster of Excellence “Cognitive Interaction Technology” (CITEC)

Title: TDCS in a verbal recognition task: Boosting older participants' word recognition time

Authors: *L. S. BALDUIN-PHILIPPS, S. WEISS, H. M. MUELLER
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Abstract: Transcranial direct current stimulation (tDCS) may elicit an improvement of memory functions in healthy individuals of different age after multi-day anodal stimulation of temporoparietal regions. Furthermore, anodal tDCS of these areas facilitates memory and recognition processes in participants with memory deficits. Therefore, our study deals with the question to which extent even a single session of anodal tDCS applied to the left temporal cortex influences recognition performance in a verbal recognition task in older healthy subjects.
In this sham-controlled and double-blinded experiment 19 healthy, elderly participant (14 female, M∅ = 69.5y, SD = 5.2y, 64-80y) underwent a verbal recognition task. All participants were right-handed (EHI M∅ = 96.3, SD = 6.9, 80-100) and monolingual native speakers of German, and had no verified memory deficits. Participants completed two sessions (sham/anodal

tDCS), counterbalanced between subjects, with a wash-out period of at least ten days. They had to memorize auditorily presented words in a learning phase. After that, they had to recognize single words out of several distractors (semantically or phonologically related words) via button press. 20 minutes of 1.5 mA anodal tDCS was applied on the left temporal cortex including both the learning and the recognition phase. We hypothesized that already a single anodal tDCS session would lead to improved word recognition performance compared to sham.
First results indicate a positive influence of anodal tDCS compared to sham. Word recognition times are significantly shorter in the tDCS compared to the sham condition (F (1, 18) = 7.277, p = .015). Pure motor reaction times are not influenced by tDCS (F (1, 18) = .013, p = .912). Hence, a single session of anodal tDCS to the left temporal cortex selectively improves word recognition speed in healthy older individuals, but not motor reaction time. Therefore, these results are promising for further studies on patients with memory and recognition deficits.

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Poster

608. Human Cognition and Behavior: Cognitive Aging III

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 608.06/JJJ19

Topic: H.02. Human Cognition and Behavior

Title: Frequency oscillations in error processing: An EEG study on age-related effects

Authors: *J. KRAUSS[1], E. NIESSEN[1], N. ROSJAT[1], S. DAUN[2], J. STAHL[2], G. R. FINK[1], P. H. WEISS[1]
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Abstract: Event-related oscillations in different frequency bands are related to different motor cognitive functions: theta oscillations are linked to inhibition of movements, whereas alpha and beta oscillations are associated with different movement processes. Both theta and alpha oscillations are known to be influenced by error processing.
Here, we comprehensively examined error processing and its underlying neural correlates in two age groups. Brain oscillations in the theta (4-7 Hz), alpha (8-13 Hz) and beta (14-30 Hz) frequency bands measured by EEG were analysed in 22 young (age 25 ± 4 years) and 20 older (age 58 ± 6 years) adults in terms of event-related spectral perturbation (ERSP) using a previously established Go/Nogo task.
In young adults, significant changes in theta power were found between Go and (correct and incorrect) Nogo trials, suggesting a role of theta oscillations in processing infrequent stimuli. The occurrence of errors modulated alpha power by creating the exact inverse pattern as compared to correct responses and correct withholds. An increase in power was present in the beta frequency band after movement in correct Go and Nogo trials, but not in error trials. In stark contrast, none

of these condition specific effects were observed in the older adults. In fact, for the three frequency bands no significant differences between the conditions emerged in the older group. Data suggest a differential modulation of the three frequency bands during error processing in young adults. In older adults, the activation pattern within the frequency bands did not reveal any differences between conditions. The finding that the older subjects nevertheless showed similar behaviour as the young subjects suggests compensatory mechanisms in older adults.

Disclosures: J. Krauss: None. **E. Niessen:** None. **N. Rosjat:** None. **S. Daun:** None. **J. Stahl:** None. **G.R. Fink:** None. **P.H. Weiss:** None.

Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 608.07/JJJ20

Topic: H.02. Human Cognition and Behavior

Support: Iowa State University College of Human Sciences Graduate Scholarship

Title: Behavioral and neurophysiological differences in cognitive and motor inhibition of aging musicians and non-musicians

Authors: *P. IZBICKI[1], E. L. STEGEMOLLER[2]
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Abstract: Older adults experience a decline in the domains of cognitive and motor inhibitory control. These declines have been implicated in instrumental activities of daily living. However, studies have revealed that older musicians have behavioral and neurophysiological enhancements in various motor and cognitive domains as compared to non-musicians. This suggests that music training may delay the decline in cognitive and motor inhibition with aging. Yet, cognitive and motor inhibition has not been studied across the lifespan in musicians and non-musicians. Thus, the aim of this study was to investigate the behavioral differences in cognitive inhibition and motor inhibition in aging musicians and non-musicians. Healthy young adult (HYA) musicians and non-musicians and healthy older adult (HOA) musicians and non-musicians were recruited for the study. To measure cognitive inhibition**,** the Stroop task was performed. Participants were asked to name the color of a word presented in either red, green, yellow, or blue. Three conditions were presented randomly: neutral (infrequent words sol, helot, eft, and abjure presented in different colors), congruent (color of word matches the word), and incongruent (color of the word does not match the word itself). Accuracy and reaction time were recorded using E-Prime 2.0 (Psychology Software Tools, Pittsburgh, PA). To measure motor inhibition, participants were asked to perform an index finger flexion-extension movement (i.e., finger tap) in sync with an auditory tone (i.e., synchronized) and between auditory tones (i.e., syncopated)

presented at 1 Hz. The forearm, wrist, thumb, and fingers 2-4 were supported with a brace maintaining the forearm in a pronated position with the elbow flexed at 90 degrees. The index finger remained unconstrained to allow for full range of motion without touching a surface. Accuracy was recorded using a goniometer. For cognitive inhibition, results revealed that both HYA and HOA musicians showed faster reaction time on the Stroop task than non-musicians. For motor inhibition, results revealed that HYA and HOA musicians demonstrated greater accuracy in the syncopation task than non-musicians. Overall, these results suggest that HYA and HOA musicians display greater cognitive and motor inhibition than HYA and HOA non-musicians, respectively. Future studies will explore the neurophysiological measures associated with the tasks. At the conclusion of the study, results will demonstrate a clearer understanding of whether music training contributes to greater cognitive and motor inhibitory control during the aging process, thus, enhancing health and quality of life in older adults.

Disclosures: P. Izbicki: None. **E.L. Stegemoller:** None.

Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 608.08/JJJ21

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R00 AG-036818-05
NIH Grant R01 AG-56535-02
NIH Grant R00 AG-036848-05

Title: Fronto-parietal functional connectivity during an n-back task decreases across the adult lifespan and predicts working memory performance

Authors: *E. E. PONGPIPAT, C. M. FOSTER, K. M. KENNEDY, K. M. RODRIGUE
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Abstract: Working memory (WM), an age-vulnerable process, is associated with activity in fronto-parietal association cortices. BOLD activation and modulation to task difficulty decreases with aging and has been shown to relate to task performance and cognitive ability. Differential, age-related changes in the patterns of cross-connectivity between frontal and parietal regions during WM tasks likely support proper performance. However, the nature of the aging findings are mixed and lifespan data are limited. In the present study, we examined frontal and parietal functional connectivity (FC) during an *n*-back task (i.e., 0-, 2-, 3-, and 4-digits back) and the relationship between age related alterations in FC and WM performance. Participants included 170 healthy adults (aged 20-94 years) who completed cognitive assessment sessions along with

an MRI session. FC was analyzed and measured using psychophysiological interactions (PPI) with seed regions of interest selected from local peak maxima in the frontal and parietal cortices from each hemisphere. The *n*-back contrast of interest was 2, 3, 4-back vs. 0-back from three functional runs of pseudo-counterbalanced blocks of the digits back. We observed the expected increase in fronto-parietal FC as WM load increased. We also found that FC with the two parietal seeds decreased with age but that FC with the two frontal seeds was age-invariant. To gauge the effects of decreased FC on performance, we tested models with positive FC, negative FC, age, and their interactions on a multivariate variable composed of task accuracy, Digit Span (i.e., forward, backward, and sequencing), and absolute Listening Span for each seed PPI. We found that decreased right parietal positive and negative FC was associated with poorer WM performance, as was left parietal positive FC, and left frontal positive FC, all beyond the effects of age on WM. These results suggest that fronto-parietal FC during WM decreases linearly across the adult lifespan and this age-related decreased FC is associated with poorer WM ability.

Disclosures: E.E. Pongpipat: None. **C.M. Foster:** None. **K.M. Kennedy:** None. **K.M. Rodrigue:** None.

Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 608.09/JJJ22

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant AA021187
NIH Grant AG054067
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NIH Grant AG028507
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UC San Diego Clinical Research Fellowship

Title: Hearing impairment and cognitive decline among older, community dwelling adults

Authors: A. A. ALATTAR[1], J. BERGSTROM[1], G. A. LAUGHLIN[1], D. KRITZ-SILVERSTEIN[1], E. RICHARD[1], E. T. REAS[1], J. HARRIS[1], E. BARRETT-CONNOR[1], *L. K. MCEVOY[2]
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Abstract: Hearing impairment is an emerging risk factor for cognitive impairment in older adults. Several cross-sectional studies have shown associations between hearing impairment and poorer cognitive performance. Few prospective studies have examined whether hearing impairment is associated with a faster rate of cognitive decline with age, with mixed results. We

investigated the association between hearing acuity and cognitive function over a 24-year follow-up in a large, well-characterized sample of community-dwelling older adults. Between 1992-1996 participants of the Rancho Bernardo Study of Healthy Aging (n=1164, mean age 73·5 ±9.3 years; 64% women) had hearing thresholds measured and cognitive function assessed. Participants returned for reassessment of cognitive function up to 5 times, at approximate 4-year intervals. Participants were classified into 3 groups based on pure tone average (PTA) threshold in the better hearing ear: normal hearing, PTA<25 dB, N= 388 (33.3%); mild hearing impairment, PTA 25-40 dB, N=580 (49.8%); and moderate-or-greater impairment, PTA>40 dB, N=196 (16.83%). Multivariable mixed-effects linear regression, adjusting for age, education, cardiovascular risk factors and health behaviors, was used to assess group differences in cognitive function and rate of cognitive change over time. Hearing impairment was associated with poorer performance and steeper decline over time on the MMSE (p=0.002) and Trails B (p=0.001). Associations did not differ by sex, APOE-ϵ4 status, or after further adjustment for social engagement. Associations were modified by education (p=0.037): mild hearing impairment was associated with steeper decline on the MMSE among participants with high school education or less; but not among those with at least some college. Moderate or greater hearing impairment was associated with steeper MMSE decline relative to normal hearing adults regardless of education level. Differences in rates of decline by hearing group remained significant when the effect of informative drop-out due to death was accounted for with joint longitudinal models, indicating that the differences were not due to survival bias. This study shows that among older adults, hearing impairment is associated with a faster rate of cognitive decline, and that higher education may protect against cognitive decline associated with mild hearing impairment. Screening for hearing impairment may be important for identifying older adults at risk for accelerated cognitive decline.

Disclosures: A.A. Alattar: None. **J. Bergstrom:** None. **G.A. Laughlin:** None. **D. Kritz-Silverstein:** None. **E. Richard:** None. **E.T. Reas:** None. **J. Harris:** None. **E. Barrett-Connor:** None. **L.K. McEvoy:** None.

Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 608.10/JJJ23

Topic: H.02. Human Cognition and Behavior

Support: NIH 5RO1AG04936903

Title: Remediating age-related cognitive decline in older adults through exercise and mindfulness

Authors: H. RIPPERGER[1], K. AHERN[2], N. HARPER[2], M. YINGLING[2], *J. A. SCHWEIGER[3], E. LENZE[2]
[1]Psychiatry, [2]Washington Univ. Sch. of Med., Saint Louis, MO; [3]Dept Psychiatry, Washington Univ. Sch. Med., Saint Louis, MO

Abstract: Participants were enrolled in an 18-month pilot study of exercise and mindfulness-based stress reduction (MBSR) to test whether these interventions can remediate age-related cognitive decline.
Previous research studies have shown that MBSR appears to produce neurocircuitry changes that are the reverse of those seen in age-related cognitive decline, and it also alters stress-related biological pathways that contribute to cognitive changes in older adults. Other studies have demonstrated that exercise also appears to affect brain structure and function and improves cognition.
Participants attended exercise classes twice weekly for 6 months, and then once weekly for the next year. They completed an additional 120 minutes of exercise per week at home. They also attended MBSR classes once weekly for 10 weeks, and then once monthly for the remainder of the 18 months. They were asked to practice MBSR independently up to 1 hour per day. A comprehensive cognitive battery was administered at baseline, 3-month, 6-month, and 18-month time points.
Participants in this pilot group were community-residing adults between the ages of 65 and 84 who reported a decline in their memory or concentration, but did not have a diagnosis of MCI, dementia, or Alzheimer's. In this pilot group of individuals (n=29), the average age of respondents was 71.8 years (SD=5.7), 65.5% (n=19) were female, 96.6% (n=28) were white, 6.9% (n=2) were Hispanic or Latino.
We created a memory composite (using a paragraph and word recall task, and picture sequence task) and cognitive composite (using Stroop, Flanker, CVOE, DCCS, and List Sorting). The average baseline memory composite score was 0.00 (SD = 0.79). In the overall group, the mean change in memory composite score from baseline to 6 months was 0.41 (SD=0.42). The average baseline cognitive composite score was 0.00 (SD=0.65). In the overall group, the mean change in cognitive composite score from baseline to 6 months was 0.05 (SD=0.37). These data indicate that participation in exercise and MBSR was significantly correlated with improvements in memory, but not cognitive control.
These results are part of a larger, ongoing randomized clinical trial testing the effectiveness of exercise and MBSR as a treatment for age-related cognitive in older adults. In the larger RCT (n=600), participants are randomly assigned to either MBSR, exercise, a combination of MBSR and exercise, or health education (an active control group). They will undergo a number of assessments, including structural and functional MRI. This will be a comprehensive data set that will give us more clarity on changes in memory and cognitive control as a result of MBSR and/or exercise training.

Disclosures: H. Ripperger: None. **K. Ahern:** None. **N. Harper:** None. **M. Yingling:** None. **J.A. Schweiger:** None. **E. Lenze:** A. Employment/Salary (full or part-time):; Washington University School of Medicine. 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.; Takeda, Lundbeck. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NIMH, NIA, NCCIH, OBSSR, FDA, PCORI, MCKnight Brain Research Foundation, Taylor Family Institute for Innovative Psychiatric Research, Barnes Jewish Hospital Foundation. F. Consulting Fees (e.g., advisory boards); Aptinyx, Alkermes.

Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 608.11/JJJ24

Topic: H.02. Human Cognition and Behavior

Support: MOST104-2410-H-006-021-MY2
MOST106-2410-H-006-031-MY2

Title: Age-related differences in some but not all cognitive control functions are mediated by frontal lobe markers

Authors: *S. HSIEH, M.-H. YANG
Natl. Cheng Kung Univ., Tainan, Taiwan

Abstract: This study aimed at examining the relationships among age, cognitive functioning, and brain structure and functioning. We tested a brain-mediating model in 156 healthy participants (74 females) 20 to 78 years old by examining whether multi-modal frontal lobe variables (gray matter volume, white matter fractional anisotropy, and resting-state functional connectivity) can be mediators of age-related decline in cognitive control function. Cognitive control function was measured by the task-switching paradigm, flanker, n (1&2)-back and stop-signal tasks. The results show that although these tasks are classified as cognitive control tasks, they can be differentiated and their scores do not necessarily decline with age. In addition, although the 2-back task's sensitivity and stop-signal reaction time (SSRT) are sensitive to age, their age-related variance was not mediated by the same sets of brain structures and/or functioning. The current multimodal neuroimaging data combined with psychometric mediation models provide evidence in supporting a multi-factorial theory of cognitive control deficit in aging.

Disclosures: S. Hsieh: None. **M. Yang:** None.

Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 608.12/DP14/JJJ25

Topic: H.02. Human Cognition and Behavior

Support: NIH grant R01AG038465

Title: Optimized multi-modal prediction of cognitive function from brain data for different age ranges

Authors: *C. G. HABECK[1], Y. STERN[2]
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Abstract: Cognition can be broadly characterized by 4 domains: episodic memory (=MEM), fluid reasoning (=REASON), perceptual processing speed (=SPEED), and vocabulary. We investigated the predictive utility of structural and functional brain data for performance in MEM, REASON, and SPEED beyond demographics in 451 participants aged 20-80, similarly to (Hedden et al. 2014). We used gray-matter volume and cortical thickness acquired in 68 regions of interest (ROIs), and fractional anisotropy for 18 major white-matter tracts. These 3 modalities represented all brain-structural independent variables, and yielded 2*68+18=154 features in total. Further, resting-state fMRI was collected in 264 regions of interest, making up the brain-functional independent variables in form of 34,716 connectivity ROI-pairs. Demographic independent variables were constituted by age, years of education and verbal intelligence (=NARTIQ). We ran simulations with 1,000 iterations for which the data were randomly split into a training and test set. Principal-component regression was run in the training set to estimate a best-fit model to predict cognitive outcome, and this model was then applied to the held-out test data set. The prediction in the test-set was correlated with the actual values of the cognitive outcome, and the logarithmic P-value was recorded as a measure of prediction success. We were particularly interested in comparing predictive utility of brain and demographics variables between the 3 cognitive outcomes and 2 age ranges (20-50 and 50-80). The number of participants in both training and test sets was 60 for all age ranges and cognitive outcomes, to avoid differences in statistical power. Several points emerged from these analyses: (1) Demographics outperformed both structural and functional brain data substantially, regardless of cognitive outcome or age range; (2) combining brain + demographics together, however, did usually achieve even better predictive success; (3) REASON showed the best predictive utility for all independent variables for both age ranges, with the best overall prediction for the young age range where the brain + demographics model achieved 100% predictive success at $p<0.01$; (4) MEM showed the worst predictive success for either demographics or brain variables, with

demographics achieving $p<0.01$ success in 18% of the iterations, while the brain variables failed to exceed the expected false-positive rate; (5) SPEED proved intermediate with $p<0.01$ prediction success for 70% of iterations of the brain + demographics model for both age ranges. These results show significant differences in brain-cognition relations between cognitive domains.

Disclosures: C.G. Habeck: None. **Y. Stern:** None.

Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 608.13/JJJ26

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant AG054719
NIH Grant AG043552-05
Alzheimer's Association NIRG-339422

Title: Dendritic spine structural remodeling accompanies Alzheimer's disease pathology in cognitively normal human aging

Authors: *J. H. HERSKOWITZ[1], B. D. BOROS[2], K. GREATHOUSE[3], M. GEARING[4]
[1]Neurol., The Univ. of Alabama At Birmingham, Birmingham, AL; [2]Univ. of Alabama At Birmingham, Birmingham, AL; [3]Univ. of Alabama at Birmingham, Birmingham, AL; [4]Emory Univ., Atlanta, GA

Abstract: Subtle alterations in dendritic spine morphology can induce marked effects on connectivity patterns of neuronal circuits and subsequent cognitive behavior. Past studies of rodent and non-human primate aging revealed reductions in spine density with concomitant alterations in spine morphology among pyramidal neurons in the prefrontal cortex. In this report, we visualized and digitally reconstructed the three-dimensional morphology of dendritic spines from the dorsolateral prefrontal cortex in cognitively normal individuals aged 40-94 years. Linear models defined relationships between spines and age, Mini-Mental State Examination (MMSE), APOE ε4 allele status, and Alzheimer's disease (AD) pathology. Similar to findings in other mammals, spine density correlated negatively with human aging. Reduced spine head diameter as well as morphologic changes in thin spines associated with higher MMSE scores. Individuals harboring an APOE ε4 allele displayed greater numbers of dendritic filopodia and concomitant structural alterations in thin and mushroom spines. The presence of AD pathology correlated with increased spine length, reduced thin spine head diameter, and increased filopodia density. Our study reveals how spine morphology in the prefrontal cortex changes in human

aging and highlights key structural alterations in selective spine populations that may promote cognitively normal function despite harboring the APOE ε4 allele or AD pathology.

Disclosures: J.H. Herskowitz: None. **B.D. Boros:** None. **K. Greathouse:** None. **M. Gearing:** None.

Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

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Program #/Poster #: 608.14/JJJ27

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant K01AG051777
NIH Grant R01AG038465

Title: Differences in functional activation and fractional anisotropy between an age-stable ability, vocabulary, and an age-declining ability, perceptual speed, provides support for greater resilience in neural processes for vocabulary

Authors: *Y. GAZES[1], C. G. HABECK[1], Q. R. RAZLIGHI[2], P. LI[3]
[1]Taub Inst., [2]Neurol., Columbia Univ., New York, NY; [3]Col. of Physician and Surgeon, Neurol., Columbia Univ. Med. Ctr., New York, NY

Abstract: Cognitive tasks known as crystallized abilities remain stable with older age while other tasks, fluid abilities, decline as early as the third decade, which is puzzling considering these are manifested in the same brains. This cross-sectional study is a novel first step to examine whether regional distribution of processes supporting the two ability types contributes to the differential age effects. We administered tasks for a typical crystallized ability, vocabulary (V), and a typical fluid ability, perceptual speed (PS), within the same set of 316 subjects, enabling within-subject comparisons of the two abilities. ROIs were extracted based on unique functional activations for each ability and used as seeds to extract white matter tracts connecting these ability-unique regions. Mean parameter estimates were also extracted from these ability-unique ROIs as well as from regions commonly activated by both abilities. We examined within-subject ability differences in ability-common and -unique activations and in the fractional anisotropy (FA) of the white matter tracts connecting the ability-unique ROIs. Ability-common regions consisted of visuomotor areas. Vocabulary-unique regions included the left inferior frontal gyrus (BA44 and 45) and left superior temporal gyrus, and speed-unique regions included bilateral dorsolateral prefrontal gyri (BA 9) and the supramarginal gyri (BA 40). While vocabulary activations in regions common to both abilities showed strong age effects (V: $r = .316$; PS: $r = .386$; both $p<.01$), activations in vocabulary-unique areas did not correlate with age ($r = .062$, $p > .05$) whereas PS-unique activations did correlate with age ($r = .326$, $p < .01$). The

within-subject differences in ability-unique activations correlated positively with age, such that speed-unique activations became increasingly greater than vocabulary-unique activations with older age ($r = .235$, $p < .01$) whereas the age association for the differences between the common regions was not significant ($r = .107$, $p > .05$). In fact, Steiger test showed that the two correlations was significantly different ($Z=-2.64$ with Common-Unique, $p < .01$). Furthermore, FA of the white matter tract connecting vocabulary-unique ROIs showed greater FA than tracts connecting the speed-unique ROIs ($t=27.6$, $p<.001$). Together, these results provided support for the possible resilience of vocabulary-related brain structures against age relative to those involved in the processing of perceptual speed.

Disclosures: Y. Gazes: None. **C.G. Habeck:** None. **Q.R. Razlighi:** None. **P. Li:** None.

Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 608.15/JJJ28

Topic: H.02. Human Cognition and Behavior

Title: Mind over matter, understanding the relationship between memory self-efficacy, cognition and activation in older adult women with probable mild cognitive impairment

Authors: *B. R. HORST[1], L. S. NAGAMATSU[2]
[1]Neurosci., [2]Kinesiology, Western Univ., London, ON, Canada

Abstract: In our aging population, cognitive decline and brain health are critical areas of concern for healthy aging. Evidence has shown that certain memory performance measures such as associative memory, one's ability to remember connections between distinct items, is linked with conversion to dementia in those already affected by Mild Cognitive Impairment (MCI). The need to protect memory performance is clear. Yet, memory performance is a complex construct, therefore recognizing influential variables is important to improve or maintain performance. Global cognition, functional activation of medial temporal lobe structures, and self-efficacy have each been identified as independent predictive variables of memory performance. However, it is unknown how these variables compare in their predictive value against each other. The aim of our study is to compare the predictive values of these variables. We predict that memory self-efficacy, a psychosocial construct of one's perceived memory ability, may be a significant predictor of associative memory performance beyond physiological variables. Using a cross-sectional design, community dwelling older women (age 65-80) with probable Mild Cognitive Impairment (MCI) were asked to evaluate their memory self-efficacy using the Multifactorial Memory Questionnaire (MMQ) in addition to standardized cognitive tests. T1 weighted structural imaging and BOLD signal fMRI, during an associative-memory task, was obtained using a 3T scanner. Multiple linear regression models were constructed for the prediction of

memory performance outcomes using independent variables of MSE, functional activation, and global cognitive status; co-varying for age, physical activity level, and neural structural volumes. Our results found that the memory self-efficacy added significant predictive value to the models beyond global cognition and functional activation for performance on an associative memory task. Based on these results it appears that one's perceived feelings and contentment about their memory ability is associated with how they will perform on a memory task regardless of cognitive status or physiological differences. Based on this data our research has the potential to progress into a longitudinal study of observing the relationship between changes in memory self-efficacy, brain health and cognition, as well as progression to collaborative clinical studies in memory self-efficacy modification for healthy aging

Disclosures: B.R. Horst: None. **L.S. Nagamatsu:** None.

Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 608.16/JJJ29

Topic: H.02. Human Cognition and Behavior

Title: Age differences in spatial navigation: Allocentric versus egocentric strategies

Authors: *M. FRICKE, O. L. BOCK
German Sport Univ., Koeln, Germany

Abstract: Spatial navigation is a complex cognitive ability, which can generally be divided into two components. One of them uses an ego perspective and includes the use of directional codes and landmarks along the way (e.g., "when I reach the pharmacy, I turn right"). It is referred to as "egocentric". The other component is independent of one's own body position and involves the use of a mental bird's eye view of the environment, called 'cognitive map'. This "allocentric" strategy is indispensable for finding shortcuts or ways around a roadblock.
Previous research showed that spatial navigation abilities decline with advancing age, prompting older adults to limit their physical and social activities and thus to reduce independence and quality of life. It has been argued that age-related decline affects mainly allocentric rather than egocentric navigation, but direct experimental evidence for this view is still missing. The present study was designed to provide such evidence.
Participants were 61 healthy volunteers (32 young, 18 - 35 years, 17 male; 29 older, 63 - 81 years, 16 male). They were tested in two game-like computer environments developed with Unreal Engine (Epic Games®). Both tests were presented in a virtual reality setting on a standard computer monitor. Participants had to find a goal by pursuing a route with 5 decision points, each with 4 branches. Test Ego provided only local landmarks (characteristic buildings in front of a featureless horizon), and test Allo only global landmarks (mountains and castles in the

distance, with all decision points looking alike). Participants performed each test eight times, with test order balanced between individuals. Performance was quantified as time to completion, distance covered and number of wrong turns.
Analyses of variance yielded significant interactions of the between-factor Age Group and the within-factor Test: older adults performed similarly to young ones on the egocentric test, but performed less well than young ones on the allocentric test. We thus now have direct experimental evidence that allocentric but not egocentric navigation declines in older age.

Disclosures: M. Fricke: None. **O.L. Bock:** None.

Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

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Program #/Poster #: 608.17/JJJ30

Topic: H.02. Human Cognition and Behavior

Support: This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie Actions, Individual Fellowship (MEMORAGE 702483) to IW.

Title: Hybrid foraging in healthy aging

Authors: *H. SCHILL[1,2], I. WIEGAND[3], C. SEIDEL[4], J. WOLFE[5]
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Abstract: "Hybrid foraging" tasks are those where observers (Os) look for multiple instances of multiple types of target; for example, rummaging through the Lego box for small red bricks, long blue bricks, and alligators. Wolfe et al. (2016) created an analogous task in the lab by teaching Os a set of N targets that they held in memory and asking Os to collect instances of those targets as quickly as possible from displays containing multiple instances. Os pressed a 'next' button whenever they wished to move to a new patch of targets and distractors. Wolfe et al. (2016) found that their young adults' decisions to move to the next 'patch' followed the Marginal Value Theorem (MVT) which states that foragers will leave a patch for a new one when the instantaneous rate of return from the current patch drops below the average rate of return over all patches. Previous literature has reported that healthy older adults (OA) often favor exploitative over explorative behavior (Chin et al. 2015), suggesting their behavior in a foraging task might be more conservative than what MVT would predict. Using the hybrid foraging task, we found that this is indeed the case: OA left the patch only after the instantaneous rate of return had fallen well below the average rate, which made their foraging behavior less efficient. With young

adults, response time increases in a logarithmic fashion with increases in memory set size. Interestingly, we found a very similar pattern in OA. OAs were slower overall, but they appeared to search through memory with no sign of a qualitative age-related decline. Within a patch, younger adults were also more likely than chance would predict to select another instance of the same item that they just selected. Thus, they tended to pick in 'runs.' This priming-related guidance to the previously selected target speeded foraging. OAs showed an almost identical tendency to pick up items in 'runs,' suggesting that these priming effects are preserved into older age. To conclude, OA performance on hybrid search tasks suggest that age deficits in foraging are ultimately due to strategic changes, while basic memory and visual search processes seem to remain largely intact.

Disclosures: H. Schill: None. **I. Wiegand:** None. **C. Seidel:** None. **J. Wolfe:** None.

Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 608.18/JJJ31

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R00-AG036848
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NIH Grant R01-AG-56535-02

Title: Age-related differences in executive function are mediated by white matter integrity and underlying white matter hyperintensity burden

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Abstract: White matter health is critically important for maintaining cognitive abilities in the aging brain. Degradation of white matter integrity has been associated with cognitive performance across many domains, but particularly with aspects of executive function (EF) and processing speed (PS). However, the impact of underlying brain and health factors, such as white matter hyperintensities and pulse pressure (PP), on overall white matter health is poorly understood. We explored these associations in a lifespan sample of 183 participants (20-94 years) using a cognitive task battery assessing EF and PS, combined with Diffusion Tensor Imaging (DTI) and T2 FLAIR scans. Demographic and health information were also collected, which included sex, education, and PP (calculated from multiple blood pressure readings). Fractional anisotropy (FA) was estimated in three white matter tracts: Superior Longitudinal Fasciculus, Inferior Fronto-Occipital Fasciculus, and Genu of the corpus callosum. White matter hyperintensity volume (WMHv) was estimated via a semi-automated lesion segmentation

pipeline. We used structural equation modeling to explore the relationship between age and cognition by investigating putative mediating factors of mean tract FA, WMHv, and PP, while also accounting for PS, a major component of EF. We specified a model in which age simultaneously predicted declines in EF and PS but was mediated by the serial progression of PP, WMHv, and white matter FA, with education and sex included as covariates. We found that FA in these tracts mediates the relationship between age and EF. Overall WMH burden, while not directly related to cognitive performance, predicts changes in tract FA, and indirectly mediates the relationship between age and EF. PP was not directly related to either WMH or FA; thus, the final fitted model included PP as a significant path from age only. Importantly, when reversing the originally estimated path to lead from FA to WMHv, the mediation was non-significant, indicating a temporal order of WMHv effects on tract FA. These results illustrate that while age leads to declines in both EF and PS, associations with EF are driven by both direct effects of fronto-parietal FA, and the effect of WMH on FA. Although white matter integrity alone is crucial to maintaining cognitive abilities in aging, these results suggest that WMH lesions play an important role in the neural changes that negatively impact cognition. Understanding the influence various insults have on white matter will help further disentangle key mechanisms in the aging brain underlying cognitive decline.

Disclosures: D.A. Hoagey: None. **L.T.T. Lazarus:** None. **K.M. Rodrigue:** None. **K.M. Kennedy:** None.

Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

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Topic: H.02. Human Cognition and Behavior

Support: N Persson: VR grant 2017-00281
L-G Nilsson: Grants D1988-0092, D1989-0115, D1990-0074, D1991-0258, D1992-0143, D1997-0756, D1997-1841, D1999- 0739
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L Nyberg: Wallenberg-scholar grant 2009

Title: HIPPOCAMPAL volume, and sub regions along the anterior-to-posterior axis contributes to maintenance of episodic recall and recognition over five years: Longitudinal findings from the Betula study

Authors: *N. PERSSON
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Abstract: Individuals maintaining memory functions may have preserved structural brain volumes and integrity. However, there is a need to further disentangle potential leading or lagging relationships in brain-cognition links over time. Furthermore, specific forms of memories for episodes (EM), as recall of verbal items (RC), and more associative memories as recognition (RN) of a piece of information, involving associating different stimuli as e.g. face with a name, may depend on distinct parts of the anterior-to-posterior hippocampal axis. How these specific forms of EMs: RC, and RN, relate to anterior (aHC) and posterior hippocampal (pHC) volumes over time is largely unknown. This study investigated longitudinal relations between hippocampal (HC) volumes, and EM, in 362 community-dwelling adults (52%♀; 20-80 yrs. at baseline, 223 returned at follow-up). A series of univariate and bivariate latent change score models were specified to assess, 1) mean change, and individual differences; and 2) leading and lagging relationships between 5-year changes in HC, and EM. Measurements included a wide range of EM tasks, and FreeSurfer derived volumes from the entire HC, as well as aHC, and pHC. Chronological age, sex, and years of education were treated as covariates. Maintained structural HC integrity at baseline slowed subsequent decline in RC, and RN. Larger baseline aHC volumes selectively slowed subsequent RN loss, and larger pHC volumes hampered 5-year RC decline. Volumes of aHC was not related to subsequent change in RC, nor was pHC linked to changes in RN. No support was found for reversed causality, across models. The covariates examined, age, sex, and education, had some cross-sectional influence, but only limited longitudinal effects. To exemplify, older adults showed smaller baseline HC, and greater volume loss, in addition to lower EM scores. Higher education was associated with greater baseline EM scores, but not change. The findings inform about potential relevance of distinct neural correlates along the anterior-to-posterior HC axis of RC, and RN. These findings may further serve as a base to inform interventions to maintain HC and EM, as HC atrophy, is a major vulnerability factor for conversion to Alzheimer's disease.

Disclosures: N. Persson: None.

Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 608.20/JJJ33

Topic: H.02. Human Cognition and Behavior

Support: DFG Grant SFB 940/2 B7

Title: Cost-benefit arbitration between model-free and model-based learning strategies in human aging

Authors: *F. BOLENZ[1], W. KOOL[2], A. M. F. REITER[1], S. J. KIEBEL[1], B. EPPINGER[3]
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Abstract: To efficiently meet the challenges of our complex world, people constantly have to decide on how much cognitive effort they invest into a task. For this purpose, they weigh up the costs of cognitive effort against its potential outcomes, a form of meta-control that has been linked to the dorsal anterior cingulate cortex (dACC; Shenhav et al., 2013, *Neuron*). While dACC is part of fronto-striatal circuits that undergo extensive functional and structural changes with human aging, the impact of these aging-related changes on meta-control remains poorly understood.
We tested younger and older adults on a sequential decision-making task that provides a measure of the relative impact of a simple but inflexible "model-free" learning mechanism and a cognitively more effortful but also more accurate "model-based" learning mechanism (Kool et al., 2017, *Psychol Sci*). Across trials, we manipulated reward magnitude (leading to different pay-offs of model-based behavior) and whether the state transition structure of the task was stable or subject to changes (leading to different costs for model-based behavior).
We replicate findings from previous studies that showed reduced model-based learning in older adults compared to younger adults. Moreover, as has been shown earlier, younger adults adapted their learning strategies to reward magnitude by increasing their reliance on model-based learning when rewards were temporarily amplified. However, this effect was only observed with stable state transitions, not when subjects had to adapt to changes in the state transition structure. In older adults we found no evidence for an increased reliance on model-based control as a function of reward magnitude in either of the transition conditions.
Our results show that ageing leads to reduced model-based control during learning and decision-making. Beyond this, we find evidence for reduced meta-control of learning strategies in older adults. This suggests aging-related difficulties in trading off cognitive effort against potential outcomes.

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Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

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Topic: H.02. Human Cognition and Behavior

Support: NSERC Discovery grant (Grant No. 418454-2013] awarded to ABP
Canadian Institutes of Health Research (CIHR) Operating Grant (Grant No. 126105) and Alzheimer's Society of Canada Research Program Grant (Grant No. 1435) awarded to MNR
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NSERC (PGS D) scholarship and Izaak Walton Killam Pre-Doctoral Scholarship awarded to FB

Title: Age-related differences in the variability of BOLD signal manifest differently across tasks, and influence information processing capacity

Authors: H. WANG[1], M. N. RAJAH[2], F. BURLES[1], S. PASVANIS[3], *A. B. PROTZNER[1]
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Abstract: Brain signal variability is an important measure of brain function that reflects information processing capacity and functional integrity. However, little is known about how age-related differences in variability are influenced by task, and how these differences relate to task performance. We measured variability with fMRI during encoding and retrieval phases of spatial and temporal source memory tasks in 128 healthy adults aged 19-76 yrs of age (mean age = 46.96 yrs, 85 females, mean EDU = 15.68 yrs). We quantified variability as the standard deviation (SD) of BOLD signal (Garrett et al., 2010), and studied the relation between BOLD SD, age, and task performance using a data-driven, multivariate analysis technique, Partial least squares (PLS, McIntosh et al., 1996). We examined 1) if there were age dependent differences in BOLD SD changes between conditions, and 2) if there were age and condition dependent differences in the association between BOLD SD and performance as measured by accuracy and RT. Based on previous findings that the effects of age and performance on brain activity were mainly memory phase (encoding versus retrieval) specific (Ankudowich et al., 2016, 2017), we expected that the influence of task on age-related differences in variability would be greatest for memory phase. We also expected to find age-associated increases in variability in subcortical regions, and decreases in neocortical regions, which would be associated with worse performance (Garrett et al., 2011; Guitart-Masip et al., 2016). Consistent with these expectations, we found age-related differences in brain signal variability between encoding and retrieval phases, but not between spatial and temporal conditions, or between easy and difficult versions of the source memory tasks. At encoding, variability increased with age in subcortical regions such as thalamus and parahippocampal gyrus, but decreased with age in the superior parietal lobule and postcentral gyrus ($p < .001$). Retrieval results differed from previous work, in that variability increased with age throughout the brain ($p < .001$). All age-related variability differences during encoding ($p < .001$) and retrieval ($p < .001$) were associated with longer response time and decreased accuracy. These results suggest that age-related brain changes in variability manifest differently across tasks, and affect the information processing capacity of the brain.

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Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 608.22/JJJ35

Topic: H.02. Human Cognition and Behavior

Support: R01AG034570

Title: Relationships between hippocampal volume, subjective cognition, and cognitive performance in healthy older adults

Authors: *L. FENTON[1], S. LANDAU[2], W. JAGUST[3]
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Abstract: Background: Subjective cognitive impairment may be an early manifestation of neurodegenerative disease. Therefore, it is important to understand the relationships between subjective cognition, objective memory, and neurobiological mechanisms of cognitive decline.
Objectives: Examine the relationship between hippocampal volume, subjective cognition, and objective cognitive performance in healthy older adults.
Methods: We compared offline and online subjective cognitive monitoring, episodic memory scores, average performance across 7 neuropsychological tests (Digit Span, CVLT, Visual Reproduction, Stroop, Verbal Fluency, Category Fluency, and Listening Span), and hippocampal volume of healthy older adults (N=166, M=69, F=97, mean age=73.69). Offline monitoring consisted of 2 questions asking subjects to assess their memory in relation to other people their age, and to themselves 20 years ago on a 4-point scale. Online monitoring occurred after the completion of each of the 7 tests by asking subjects to estimate their performance on a percentile scale in relation to other people. Accuracy of online monitoring was calculated by converting scores to percentile ranks, and subtracting subjects' average percentile score from their average estimated score. Sex, education, and age were controlled for across all analyses. Geriatric Depression Scale (GDS) scores were controlled for when looking at hippocampal volume, subjective cognition, and objective performance.
Results: As depressive symptoms (GDS) increased, subjects' subjective cognition ratings on both off-line and online measures significantly decreased ($p<.01$), as did their accuracy of online cognitive appraisals ($p<.05$). Subjects with higher online ratings had better performance on all 7 tests ($p<.01$), better episodic memory scores ($p<.01$) and marginally larger hippocampal volume ($p=0.06$). Hippocampal volume was significantly correlated with episodic memory scores ($r=.279$, $p<.01$), and the relationship between hippocampal volume and average performance on the 7 tests approached significance ($r=.146$, $p=.06$). Relationships between offline cognitive

monitoring, objective cognitive measures, and hippocampal volume were not significant. **Conclusions:** These findings indicate that, while depressive symptoms are associated with less accurate assessments of increasing subjective cognitive impairment, online assessments of cognition accurately reflect cognitive ability and accepted neurobiological mechanisms. Online cognitive self-assessment therefore reflects both accurate, biologically driven self-perception, and less accurate affective components.

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Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 608.23/JJJ36

Topic: H.02. Human Cognition and Behavior

Support: R00-AG-036818-05
R00-AG-036848-05
R01-AG-56535-02

Title: Cortical thickness mediates the relationship between the drd2 c957t polymorphism and executive function across the adult lifespan

Authors: *G. G. MIRANDA, K. M. RODRIGUE, K. M. KENNEDY
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Abstract: It is well-established that both brain structure and cognition are highly heritable and partly under genetic control. Several single nucleotide polymorphisms (SNP) that regulate dopamine have been shown to influence cognitive performance, especially executive function (EF). DRD2 C957T (rs6277), which influences postsynaptic D2 dopamine availability, negatively influences executive function performance in older and younger individuals, with C allele homozygotes (who have lower dopamine availability), performing more poorly than their T carrying counterparts (who have greater dopamine availability). It is unclear through which neural mechanisms this genetic predisposition exerts it influence on cognitive performance. Here, we sought to investigate whether the effect of DRD2 polymorphism on EF was due in part to reduced cortical thickness in target regions of dopaminergic pathways. A lifespan sample of 176 healthy participants aged 20-94 (DRD2 CC *n*=51, CT *n*=76, TT *n*=49) underwent MRI and cognitive sessions in which we obtained hi-res T1-weighted MPRAGE scans and multiple measures of executive functioning. Cortical thickness was estimated using FreeSurfer, visually inspected and manually edited, before extracting parcel thicknesses. Of the frontal and parietal

parcels, we found a significant effect of DRD2 on regional thickness in the anterior cingulate, superior parietal, and precuneus, where CC homozygotes had thinner cortex than T carriers. We formed a standardized z-score cortical thickness construct from these averaged regions. An EF construct was created from the average of Stroop, Wisconsin Card Sorting Task, Trails, and Verbal Fluency tests. Using these constructs, we specified a mediation model where DRD2 group (X) predicts EF performance (Y) through the mediation of cortical thickness (M), controlling for age (i.e., X-->M-->Y) using a bootstrap estimation approach with 5000 samples. We found that DRD2 significantly predicted cortical thickness and that cortical thickness significantly predicted executive function. Importantly, cortical thickness also demonstrated significant mediation of the association between DRD2 and EF. C carriers have thinner fronto-parietal cortex and this DRD2-related thinning was associated with poorer EF. These findings help elucidate the role of brain structure as an underlying contributor to the link between risk for lower dopamine availability and poorer executive function across the adult lifespan.

Disclosures: G.G. Miranda: None. **K.M. Rodrigue:** None. **K.M. Kennedy:** None.

Poster

608. Human Cognition and Behavior: Cognitive Aging III

Location: SDCC Halls B-H

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Program #/Poster #: 608.24/JJJ37

Topic: H.02. Human Cognition and Behavior

Support: NIA P30 AG019610
NSF GRFP

Title: Differential effects of healthy aging on directed and random exploration

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Abstract: The explore-exploit tradeoff is a fundamental behavioral dilemma faced by all adaptive organisms. Should we explore new options in the hopes of finding a better meal, a better house or a better investment vehicle for our savings, or should we exploit the options we currently believe to be best? Recently, we have shown that young adults solve the explore-exploit dilemma using a mixture of two strategies: “directed exploration”, in which a competition between information seeking and ambiguity aversion drives exploration by choice, and “random exploration”, in which adaptive behavioral variability drives exploration by chance. In addition, work in adolescents has found that directed, but not random, exploration increases with age between the ages of 12 and 18. In this work we investigated whether explore-exploit behavior continues to change in old age.

Our preliminary data from older adults (n = 29, ages 65-74) suggests that explore-exploit behavior continues to change throughout the lifespan. In particular, compared to 284 healthy younger adults (ages 18-22), these data suggest that healthy aging is associated with substantial changes in explore-exploit behavior. In particular, we found that older adults showed higher ambiguity aversion overall ($p < .0001$), suggesting that they were less likely to choose an unknown, exploratory, option in general. However, we also found that older adults could overcome this ambiguity aversion through *increased* directed exploration in situations where exploration had value ($p < .05$). In contrast to this increase in directed exploration, we found a trend towards reduced random exploration, ($p = 0.07$) in older adults. The finding that ambiguity aversion changes as a function of age is consistent with previous findings in the decision-making literature. However, it is surprising that directed exploration appears to continue increasing into old age. One reason for this could be a possible relationship between directed exploration and temporal discounting which, at least in theory, suggests a negative relationship between discounting and directed exploration. It is well known that older adults discount future rewards less than young people and such an relationship could explain our effect. The finding that directed exploration has a different age dependence to random exploration is consistent with a number of recent findings suggesting that directed and random exploration rely on dissociable systems in the brain.

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Poster

608. Human Cognition and Behavior: Cognitive Aging III

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Program #/Poster #: 608.25/JJJ38

Topic: H.02. Human Cognition and Behavior

Support: NSF Grant DGE-1650044

Title: The impact of habitual sleep quality on memory-related neural oscillations in young and olde adults

Authors: *E. HOKETT[1], A. L. DUARTE[2]
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Abstract: Research has shown that sleep is essential for memory consolidation. However, the effects of habitual sleep quality on memory performance are unclear, especially regarding age-related differences in brain function. We hypothesized that sleep quality would be significantly related to retrieval-related EEG and memory performance across age groups. We investigated this relationship in young and older adults using one week of sleep data collection, a paired-

associate memory task, and retrieval-related electroencephalography (EEG). We found that memory accuracy was positively correlated with sleep quality across age groups. In addition, we found relationships between measures of sleep quality and measures of oscillatory power that supported memory performance. For older adults, there was a negative trend for sleep fragmentation (SF) and retrieval-related theta synchronization for associative hits, an index of recollection-based memory. That is, lower SF was correlated with greater theta synchronization. Both SF and theta power correlated with memory accuracy such that lower SF and greater theta power supported better memory. Moreover, we found a significant relationship across age with alpha desynchronization and memory accuracy; alpha desynchronization has been found to be associated with memory retrieval. We also found trends that suggest a relationship between sleep quality and alpha desynchronization. While our data suggests that sleep quality is important for memory performance in both young and older adults, older adults may be particularly sensitive to sleep quality. Prior research has shown that lifestyle factors such as maintaining good sleep quality and moderate physical activity may improve memory in older adults. Consistent with these findings, we found that sleep quality was positively associated with associative memory. This study extends the current literature with the finding that good sleep quality may support greater functional activity in neural correlates important for memory accuracy.

Disclosures: E. Hokett: None. **A.L. Duarte:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 609.01/JJJ39

Topic: H.03. Schizophrenia

Title: Co-localization of eqtl and gwas in schizophrenia

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Abstract: Schizophrenia is a debilitating psychiatric condition affecting roughly 0.7% of adults. It is highly heritable and polygenic. A recent genome-wide association study identified 145 loci that confer risk for schizophrenia, but the underlying mechanisms remain largely unknown. Our overarching goals in this study are to identify mechanisms that underlie genetic risk by investigating the role of disease related SNPs on regulating gene expression. Dorsolateral prefrontal cortex (DLPFC) has been demonstrated to be associated strongly with schizophrenia. In this study, we use RNA sequencing and whole exome sequencing data generated from 121 human postmortem brain DLPFC samples (85 males and 36 females; 14 African American and 107 Caucasian; 23-70 years old, mean = 58, SD = 10) originating from tissue collections of the

GTEx consortium. Gene counts were based on GENCODE Release 19 (GRCh37.p13), 56,203 transcripts in total. Genes with average RPKM < 0.01 were excluded. A total of 153,970 SNPs were retained after filtering SNPs that did not fulfill HWE at p-value < 1e-50 and MAF < 0.01. We modeled expression after transforming with log2 with an offset of 1 by liner regression. We performed eQTL analyses using the MatrixEQTL by allowing for a 1MB window around each SNP, and adjusting for age, sex, race and RIN. A total of 5,753,977 eQTLs were identified, in which 545 genes are strongly associated with SNPs (p-value < 1e-8). Then, we performed overrepresentation enrichment analysis using WebGestalt. Interestingly, pathways involved in human metabolism are significant: glutathione metabolism (PValue=2.95e-04; FDR=8.95e-02), drug metabolism (PValue=9.86e-04; FDR=0.102), and metabolism of xenobiotics by cytochrome P450 (PValue=1.27e-03; FDR=0.1.02). Using the gene sets, our human lifespan expression analysis across 6 brain regions by using BrainSpan datasets showed that they have a low abundance in fetus, but higher abundance in late infancy stage across amygdala, cerebellum, cortex, hippocampus, and striatum. Another abundant event occurs during adulthood across the hippocampus and striatum. To assess gene expression alteration by schizophrenia risk loci, we co-localized the 5 million eQTL results and 8 million schizophrenia GWAS summary statistics and obtained 4 million eQTLs that could be used for subsequent analyses. Six candidate genes (*U3*, *AL022393*.7, *HCG4*, *HLA-DQB1*, *HLA-DQB2* and *CYP2D6*) were identified after using stringent filtration on eQTL at p-value < 1e-8 and GWAS at p-value < 1e-8 for further assessment. In summary, our findings provide new targets for modeling schizophrenia risk at the molecular level.

Disclosures: L. Ma: None. **S. Chetty:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 609.02/JJJ40

Topic: H.03. Schizophrenia

Support: NIH Grant GM119831

Title: Parallel enhancer analysis in mouse brain to characterize regulatory variants in development and disease

Authors: *J. L. HAIGH[1], L. SU-FEHER[1], I. ZDILAR[1], K. J. LIM[1], D. M. QUINTERO[1], S. J. MORSE[1], T. W. STRADLEIGH[1], K. HINO[2], S. SIMO[2], L. C. BYRNE[3], A. S. NORD[1]
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Abstract: Enhancers are cis-regulatory elements with the capacity to promote gene expression both spatially and temporally. Their activity is critical in the development of the brain and sequence variation in these regions has been linked to neurological disorders including autism spectrum disorder, epilepsy and schizophrenia (SCZ). The advancement of massively parallel reporter assays has enabled the functional characterization of enhancers both in vitro and in vivo. We adapted one such assay, STARR-seq, for in vivo delivery into the mouse brain. In a plasmid using a minimal promoter the candidate sequence is placed in the 3' untranslated region of a reporter gene, as such enhancers can be identified by RNA-seq since they will be transcribed if active. We developed a pilot library of genomic candidates containing common non-coding sequence variants associated with epilepsy and SCZ. The library was delivered to postnatal mouse brain via adeno-associated virus, and genomic DNA and total RNA collected. Results from preliminary studies suggest this method is able to identify sequences capable of acting as enhancers in in vivo mouse brain. We have further developed this method to test enhancer function at embryonic time points using in utero electroporation (IUE). A SCZ-associated regulatory element in the intron of the calcium channel subunit gene CACNA1C was validated via IUE, showing that it drives gene expression in E17.5 cerebral cortex. These methods allow us to rapidly screen libraries of DNA sequences for enhancer activity in vivo, with the further potential to identify whether these variants contribute to altered gene expression in the brain. Such functional examination of enhancers will be critical toward understanding how non-coding sequence variation in human populations contributes to brain development and neurological and psychiatric disorders.

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Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 609.03/JJJ41

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant MH111099
NIH Grant GM076990

Title: Regulatory changes or alterations in cellular proportions? Re-evaluation of pathways affected in psychiatric disorders in light of cell type proportion changes

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Abstract: High-throughput expression techniques are widely used to study neuropsychiatric and neurodevelopmental disorders. A major challenge of these studies is understanding the functional impact of the identified genes and the biological pathway underlying the observed changes. At the current state of the field, the majority of studies are based on bulk tissue samples, and it was previously noted that fluctuations in cellular abundance can induce a pronounced transcriptional signature in bulk tissue data. Thus, it is crucial to understand which part of the transcriptional pattern is driven by changes in cellular abundance (e.g, due to cellular death or inflammation) and which part can be attributed to regulatory events.
We have previously reported marker-genes for multiple cell-types based on NeuroExpresso, a database of brain cell-type transcriptomes. Here, we used these genes to estimate changes in cellular abundance in 11 bulk-tissue expression datasets, representing six independent cohorts of subjects with bipolar disorder and schizophrenia. We next performed differential expression and enrichment analyses in these datasets, accounting for the estimated cellular proportion changes. We observed a robust decrease in marker-gene profiles (MGPs) of fast-spiking PV cells and an increase in marker-gene profiles of astrocytes in subjects with both psychiatric disorders. Based on analysis of mouse and human developmental data, we demonstrate that the changes in fast-spiking PV interneurons are not likely to represent defects in maturation of these cells.
We next looked at the correlation of genes previously reported to be affected in brains of subjects with schizophrenia with the estimated proportion of astrocyte and fast spiking PV cells. We found that the majority of the over- and underexpressed genes are highly correlated with astrocyte and fast spiking PV cell MGPs, respectively. Moreover, while mitochondrial genes were highly enriched among the downregulated genes in data not adjusted for MGPs, this enrichment was not observed when MGPs were included in the model. This could be explained by overexpression of mitochondrial genes in fast spiking PV cell transcriptomes, as we report based the cell-type specific data in NeuroExpresso.
Our results suggest that the pathophysiology of bipolar-disorder and schizophrenia involves changes in astrocytes and fast-spiking PV cells and highlights the need to account for cellular changes in bulk tissue expression data.

Disclosures: L. Toker: None. **O. Mancarci:** None. **S. Tripathy:** None. **P. Pavlidis:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 609.04/JJJ42

Topic: H.03. Schizophrenia

Support: Stanley Center for Psychiatric Research at Broad

Title: A protein interaction network in human neurons of risk factors incriminated by genetics in schizophrenia

Authors: *E. NACU[1], A. KIM[2], W. CROTTY[1], E. MALOLEPSZA[2], N. PETROSSIAN[2], K. LILLIEHOOK[2], J. JAFFE[2], K. EGGAN[1,2], K. LAGE[2,1]
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Abstract: Genetic studies of psychiatric disorders have provided us with putative risk factors implicated in those disorders. A 2004 GWAS study of schizophrenia has implicated 108 gene loci in the disease with some of the loci containing only a single gene and some containing multiple genes, giving estimates of more than 400 putative risk genes. Given this plethora of putative risk factors, we decided to build an interaction network of the risk proteins with the aims of (1) identifying molecular interactors of each of the risk factors, (2) uncover risk factors that are connected through their interactors and thus potentially are part of a pathological pathway, and (3) discover risk factors from multi-genic loci that are part of the network and thus potentially the relevant gene for the disease in that locus. To build the network we took the approach of performing co-immunoprecipitaton (co-IP) of risk proteins from human patterned induced Ngn2 neurons (piNs) that are cortical glutamatergic excitatory-like. We started our study with the schizophrenia GWAS hits, prioritizing risk proteins based on whether they were in a single gene locus, have been implicated in psychiatric disorders in other studies, and were expressed in iNgn2 neurons. We have performed 17 IPs of 6 different risk proteins at different time points of maturations of piNs. As an example, we identified a total of 304 interactors of CACNA1C as compared to 54 of the known interactors of CACNA1C found in InWeb. Furthermore, among the 304 interactors we identified 21 interactors implicated in other psychiatric or developmental disorders.We then created an interaction network of all identified interactors, and integrated our network with genes located in schizophrenia GWAS loci to uncover interesting direct connections between putative schizophrenia risk factors. One exciting connection is between CACNA1C and C4 that we are currently pursuing.

Disclosures: E. Nacu: None. **A. Kim:** None. **W. Crotty:** None. **E. Malolepsza:** None. **N. Petrossian:** None. **K. Lilliehook:** None. **J. Jaffe:** None. **K. Eggan:** None. **K. Lage:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

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Topic: H.03. Schizophrenia

Support: NIH Grant MH107916
LIFE Foundation
UCGNI Pilot Grant

Braun Foundation

Title: Synaptic protein-protein interactions in schizophrenia

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Abstract: Background: Mounting genetic, proteomic, and biochemical evidence indicate rare mutations, abnormal protein-protein interactions, and altered synaptic signaling pathways may be at the root of the severe phenotypes seen in patients with schizophrenia. A major area yet to be fully elucidated is the understanding of the complex synaptic protein-protein interactions in normal and pathological conditions. Increasing focus has been directed toward the NMDAR and PSD-95 protein-protein interactomes, members of which are identified as high-yield risk factors for the development of schizophrenia. PSD-95 is the most abundant scaffolding protein in the PSD, with well characterized protein-protein interactions that modulate the trafficking of glutamate receptors and other PSD constituent proteins relevant for synaptic plasticity.
Methods: Magnetic dynabeads conjugated with anti-PSD-95 antibody were used to isolate PSD-95 protein complexes from 10 control and 10 schizophrenia dorsolateral prefrontal cortex samples. The complexes were eluted from the beads and processed for data-independent acquisition (DIA) LCMS/MS analysis on an ABSciex 5600+ mass spectrometer.
Results: Bioinformatic analyses revealed increased DNA and RNA processing in schizophrenia. Additionally, the data show upregulation of protein and glucose metabolism along with increased signaling through PKA, PKC, SRC, MAPK1, and CSNK2. Pathway analyses indicate integrin signaling, glycolysis, synaptic vesicle trafficking, and cytoskeletal rearrangement are all dysregulated in schizophrenia.
Conclusions: Our data reflect cutting-edge efforts in the field of psychiatry on schizophrenia research. These data indicate significant abnormalities of synaptic protein-protein interactions which indicate the disruption of important signaling, trafficking, and metabolic pathways for normal neurological function.

Disclosures: A. Funk: None. **G. Labilloy:** None. **K. Greis:** None. **J. Meller:** None. **R.E. McCullumsmith:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

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Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 609.06/JJJ44

Topic: H.03. Schizophrenia

Support: K23MH079498

Title: Effects of risperidone on the proteome in olfactory cells from individuals with schizophrenia and at-risk for the illness

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Abstract: Olfactory dysfunction has been well characterized in patients with schizophrenia and more recently in younger subjects who are at-risk for developing psychosis. Molecular underpinnings of olfactory dysfunction, however, are largely unknown in schizophrenia or during the prodromal period. Previously, we examined odorant and neurotransmitter induced receptor-G protein coupling in olfactory neuroepithelial (ON) cells from subjects with schizophrenia and found G protein activation decreased in response to odorants but increased in response to dopamine. Subjects at at- risk for the illness did not show similar changes in G protein signaling yet showed alterations in molecules critical for odorant signaling. In this study we examined the expression of proteins that are enriched in synapse, mitochondria and signaling molecules using quantitative proteomics in vitro ON cells derived from ten schizophrenia and 8 at-risk subjects and their matched controls and tested the effects of antipsychotic treatment. Method: Olfactory neuroepithelial cells from eight antipsychotic treatment free at- risk subjects and ten schizophrenia subjects, each with controls matched for age and sex were examined. Cell cultures from all groups were treated with and without 50nM for risperidone for 7 days. Tenμg of cellular extracts after exclusion of nuclei were mixed with [13C6]lysine-labeled internal standards. Samples were trypsin-digested, and processed for LC-SRM/MS on a triple quadrupole mass spectrometer for 200+ proteins. Peak areas for “light” endogenous peptides and “heavy” standardpeptides were calculated, and ratios (l/h) between the two were used as dependent variables. Between group differences were examined for the effects of diagnosis as well as for risperidone treatment. In patients with schizophrenia, risperidone treatment significantly altered peptides representing 54 proteins and while it altered 48 proteins in control subjects. Interestingly, risperidone treatment of the same concentration and duration induced changes in a much smaller number of proteins in cell cultures from at-risk subjects and their matched controls. These together suggests differential responses to proteins expression between patients with schizophrenia and at-risk for the illness.

Disclosures: K. Borgmann-Winter: A. Employment/Salary (full or part-time):; University of Pennsylvania. 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.; NIMH. **M. Dsouza:** A. Employment/Salary (full or part-time):; University of Pennsylvania. **S. Bandyopadhyay:** A. Employment/Salary (full or part-time):; University of Pennsylvania. **N. Mirza:** None. **M. Calkins:** A. Employment/Salary (full or part-time):; University of Pennsylvania. **B. Turetsky:** A. Employment/Salary (full or part-time):; University of Pennsylvania. **C. Hahn:** A. Employment/Salary (full or part-time):; University of Pennsylvania. 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.; NIMH.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 609.07/JJJ45

Topic: H.03. Schizophrenia

Title: Abnormalities of glucose metabolism in schizophrenia

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Abstract: Schizophrenia affects approximately 1% of the world's population and is a severe mental illness associated with cognitive deficits. Alterations in the brain in schizophrenia include changes in glutamate and GABA neurotransmission, in a manner that suggests diminished energy supply in these systems. Based on prior work in our laboratory, we extended our studies of glycolysis in postmortem brain to include molecules that had not previously been evaluated in this illness. We used QPCR to assess mRNA expression for hexokinase 2 (HXK2), phosphoribosyl pyrophosphate synthetase 2 (PRPS2), and phosphorylase kinase regulatory subunit beta (PHKB) in the dorsolateral prefrontal and anterior cingulate cortices of subjects with schizophrenia (n = 16) and normal controls (n = 16). We also assessed expression of these transcripts in an animal model of schizophrenia, the GluN1 knockdown mouse. We found increased expression of HXK2 (35%, $P < 0.05$), PHKB (40%, $P < 0.05$), and PRPS2 (50%, $P < 0.05$) in the frontal cortex of the GluN1 knockdown mouse compared to wild type animals. Expression levels for these transcripts in schizophrenia will be presented. We will also present correlations between clinical dementia ratings (CDR) and mRNA expression. Our data inform the hypothesis that glycolytic pathways are dysregulated in schizophrenia, and offer insights for an animal model of schizophrenia that may be utilized to further explore the pathophysiology of this illness.

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Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

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Program #/Poster #: 609.08/JJJ46

Topic: H.03. Schizophrenia

Support: MH107487
MH094445

Title: Bioinformatic analysis of bioenergetic changes in schizophrenia

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Abstract: A growing body of evidence suggests abnormal bioenergetic function in chronic schizophrenia. Bioinformatic analyses can address important biological questions without using valuable resources, offering insights on the connectivity of biological networks in human disease. We examined glycolytic pathways in pyramidal neurons and astrocytes in the dorsolateral prefrontal cortex in control and schizophrenia subjects (n=16/group) using laser capture microdissection coupled with quantitative real-time polymerase chain reaction (t-test analysis). We next built a disease signature in the Library of Integrated Network-based Cellular Signatures web portal (iLINCS) based on selectively downregulated glycolytic enzymes in schizophrenia. We developed a discovery based workflow to identify of drug perturbagens likely to “reverse” this disease signature. Finally, we administered a candidate drug to the GluN1 knockdown model of schizophrenia (n=4-9 per group) and examined sensorimotor gating, social and anxiety related behaviors, locomotor activity, and executive function. Behavior data were analyzed using 2-way ANOVA with Bonferonni multiple comparison corrections. We found a decrease in mRNA expression of several glycolytic enzymes in pyramidal neurons in schizophrenia (phosphofructokinase muscle ($p=0.003$, 22%), phosphofructokinase liver ($p=0.010$, 27%), glucose phosphate isomerase ($p=0.015$, 26%)). We identified 12 unique drug perturbagens likely to reverse the schizophrenia signature in iLINCS. Of these perturbagens, PPAR agonists presented as promising therapeutic targets. We found that administration of the PPAR agonist pioglitazone in GluN1 knockdown animals selectively restored explicit memory ($p<0.01$). Taken together, these analyses build upon previous reports of glycolytic defects in schizophrenia, and suggest possible mechanisms to restore these deficits and cognition include PPAR agonist intervention.

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Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 609.09/JJJ47

Topic: H.03. Schizophrenia

Title: Exploratory use of machine learning to model metabolic outcomes based on genetic risk factors in patients with schizophrenia

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Abstract: Metabolic symptoms adversely affect overall health and shorten life expectancy in schizophrenia. The relative contributions of genetics, behavior, and antipsychotic drugs to metabolic risk are not well-understood. We modeled risk of various metabolic outcomes based on genetic, demographic, and clinical covariates using data collected from 306 patients recruited from the Freedom Trail Clinic at Massachusetts General Hospital. Classification trees were used to group patients into homogeneous metabolic risk categories. Classification tree analysis is a nonparametric statistical method that handles variable selection among highly correlated covariates, can accommodate complex interactions among covariates, and provides easily interpretable results in the form of a decision tree. To avoid overfitting from this small data set, we selected the most parsimonious classification tree that was within 1 standard error of the lowest error overall. Single Nucleotide Polymorphisms (SNPs) identified as putative genetic risk factors for diabetes by a previous genome-wide association study emerged as useful covariates in the grouping of patients according to risk for abdominal obesity (high waist circumference), chronic hyperglycemia (high glycated hemoglobin), and insulin resistance (according to homeostatic model assessment). The allelic variants associated with diabetes risk in previous studies also were associated with risk in our study, indicating that genetic risk for metabolic disease follows a similar pattern in the special sample of antipsychotic-treated schizophrenia patients as in the general population. In the classification tree for risk of abdominal obesity, a SNP allowed for further classification of patient risk with respect to different classes of antipsychotic drugs. Thus, our results from this exploratory study suggest that genetic screening may prove useful for personalized clinical decision making and treatment management in the

context of schizophrenia. A larger patient sample will be required to establish and validate a robust clinical decision-making tool.

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Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

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Topic: H.03. Schizophrenia

Support: USPHS grant P50-MH103222

Title: Inhibition of brain and liver kynurenine aminotransferase ll activity by N-acetylcysteine in rodent, pig and human

Authors: *K. V. SATHYASAIKUMAR[1], T. BLANCO AYALA[1], A. E. S. FOO[1], M. A. R. THOMAS[1], L. S. PIDUGU[2], R. SCHWARCZ[1]
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Abstract: Kynurenic acid (KYNA), a metabolite of the kynurenine pathway of tryptophan degradation, is increasingly understood to play an important role in the mechanism(s) underlying normal and abnormal cognitive processes, most likely by acting as an antagonist of α7 nicotinic and NMDA receptors. Specifically, elevated KYNA levels are detrimental in psychiatric diseases such as schizophrenia. KYNA is synthesized from its immediate precursor kynurenine - either by non-enzymatic oxidation or through irreversible enzymatic transamination by kynurenine aminotransferases. In the mammalian brain, kynurenine aminotransferase II (KAT II) is the principal enzyme responsible for the neosynthesis of rapidly mobilizable KYNA. KAT II therefore constitutes an attractive target for pro-cognitive interventions (Schwarcz et al., 2012). N-acetylcysteine (NAC), a brain-penetrant drug with pro-cognitive efficacy in humans, including individuals with schizophrenia, has been proposed to exert its actions by increasing the levels of the endogenous anti-oxidant glutathione (GSH) in the brain (Steullet et al., 2016). We now examined a possible alternative mechanism of NAC action, namely KAT II inhibition. Using a well-established assay (Sathyasaikumar et al., 2013), we first tested the effect of NAC on KAT II activity in liver and brain tissue homogenates from mice, rats, pigs and humans *in vitro* and observed IC_{50} values in the high micromolar to the low millimolar range. Using pure human recombinant KAT II protein, NAC was found to inhibit enzyme activity with an IC_{50} of ~500 μM, while GSH was approximately 40 times less potent (IC_{50} >20 mM). By microdialysis in the medial prefrontal cortex (mPFC) of unanesthetized adult rats, we next examined the effect of

NAC on the neosynthesis of KYNA from peripherally administered kynurenine (50 mg/kg, i.p.) *in vivo*. To this end, NAC (20 mM) was locally applied by reverse dialysis for a period of 6 h. 120 min after starting the perfusion, kynurenine was administered systemically while NAC perfusion continued for the remaining 4 h. In separate animals, NAC was administered systemically twice (500 mg/kg, i.p., each; 120 and 60 min before the administration of kynurenine). NAC reduced the *de novo* production of KYNA from its immediate precursor by ~45% and ~50%, respectively, in the two experimental paradigms. Taken together, these results raise the possibility that NAC exerts its neurobiological effects at least in part by reducing cerebral KYNA levels via KAT II inhibition.

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Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

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Topic: H.03. Schizophrenia

Support: USPHS grant P50-MH103222

Title: Effects of acute tryptophan depletion on blood kynurenic acid concentrations and reinforcement learning performance in individuals with schizophrenia

Authors: *F. M. NOTARANGELO, J. A. WALTZ, M. A. R. THOMAS, K. V. SATHYASAIKUMAR, Y. MURTAZA, A. K. WELLS, R. R. RUIZ, R. SCHWARCZ
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Abstract: An excess of kynurenines has been associated with cognitive dysfunction in schizophrenia (SZ) and other forms of mental illness. Because over 95% of dietary tryptophan is metabolized into kynurenines, such as L-kynurenine ("kynurenine") and kynurenic acid (KYNA), acute tryptophan depletion (ATD) is one potential method for manipulating kynurenine levels in human subjects. Although studies have revealed effects of ATD on learning and memory, it is not known if those effects stemmed from changes in the levels of kynurenines or serotonin, and ATD has so far been used in only a small number of studies involving SZ patients. Using a double-blind within-subject crossover design, we administered 16 SZ patients both an ATD challenge and a balanced (BAL) amino acid load (in two separate sessions, at least a week apart, in randomized order). In each session, we collected samples of saliva and blood at baseline, 90 and 180 minutes after mixture consumptions and measured tryptophan, kynurenine, KYNA and the serotonin metabolite 5-hydroxyindoleacetic acid. Participants also performed a

probabilistic reinforcement learning (PRL) task 180 minutes after administration. Following a training phase, in which two Gain stimulus pairs (involving either positive or neutral feedback) and two Loss-avoidance stimulus pairs (involving either negative or neutral feedback) were presented in an interleaved fashion for 160 trials, participants indicated their valuation of the stimuli in the absence of feedback in a Test/Transfer phase. We found that SZ patients exhibited better PRL performance under ATD [$F(1,15) = 7.014$, $p = 0.018$] than after consuming the BAL ("tryptophan-loading") drink. Furthermore, PRL performance following consumption of the BAL drink correlated significantly ($r = -0.540$, $p = 0.031$) with the intra-session change in KYNA levels, such that patients showing the worst performance displayed the greatest KYNA increases. The performance advantage shown by patients under ATD also correlated significantly ($r = 0.515$, $p = 0.041$) with the full dynamic range of KYNA levels, such that patients experiencing the greatest performance advantage showed the greatest KYNA dynamic range. These results support the idea that acute modulation of kynurenine levels in the body has subtle effects on cognitive performance, with patients deriving benefits from reductions in KYNA levels. These results point to the kynurenine pathway in the brain as a potential target for therapeutic agents.

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Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

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Program #/Poster #: 609.12/JJJ50

Topic: H.03. Schizophrenia

Support: P50 MH103222

Title: Prenatal THC exposure permanently disturbs kynurenic acid and glutamate levels and amplifies the responsivity to an acute kynurenine challenge in the rat prefrontal cortex

Authors: *S. BEGGIATO[1], L. FERRARO[1], R. SCHWARCZ[2]
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Abstract: Throughout the world, cannabis remains one of the most widely used illicit drugs during pregnancy (Porath-Waller, 2015). The main psychoactive component of marijuana (delta9-tetrahydrocannabinol, THC) passes through the placenta, and its use is correlated with early physiological effects in the offspring. Neurobehavioural and cognitive impairments have been reported in several longitudinal studies on children and adolescents prenatally exposed to

marijuana (Calvigioni et al., 2014), and a link to psychiatric disorders has been proposed (Jutras-Aswad et al., 2009; Mathews et al., 2014). Prenatal exposure to cannabinoids induces cognitive deficits in rat offspring (Ferraro et al., 2009) and is associated with alterations in cortical/hippocampal glutamate and GABA levels (Antonelli et al., 2005, Beggiato et al., 2017). Interestingly, the deleterious effects of cannabinoids on cognitive functions are similar to those observed in adult rats prenatally exposed to (L)-kynurenine (KYN), which is the direct bioprecursor of kynurenic acid (KYNA), a neuroactive metabolite of tryptophan degradation (Pocivavsek et al., 2014). We therefore investigated whether alterations in KYNA levels in the rat brain might play a role in the long-term consequences of prenatal cannabinoid exposure. Pregnant Wistar rats were treated daily with THC [5 mg/kg or vehicle (sesame oil) by oral gavage] from gestational day (GD) 5 through GD 20. One adolescent [postnatal day 35-45] and one adult male rat per litter was then used to determine the extracellular levels of KYNA and glutamate before and after a challenge with KYN (5 mg/kg i.p.) by in vivo microdialysis in the medial prefrontal cortex (mPFC). Compared to vehicle-treated controls, extracellular basal KYNA levels were higher in adolescent and adult rats that had been prenatally treated with THC ($p<0.01$; $p<0.05$, respectively). These rats also had lower extracellular glutamate levels than respective controls ($p<0.01$; $p<0.05$, respectively). Following a challenge with KYN, extracellular KYNA levels increased in both adolescent groups (i.e. vehicle- and THC-treated; $p<0.05$) Interestingly, this effect was more pronounced in adult rats which had been prenatally exposed to THC. KYN also caused a trend towards a reduction in extracellular glutamate levels in vehicle-treated adolescent and adult rats. We propose that these permanent alterations in KYNA and glutamate signalling in the mPFC of prenatally THC-exposed rats could be relevant for cognitive dysfunction. Our results are also in line with the hypothesis that a “double-hit” may precipitate psychiatric disorders such as schizophrenia later in life.

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Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

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Program #/Poster #: 609.13/JJJ51

Topic: H.03. Schizophrenia

Title: Perivascular and putative parenchymal macrophages are increased in people with schizophrenia who also demonstrate signs of cortical inflammation

Authors: *H. Q. CAI[1,2], V. S. CATTS[1,2], M. J. WEBSTER[3], C. S. WEICKERT[1,2]
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Abstract: Background: A proportion of individuals with schizophrenia are in an elevated inflammatory state and express increased levels of intercellular adhesion molecule-1 (ICAM1) in the cortex. ICAM1 is involved in leukocyte transmigration and the elevation found in the prefrontal cortex in schizophrenia suggests more peripheral immune cells could adhere to the brain endothelium and potentially enter the brain. The aim of this study was to investigate if immune cells can be identified in prefrontal cortex of people with schizophrenia using immunohistochemistry for macrophages.
Methods: Gene expression for a macrophage marker CD163 (cluster of differentiation 163) and for a microglia marker, IBA1, was measured using qPCR in the prefrontal cortex of 37 people with schizophrenia and 37 controls. We further defined "high inflammation" and "low inflammation" schizophrenia and control subgroups based on mRNA expression of inflammation-related genes in the prefrontal cortex. Fresh frozen sections of orbital frontal cortex (14µm) were obtained from 38 schizophrenia and 38 control cases. 3,3-Diaminobenzidine immunohistochemistry was used to localize CD163 while immunofluorescence was used to determine the anatomical relationship between CD163+ cells and collagen-IV, a vascular membrane marker. Immunoreactivity was qualitatively assessed blind to diagnosis by microscopically scanning an entire bank of the gyrus rectus.
Results: IBA1 mRNA levels did not differ between diagnostic groups or inflammatory groups. CD163 mRNA did not differ according to diagnosis, but was elevated in "high inflammation" schizophrenia compared to "low inflammation" schizophrenia and controls. Immunofluorescence double labelling confirmed CD163+ cells occur in blood vessels, the perivascular space and on the parenchymal side of the lumen in both schizophrenia and control brains. CD163+ cells were present in the parenchyma in close association with neurons and not clearly associated with any blood vessels in 6% of "low inflammation" controls, 9% of "low inflammation" schizophrenia cases and 43% of "high inflammation" schizophrenia brains.
Conclusion: We provide evidence that CD163+ macrophages are elevated and are more frequently found in the parenchyma in people with schizophrenia who also have increased cytokines. As CD163+ macrophages were found away from blood vessels, they may be capable of infiltrating the parenchyma and interacting directly with neurons. These peripheral immune cells residing in the cortex, in addition to microglia, may be producing inflammatory factors to further drive the inflammatory cascade by signalling between astrocytes and the endothelium.

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Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

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Topic: H.03. Schizophrenia

Support: NRF-2015M3C7A1030964

Title: Topographic biomarkers reveal defective neurovascular units in schizophrenia

Authors: *S. YEO, J. YOON, H.-J. JUNG, Y. CHOI, D. KIM, S. CHOI, Y. CHOE
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Abstract: Schizophrenia (SZ) is known as developmental alterations of synapse structures that lead to the deterioration of the neural circuit functions. Thus, drugs targeting the disease have been based on studying several neuron-specific candidate genes such as Disc1, Shank3 and Ube3b. To expand our understanding of SZ, we questioned abnormal subcellular targeting of functional proteins might represent the underlying mechanism for the disease. To reveal the topographic proteomic landscape and subcellular protein localization in SZ neurons, we utilized human SZ patient derived-olfactory epithelial cells (hOE). hOE are easy to obtain, cultivate, well polarized, and most importantly differentiate into neurons during development. We compared proteomic profiles of hOE derived from normal donors and SZ donors. To categorize subcellular localization of proteins, we analyzed fractionated hOE proteins. Interestingly, several neurovascular proteins showed significant changes in their subcellular localization. These neurovascular protein expressions were also defective in Disc1 mutant mouse brains. These results imply that the abnormal development of neurovascular structures may contribute to the SZ neural dysfunction.

Disclosures: S. Yeo: None. **J. Yoon:** None. **H. Jung:** None. **Y. Choi:** None. **D. Kim:** None. **S. Choi:** None. **Y. Choe:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 609.15/JJJ53

Topic: H.03. Schizophrenia

Support: FAPESP 2013/10350-9

Title: Plasma metabolites on first-onset psychosis: Schizophrenia and bipolar disorder biomarkers

Authors: *H. P. JOAQUIM, A. C. COSTA, L. L. TALIB, W. F. GATTAZ
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Abstract: Background: Schizophrenia (SCZ) and bipolar disorder (BD) are severe psychiatric disorders and share many characteristics and symptoms since the first-onset. Identify molecular biomarkers for psychiatric disorders can assist in the diagnosis of disease and treatment and monitoring of patients. **Methods:** Plasma metabolites were quantified with a targeted quantitative and quality controlled metabolomics approach using the AbsoluteIDQ® p180 Kit (BIOCRATES Life Science) followed by mass spectrometer operating in the MRM mode. Data analysis was performed using the MetIDQ software (Biocrates) and Metaboanalyst version 3.0. **Results:** 37 metabolites were different between the groups: 5 Lyso- phosphatidylcholines, 11 phosphatidylcholines, 6 acylcarnitines, 1 sphingomyelin, 10 amino acids and 4 biogenic amines. Analyzing the pathways, we found three metabolites alterated: Nitrogen metabolism (FDR = 4.8 x 10-3), Arginine and proline metabolism (FDR = 4.8 x 10-3) and Aminoacyl-tRNA biosynthesis (FDR = 1.66 x 10-6). In order to determine the 5 main metabolites able to differentiate the diagnosis, ROC curves were used. Considering SCZ patients and healthy controls, the area under the curve (AUC) was 0.534, applying metabolites Met-SO, Gly, LysoPCaC26:1, C16-OH; PCaaC40:3. Considering BD patients and healthy controls the AUC was 0.947, applying metabolites t4-OH Pro, Creatinine, PCaaC24:0; PCaaC26:0 and LysoPCaC26:0. Considering SCZ and BD patients the AUC was 0.921, applying metabolites t4-OH-Pro; C16:2-OH; C3-OH; PCaaC36:1 and Met. **Discussion:** Our results clearly show that different classes of metabolites are implicated in both schizophrenia and bipolar disorders comparing to healthy controls. Besides that, we observed that the metabolites implicated in each disorder are not the same since the first onset psychosis.

Disclosures: A.C. Costa: None. **L.L. Talib:** None. **W.F. Gattaz:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 609.16/JJJ54

Topic: H.03. Schizophrenia

Support: NIMH R01 MH095995
UT System BRAIN Initiative

Title: Role of the glycogen synthase kinase 3 pathway in the pathophysiology of schizophrenia

Authors: *J. DI RE[1,2], W.-C. J. HSU[3,2,4], L. STERTZ[5], K. KHANIPOV[2], Y. FOFANOV[2], H. RAVENTOS[6], C. WALSS-BASS[5], F. LAEZZA[2]
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Abstract: The mechanisms underlying schizophrenia (SZ), one of the most severe and debilitating mental health disorders, are not well understood. Studies and clinical evidence suggest that multiple environmental and genomic risk factors contribute to the risk of developing SZ. As such, preclinical animal models do not fully recapitulate the complexity of the disease and can be only used to characterize specific endophenotypes associated with disease presentation. Integrative translational approaches that include *in vitro* models and fine-tuned human genetic studies are therefore necessary to elucidate the contribution of these genomic risk factors to endophenotypes of SZ. Emerging evidence indicates that dysregulation of the protein kinase B (AKT)/glycogen synthase kinase 3β (GSK3β) pathway is a risk factor for SZ. As such, there is a need to understand the molecular targets of this pathway. We have previously shown that GSK3β regulates the complex assembly and protein:protein interactions (PPI) within the voltage-gated sodium (NaV) channel complex at the axon initial segment (AIS), the molecular determinant of neuronal excitability. Based on this premise we hypothesized that dysfunction of the GSK3/AKT pathway could disrupt PPI of the AIS and excitability that could recapitulate molecular endophenotypes of SZ. Using a split-luciferase in-cell assay we have reconstituted the PPI complex between neurofascin, an important AIS cell adhesion molecule, and NaV channels and found that this interaction increases by increasing the level of active GSK3. By integrating genomic and functional studies neurons differentiated from induced pluripotent stem cells (iPSCs) from a small, homogeneous population with SZ we have also found a decrease in the mRNA level of GSK3β in SZ patients ($p<.05$, $n=11$, T-test with Welch's Corrections) compared to controls. We also identified a missense mutation in the NFASC protein associated with the disease in a combined cohort including patients from the NIMH Human Brain Collection Core ($p<.01$, $n= 424$, 1-sample test of proportions). We are currently evaluating whether changes associated with SZ and GSK3β distribution and intensity of neurofascin and Nav channels could be identified in neurons derived from patient iPSCs compared to unaffected relatives, which may underlie changes in intrinsic excitability that have previously been linked to SZ. Overall, these studies might help elucidate new endophenotypes associated with SZ due to dysregulation in the GSK3 pathway that could lead to a biological based classification of the disease and future targeted therapeutics.
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Disclosures: J. Di Re: None. **W.J. Hsu:** None. **L. Stertz:** None. **K. Khanipov:** None. **Y. Fofanov:** None. **H. Raventos:** None. **C. Walss-Bass:** None. **F. Laezza:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 609.17/JJJ55

Topic: H.03. Schizophrenia

Support: R01MH095995

Title: Disruption of the axonal initial segment composition in schizophrenia

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Abstract: The axon initial segment (AIS) is the site of action potential initiation in neurons and a critical determinant of neuronal excitability. This highly specific subcellular domain is composed of ion channels and scaffolding proteins that include ankyrin-G, beta-IV spectrin and intracellular fibroblast growth factor 14 (FGF14). Growing evidence indicates that the appropriate recruitment of the AIS macromolecular complex is essential for synchronized firing. Studies have also shown that disruption of the AIS structure and/or mutations in its molecular components are linked to the etiology of neuropsychiatric disorders, including schizophrenia (SZ). However, until now, a phenotypic description of the AIS structure in SZ patients has remained elusive. Here we applied confocal imaging to interrogate whether any potential changes in the AIS could be identified as an endophenotype of the disease. Quantification of immunofluorescence images from the dorsolateral prefrontal cortex (DLPFC) deep layer III of post-mortem human brain tissue of SZ (n=4) versus control subjects (n=5) revealed significantly reduced (p=<0.05) expression of ankyrin-G in the soma of NeuN positive neurons in SZ (n=132 neurons) vs control (n=117). In addition, in these cells ankyrin-G and FGF14 were both significantly upregulated in SZ (n=42 AIS p=<0.05) vs control patients (n=58 AIS). This study provides the first direct evidence of previously undescribed structural changes of the AIS macromolecular complex in the human brain as a potential causative link to the biology of SZ.

Disclosures: M.A. Alshammari: None. **T.K. Alshammari:** None. **J. Di Re:** None. **F. Laezza:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 609.18/JJJ56

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

Title: Dysbindin regulates axonal mitochondrial movement

Authors: *B. SUH[1], S.-A. LEE[2], C. PARK[3], S. LEE[1], S. PARK[1]
[1]Dept. of Life Sci., Postech, Pohang, Korea, Republic of; [2]SK Biopharmaceuticals, Seongnam, Korea, Republic of; [3]UCSF Sch. of Med., San Francisco, CA

Abstract: Neurons have a fine system for mitochondrial movement regulation to meet high demand of energy and calcium buffering. Therefore, specialized machineries are required to distribute mitochondria to the appropriate cellular locations through the transport system. Defects in mitochondrial transport have been reported to cause neuronal disorders, however, a detailed mechanistic link to the pathogenesis is not fully understood. We found axonal mitochondrial movement was significantly decreased in Dysbindin-deficient Sandy mice. Dysbindin, a schizophrenia susceptibility factor, shows interaction with a motor protein complex. Dysfunction of the motor protein complex resulted in reduced mitochondrial movement in neurons and it is partially rescued by Dysbindin overexpression. In addition, abnormal local calcium homeostasis was observed in neurons from Sandy mice or neurons with defects in the motor complex. These results and further investigation of the mechanism will collectively suggest that Dysbindin is involved in regulation of axonal mitochondrial movement cooperating with the transport machinery and support a potential link between mitochondrial movement and schizophrenia pathogenesis.

Disclosures: B. Suh: None. **S. Lee:** None. **C. Park:** None. **S. Lee:** None. **S. Park:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

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Program #/Poster #: 609.19/JJJ57

Topic: H.03. Schizophrenia

Support: NIH Grant R01MH094358 (RPS)
NIH Grant UL1TR002003 (JKM)

Title: Targeted and genome-wide approaches reveal alterations to the JAK-STAT1 transcriptional signature in psychosis

Authors: *J. K. MELBOURNE, B. FEINER, Y. PANG, M. PARK, C. ROSEN, R. P. SHARMA
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Abstract: Changes in immune activity are widely reported in individuals with psychotic disorders. These findings, in conjunction with epidemiological data and recent research demonstrating the influence of the immune system in modulating brain activity and behavior, suggest that these alterations may be related to symptom development and exacerbation. Peripheral immune cells, particularly monocytes and macrophages, are proposed to contribute to increased levels of inflammation in psychosis. While activation of the JAK-STAT1 signaling pathway by IFN-γ is understood to induce and stabilize the monocyte and macrophage proinflammatory phenotype, there a scarcity of data on this pathway and its relation to clinical

measures in the literature. Therefore, five genes (IFNG, CXCL10, IRF1, STAT1 and TLR4) were selected as measures of the JAK-STAT1 transcriptional signature, and expression was measured in peripheral blood mononuclear cells from a cohort of 89 participants with psychosis and 44 non-psychiatric controls. These measures were assessed in relation to clinical characteristics such as illness duration and acuity. Results demonstrated a suppressed JAK-STAT1 transcriptional signature earlier in illness and with greater acuity, that increased with illness duration. Additionally, the immune modifying effects of risperidone on the JAK-STAT1 transcriptional signature were assessed using a monocyte cell line. Treatment resulted in an increase in JAK-STAT1 signature gene expression in these cells, and thus potentially contributes to the increase in expression seen with illness duration. Finally, RNA sequencing was carried out using isolated monocytes from an independent cohort of 14 participants with schizophrenia (divided into 2 illness duration categories) and 14 non-psychiatric controls. Participants in the shorter illness duration category once again had a decreased enrichment of IFN-γ response genes (JAK-STAT1 signature) compared to participants with a longer illness duration. Interestingly, an opposing pattern was seen for TNF-α via NF-κB response gene enrichment, another important proinflammatory pathway, indicating a dichotomy between these signaling systems in monocytes in psychosis that warrants further investigation.

Disclosures: J.K. Melbourne: None. **B. Feiner:** None. **Y. Pang:** None. **M. Park:** None. **C. Rosen:** None. **R.P. Sharma:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 609.20/JJJ58

Topic: H.03. Schizophrenia

Support: R01MH107487

Title: Characterization of foxo1 in the anterior cingulate cortex in schizophrenia

Authors: *E. A. DEVINE[1], S. M. O'DONOVAN[2], C. R. SULLIVAN[3], R. E. MCCULLUMSMITH[4]
[1]Psychiatry and Behavioral Neurosci., Univ. of Cincinnati Col. of Med., Cincinnati, OH; [2]Psychiatry and Behavioral Neurosci., [3]Psychiatry, [4]Univ. of Cincinnati, Cincinnati, OH

Abstract: Schizophrenia is a devastating neuropsychiatric disorder that affects approximately 1% of the world's population. Its pathology involves the dysregulation of bioenergetics including the disruption of glucose metabolism as well as changes in the AKT pathway. This decrease in glucose metabolism has been linked to cognitive deficits, a primary symptom of schizophrenia. Previous studies have shown an increase in AKT kinase activity in postmortem schizophrenia in

the anterior cingulate cortex (ACC). We hypothesize that altered AKT kinase activity will lead to dysregulation of downstream signaling targets and bioenergetic pathways, contributing to cognitive deficits associated with schizophrenia. Forkhead box O1 (FOXO1) is a transcription factor involved in the regulation of cell metabolism and promotion of gluconeogenesis. Activated FOXO1 binds to the promoter for glucose-6-phosphatase, which in turn, promotes gluconeogenesis. FOXO1 is directly phosphorylated by AKT resulting in its translocation from the nucleus into the cytoplasm, thus preventing the activation of FOXO1 and leading to the suppression of gluconeogenesis. We propose that increased AKT kinase activity in schizophrenia will lead to an increase in FOXO1 phosphorylation, reducing FOXO1 levels in the nucleus causing dysregulation of gluconeogenesis pathways leading to cognitive deficits in illness. Preliminary in silico analysis has identified cell-specific decreases in FOXO1 in neurons in a model of schizophrenia. Using postmortem ACC (Bronx-Mt. Sinai Brain Bank) from schizophrenia and control subjects (n=20 per group), we will characterize mRNA and protein expression of FOXO1 and other AKT pathway components at the region-level and cell-level in enriched populations of astrocytes and pyramidal neurons. qPCR and in-situ hybridization will be used to assay mRNA expression and localization, and Western immunoblotting and immunohistochemistry will be used to analyze protein expression and localization.

Disclosures: E.A. Devine: None. **S.M. O'Donovan:** None. **C.R. Sullivan:** None. **R.E. McCullumsmith:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 609.21/JJJ59

Topic: H.03. Schizophrenia

Title: Computational analysis of genetic and transcriptional landscapes of the caudate nucleus in schizophrenia

Authors: *K. J. BENJAMIN, A. PAQUOLA
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Abstract: Schizophrenia, affecting 1% of the population worldwide, is a neurodevelopmental disorder arising from altered connectivity and plasticity. Currently it is established that there are numerous and heterogeneous risk factors that contribute to schizophrenia including genetic and environmental factors. Genome-wide association studies (GWAS) have determined more than hundred independent loci that contribute to schizophrenia risk. As such, the importance of large-scale studies has increased in recent years. The first effective treatments for schizophrenia, developed in the late 1950s, where shown to affect dopamine D_2 receptors. Interestingly, dopamine and dopamine D_2 receptors are known to have upregulated expression in schizophrenic

brains compared to neurotypical controls, and more specifically, the caudate nucleus. While decades of research have pointed to a major role of the dopaminergic pathway in the caudate nucleus with regards to schizophrenia, there are no large scale studies that investigate this relationship.
Here we investigate the caudate nucleus transcriptome using post-mortem samples from hundreds of individuals diagnosed with schizophrenia and neurotypical controls. We use genetic information on these individuals to assess whether genotypes that confer increased risk of schizophrenia are associated with transcriptional changes in the caudate nucleus, and which expression quantitative trait loci (eQTLs) are detected in the caudate nucleus. We apply machine learning techniques to build predictive models of primary diagnosis and other clinical outcomes (eg hallucinations), and identify which transcriptional and genetic features contribute most to prediction. We compare these findings with those from dorsolateral prefrontal cortex and hippocampus and identify those that are caudate nucleus-specific. Finally, we conduct a gene network and pathway study to investigate what predictive features are related to dopaminergic pathways. Altogether, our work presents a comprehensive analysis of caudate nucleus transcriptional and genetic landscapes and identifies predictive models of schizophrenia that could lead to new insight into its molecular nature and new lines of treatment.

Disclosures: K.J. Benjamin: None. **A. Paquola:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

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Program #/Poster #: 609.22/JJJ60

Topic: H.03. Schizophrenia

Title: Novel, non-catechol dopamine d1 receptor agonists exhibit g protein biased, beta-arrestin independent signaling

Authors: *A. N. NILSON, D. E. FELSING, P. WANG, J. ZHOU, J. A. ALLEN
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Abstract: The dopamine D1 receptor (D1R) is essential for many neurological functions including voluntary movement, working memory, attention and reward. The D1R canonically activates Gs/olf proteins to increase cyclic adenosine monophosphate (cAMP) production and increases neuronal excitability. However, the D1R also engages β-arrestin proteins which may facilitate receptor endocytosis and limit canonical receptor signaling. We recently discovered the first non-catechol D1R selective agonists and many of these ligands activated the D1R via G proteins without activating β-arrestin (Gray, Allen et al *Nature Comm., vol* **9**: 674, 2018). Here we further evaluate these non-catechol D1R agonists for their G protein and β-arrestin dependent signaling activity. The non-catechol D1R agonists PF-1119, PF-6142 and PF-2334 dose-

dependently increased D1R/G protein/cAMP signaling in HEK293 cells similar to dopamine using the Glosensor assay, but did not activate D1R/β-arrestin2 interactions using the Tango assay. To investigate D1R endocytosis and involvement of β-arrestin, we conducted confocal imaging and ELISA-based D1R endocytosis assays in wildtype HEK293 cells or cells lacking β-arrestin 1 and 2 using CRISPR/Cas9 genome editing. The catechol agonists dopamine and A77636 induced robust D1R endocytosis, but this trafficking was entirely blocked by knockout of β-arrestin. In stark contrast, the non-catechol agonists PF-6142 and PF-1119 did not induce D1R endocytosis. To further define D1R β-arrestin signaling outcomes, we conducted D1R desensitization assays by treating cells with agonists for 30 to 240 minutes followed by detection of D1R cAMP production using wildtype or β-arrestin knockout cells. The catechol agonists dopamine and A77636 significantly desensitized D1R cAMP signaling but this was prevented by knockout of β-arrestin 1 and 2. However, non-catechol agonists PF-6142 and PF-1119 did not induce D1R desensitization. Taken together, these results indicate that β-arrestins are required for D1R endocytosis and desensitization and that non-catechol D1R agonists signal independently from β-arrestin. Future studies will further investigate the mechanisms underlying this G protein biased signaling by non-catechol agonists. In addition, discovery of these non-catechol D1R agonists with limited desensitization may provide therapeutic value for neurological diseases involving reduced dopaminergic signaling.

Disclosures: A.N. Nilson: None. **D.E. Felsing:** None. **P. Wang:** None. **J. Zhou:** None. **J.A. Allen:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

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Program #/Poster #: 609.23/JJJ61

Topic: H.03. Schizophrenia

Support: K08MH077220
R21TW007882

Title: Dopaminergic networks bias phenotypic expression and genetic risk for schizophrenia

Authors: *J. MOLINA[1], J. ARNEDO[2], D. KAMIS[3], I. ZWIR[4], M. CORAL DE VAL MUÑOZ[5], C. CLONINGER[4], M. CALVO[6], E. PADILLA[6], G. GONZALEZ ALEMAN[6], J. TORANZO[2], M. SEDO[6], N. V. FLORENZANO[6], G. A. DE ERAUSQUIN[2]
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Abstract: Schizophrenia is a heterogeneous, neurodevelopmental disorder associated with poor functional outcomes. Despite it's heritability, uncovering the mechanisms of inherited risk in schizophrenia has proven challenging. Previous work has suggested that schizophrenia may actually represent a group of neurobiologically distinct disorders with variegated phenomenological subtypes. We tested the hypothesis that a primary dopaminergic deficit underlies vulnerability to schizophrenia, incorporating machine learning and optimization research to solve complex combinatorial problems not approachable with traditional statistics. We studied a unique ethnocultural sample (n=288) of subjects with chronic, never-treated schizophrenia, their unaffected relatives and matched controls who were evaluated blindly for measures of motor function, cognitive performance, personality traits, and transcranial ultrasound of the brainstem. A broad-coverage genome wide scan was also obtained. Following multi-step clustering and optimization, we combined genetic and phenotypic information in an unbiased fashion and uncovered nonlinear relations that predicted risk status (affected, relatives or controls). The function underlying this prediction is the first demonstration of a map of schizophrenia risk, where independent genomic clusters relate to distinct clinical phenotypes. Pathway analysis of the genomic clusters implicates molecular networks with known functional significance to neural development and synaptic function. Our data suggests that genetic risk for schizophrenia may act primarily by modulating dopaminergic pathways and biasing phenotypic plasticity.

Disclosures: J. Molina: None. **J. Arnedo:** None. **D. Kamis:** None. **I. Zwir:** None. **M. Coral de Val Muñoz:** None. **C. Cloninger:** None. **M. Calvo:** None. **E. Padilla:** None. **G. Gonzalez Aleman:** None. **J. Toranzo:** None. **M. Sedo:** None. **N.V. Florenzano:** None. **G.A. de Erausquin:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 609.24/JJJ62

Topic: H.03. Schizophrenia

Title: Transcriptional regulation of dopamine D1 receptor by DISC1-DRRF repressor complex

Authors: *Y. SUH[1], S. LEE[2], S.-J. NOH[1], S. KIM[1], S. PARK[1]
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Abstract: Dopaminergic system is important for motor functions and cognitive processes and its abnormality is linked to several neuropsychiatric disorders. Accumulating evidence suggests that Disrupted-in-schizophrenia 1 (DISC1) modulates dopamine mediated function and is involved in

gene transcription in the brain. In this study, we report a DISC1-involved transcriptional repressor complex playing a role for the expression of dopamine D1 receptor (DRD1). First, we found that DISC1 mutant mice showed increased level in DRD1 transcription. Scrutinizing the underlying molecular mechanisms, we identified a novel co-repressor complex for the DRD1 gene locus composed of DRRF and DISC1. First, we observed physical interactions between DISC1 and DRRF in the nucleus and subsequently among mSin3A binding with DRRF and DISC1. The interaction between DRRF and mSin3A was significantly altered by DISC1 co-expression. In chromatin immunoprecipitation assays, these potential co-repressor complex repressed the transcription of DRD1 and DISC1 seemed to strengthen the association of the complex to the promoter region. Finally, we confirmed the participation of DISC1 in DRD1-related dopaminergic system, by measuring the basal level of cAMP and p-ERK in primary cultured neurons and analyzing behavior patterns of DISC1 mutant mice. Collectively, our study provides a novel epigenetic mechanism in dopamine receptor-mediated signaling toward further understanding molecular views of psychiatric disorders.

Disclosures: Y. Suh: None. **S. Lee:** None. **S. Noh:** None. **S. Kim:** None. **S. Park:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 609.25/JJJ63

Topic: H.03. Schizophrenia

Support: MH087752
MH094445

Title: Altered subcellular EAAT2 localization in the DLPFC in schizophrenia

Authors: *S. M. O'DONOVAN[1], R. C. ROBERTS[5], J. ROCHE[6], C. DORSETT[6], C. R. SULLIVAN[2], K. A. HASSELFELD[3], R. KOENE[4], E. DEVINE[2], R. MEEKS[3], R. E. MCCULLUMSMITH[3]
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Abstract: Schizophrenia is a major mental illness with complex pathology, including abnormalities in the glutamate system. Glutamate is rapidly removed from the synapse by a family of membrane excitatory amino acid transporters (EAATs). To prevent glutamate spillover, EAATs must be expressed at high levels on the astrocytic plasma membrane and be localized adjacent to the synapse. Postmortem cortical tissue (dorsolateral prefrontal cortex (DLPFC)) was obtained from the Maryland Brain Collection from control and schizophrenia

subjects. EAAT2 protein expression (Western immunoblot n=10/group), activity (glutamate uptake assay n=10/group) and EAAT2-related gene expression (qPCR, n=16/group) were characterized at the region-level and/or cell-level in enriched populations of astrocytes and pyramidal neurons. Electron microscopic examination of EAAT2 subcellular localization was assayed in a subset of subjects (n=9-10/group). EAAT2-related gene expression was significantly reduced (Student's t-test, $p < 0.05$) in schizophrenia in the DLPFC in a region and cell-subtype specific manner, including a decrease in EAAT2 mRNA in an enriched population of astrocytes. Electron microscopy analysis found that the mean distance from EAAT2 labeling in perisynaptic astrocytic processes to the nearest edge of asymmetric synapses was significantly ($p<0.05$) further in schizophrenia cases ($0.440 \pm 0.072\mu m$) than in controls ($0.290 \pm 0.05\mu m$). EAAT2 labelling was also increased in the post-synaptic density in schizophrenia subjects ($22.3 \pm 10.5\%$) compared to controls ($11.4 \pm 6.6\%$). These studies suggest that glutamate transporter expression and localization is significantly altered in the DLPFC in schizophrenia. Such changes in EAAT2 could lead to diminished buffering and reuptake of glutamate at the synaptic cleft, leading to increased spillover of glutamate into the extrasynaptic space. Our findings suggest that altered ultrastructural localization of EAAT2 is part of a pathophysiological mechanism contributing to deficits of synaptic plasticity that underlie cognitive symptoms found in schizophrenia.

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Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

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Program #/Poster #: 609.26/JJJ64

Topic: H.03. Schizophrenia

Support: TEKES
UTUGS

Title: JNK1 provides a point of convergence for schizophrenia polygenes and controls cell surface availability of NMDA receptors

Authors: *Y. HONG[1], A. VARIDAKI[2], P. CIFANI[3], R. MYSORE[2], L. ELO[4], P. JAMES[3], E. T. COFFEY[5]
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Abstract: Schizophrenia is a polygenic disorder where no single major risk factor has been found in a large number of patients making it difficult to understand molecularly, though an imbalance of inhibitory and excitatory neurotransmission is a hallmark. Human genetic studies have linked MAPK regulators (*TAOK2, MKK4, MKK7, TNIK, TAK1* and *ULK4*) with schizophrenia, implicating involvement of the JNK cascade, however no mechanism is known. Here we characterize the brain phosphoproteome of *Jnk1-/-* mice over a lifetime. We show that *Jnk1* deletion alters 12 % of brain phosphoproteins and impacts previously unrelated signaling. This dataset is enriched for schizophrenia risk proteins and regulators of NMDA receptor trafficking. Consistent with bioinformatics analysis, we demonstrate that genetic deletion of *Jnk1* induces diametrically opposed regulation of excitatory NMDA. This is partly mediated by PKC which is activated in *Jnk1-/-* cortex. We show that the JNK1 pathway comprises a signaling framework for over 100 schizophrenia polygenes, to control inhibitory/excitatory balance.

Disclosures: Y. Hong: None. **A. Varidaki:** None. **P. Cifani:** None. **R. Mysore:** None. **L. Elo:** None. **P. James:** None. **E.T. Coffey:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 609.27/JJJ65

Topic: H.03. Schizophrenia

Support: NCSF 81771435
NCSF 81371473
NCSF 81171262

Title: The role of HINT1 in several neuropsychiatric diseases

Authors: *Y. DANG[1], P. LIU[2], G. LEI[2]
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Abstract: Although many studies have investigated the functions of histidine triad nucleotide binding protein 1 (HINT1), its roles in neurobiological processes remain to be fully elucidated. Accumulating clinical and pre-clinical evidence suggests that HINT1 may play an important role as a mediator in neuropsychiatric diseases, such as schizophrenia, mood disorders, drug addiction and so on. Therefore, we investigated the function of HINT1 in several neuropsychiatric diseases related animal models. We found that HINT1 plays a role in social isolation (SI) mouse model, characterized by behavioral abnormalities similar to those in schizophrenia. We also investigated the function of HINT1 in knockout mice. Both male and female HINT 1 knockout (KO) and heterozygosity (HT) mice had a trend of anxiolytic like behavior and anti-depression like behavior at control group. In regard of the researches on pain,

hot-plate test and formalin test showed that HINT1 KO mice showed higher pain sensitivity in the basal state, with no gender differences. We also investigated the role of HINT1 in methamphetamine (METH) and morphine addiction. In nucleus accumbens (NAc) of the METH-induced conditioned place preference (CPP) group mice, the HINT1 expression level initially increased after acquisition phases, and then dropped to the normal level after extinction phase, and again increased after reinstatement phase. In the METH-induced behavioral sensitization model, the HINT1 expression level increased in the prefrontal cortex (PFC) after the development phase. In addition, the HINT1 KO mice seem to be more sensitive in METH-induced behavioral sensitization, but only during the development phase. Interestingly, the situation of morphine addiction is just the opposite. The mRNA and protein expression level of HINT1 did not show any difference during the development phase, but both became higher in PFC in the morphine-induced behavioral sensitization group than the saline control group during the expression phase of this model. The HINT1 KO mice show less sensitivity in this addition model, and statistically difference emerges during the expression phase. In another model, mentioned as CPP, expression level of HINT1 protein of morphine-paired group in NAc significantly decreased during the acquisition phase. For the HINT1 KO mice, they need more training times than the wild-type control mice for the acquisition and extinction phases, but show no difference during the reinstatement test.

Disclosures: Y. Dang: None. **P. Liu:** None. **G. Lei:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 609.28/JJJ66

Topic: H.03. Schizophrenia

Support: NIH Grant R01 MH107487

Title: AMPK dysregulation in a human induced pluripotent stem cell model of DISC1-related schizophrenia

Authors: *E. BENTEA[1], S. O'DONOVAN[1], E. DEPASQUALE[2], J. MELLER[2], C. XU[3], Z. WEN[3], R. MCCULLUMSMITH[1]
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Abstract: Disrupted-in-schizophrenia 1 (DISC1) is a well-known genetic risk factor for severe mental illness including schizophrenia. A large array of animal studies supports an etiopathogenic role of DISC1, by linking it with regulation of processes such as synapse

formation and neuronal development. However, much less is known regarding the involvement of DISC1 in human neurons. Induced pluripotent stem cells (iPSCs) generated from patients have emerged as powerful tools to study cellular dysfunction in a disease-relevant context. In this study, we investigated serine/threonine kinase networks in a human iPSC model of DISC1-related schizophrenia. Using PamChip kinome arrays, we mapped the serine/threonine sub-kinome of neuronally differentiated iPSCs generated from a patient with schizophrenia presenting with a 4-bp deletion in DISC1, an unaffected family member without the mutation, and isogenic iPSC lines in which the mutation was either introduced in the control cell line or corrected in the DISC1 cell line. Arrays were run in triplicate and the results of the three chips averaged. Using a novel bioinformatics workflow, we identified kinases that were commonly changed in the DISC1 cell line, the control cell line with the DISC1 mutation introduced, and the DISC1 cell line which was genetically rescued, in order to identify disease pathways that are causally linked with the DISC1 mutation. This analysis highlighted 5' adenosine monophosphate-activated protein kinase (AMPK) as a common node of kinase dysregulation. AMPK is a subfamily of the CAMKL family of kinases involved in regulating cellular energy metabolism, and may link bioenergetic perturbations with changes in synaptic plasticity. To confirm the involvement of this signaling pathway, we screened for mRNA expression changes of 84 targets linked with AMPK signaling, using a previously generated RNA-Seq database of DISC1 cells. This approach confirmed significant AMPK dysregulation in DISC1 cells, with a wide range of targets linked with AMPK signaling differentially expressed at mRNA level. Our unbiased, and combined kinomics and transcriptomics approach supports evidence of AMPK dysregulation in a human iPSC model of DISC1-related schizophrenia. These findings provide further support of kinase network dysregulation in schizophrenia and may open new avenues for treating this highly disabling neuropsychiatric disorder.

Disclosures: E. Bentea: None. **S. O'Donovan:** None. **E. Depasquale:** None. **J. Meller:** None. **C. Xu:** None. **Z. Wen:** None. **R. McCullumsmith:** None.

Poster

609. Schizophrenia: Genetic, Cellular, and Molecular Features

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 609.29/JJJ67

Topic: H.03. Schizophrenia

Support: R01 Grant MH107487
R01 Grant MH094445

Title: Characterization of adenosine kinase in schizophrenia

Authors: *C. MOODY[1], A. FUNK[1], R. E. MCCULLUMSMITH[1], S. M. O'DONOVAN[2]
[2]Psychiatry and Behavioral Neurosci., [1]Univ. of Cincinnati, Cincinnati, OH

Abstract: Schizophrenia is a devastating neuropsychiatric disorder characterized by positive, negative, and cognitive symptoms. Hyperfunction of the dopamine system contributes to positive symptoms and hypofunction of the glutamate system contributes to negative and cognitive symptoms. Adenosine is a neuromodulator that regulates both the glutamate and dopamine systems. The adenosine hypothesis of schizophrenia states that hypofunction of the adenosine system, driven by overexpression of adenosine kinase (ADK), disrupts the role of adenosine as a neuromodulator and contributes to the pathophysiology of schizophrenia. This hypothesis has yet to be tested in postmortem schizophrenia. Postmortem tissue from the dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) of schizophrenia subjects and age and sex matched controls were obtained from the Maryland Brain Collection and Bronx-Mt.Sinai Neurobiobank, respectively. ADK protein levels were measured using Western immunoblot in the DLPFC. *In silico* analysis of ADK expression in postmortem schizophrenia databases was used to examine the effects of antipsychotic medication on ADK. There was no significant change in the expression of the long (ADKL; n=15-16/group, Student's t-test p=0.2). or the short (ADK S; n=16/group, Student's t test p=0.8) variant in schizophrenia compared to control. All samples were normalized to housekeeping gene beta actin. ADK protein expression was not significantly altered in schizophrenia in the DLPFC in tissue from the Maryland Brain Collection (n=17-18/group, p=0.7), or the NIH brain bank (n=35-36/group, p=0.1). ADK protein expression was also not altered in the ACC in tissue from the Bronx-Mt Sinai Neurobiobank (ANCOVA $F_{(1,21)}$ =1.09, n=12/group, p=0.31). ADK was normalized to beta tubulin prior to analysis. *In silico* analysis of the Stanley Online Genomics Database found an effect of antipsychotic administration on ADK gene expression, fold change (FC) >1, p=0.00046 in schizophrenia (SCZ) subjects on antipsychotics compared to off antipsychotics. However, there was no significant difference in ADK expression due to lifetime alcohol (EtOH) consumption (p=0.136), drug abuse (p=0.763), or sex (p=0.438). ADK protein expression is not significantly changed in the DLPFC or ACC in schizophrenia. The adenosine hypothesis of schizophrenia postulates that overexpression of ADK is central to dysregulation of this system in disease. Our results suggest that dysregulation of other components of the adenosine system contribute to the pathophysiology of schizophrenia.

Disclosures: A. Funk: None. **R.E. McCullumsmith:** None. **S.M. O'Donovan:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.01/JJJ68

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Easy tissue clearing mediated imaging of the brain and tissues using sunhyun 3dimensionalimage kit

Authors: *S. PARK[1], K.-S. KIM[2]
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Abstract: Despite the recent advances in tissue clearing methods, it remains challenging to reproducibility and simplification due to the complexity of the method and the limit of the cost. We have developed the SunHyun method to overcome current problems by simplifying the process of the tissue clearing, increasing reproducibility, and lowering costs. SunHyun method was able to protect protein loss without using a nanoporous hydrogel-hybridized form. This method also made the undamaged tissue transparent without using organic solvents or harsh detergents or conditions. Finally, SunHyun method preserved the fluorescent proteins over several months, and overcame the working distance of the microscope reducing the size of the transparent tissue. We use SunHyun method to image the transparent whole brain by light-sheet microscope and show important differences in the distribution of excitatory and inhibitory neurons in the hippocampal area. We were also able to quickly discover the degree of drug diffusion and side effects of the brain in a drug delivery system. Therefore, this method can be applied to read the latest research on diseases and therapeutic drug development.

Disclosures: S. Park: None. **K. Kim:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.02/JJJ69

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NSF Grant 1645170

Title: Viral-mediated transgenesis in the brain as a method to determine molecular mechanisms of aggression in stickleback fish

Authors: *N. JAMES[1], A. BELL[2]
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Abstract: Establishing a causal relationship between genes and social behavior is challenging since gene expression is dynamic. One gene may have drastically different effects when expressed in different parts of the social behavior network (areas in the fore- and mid-brain) or at different times. Thus, to determine the causal relationship between a gene of interest and a behavior, it is crucial to have a method for manipulating gene expression at a specific time and location. We developed one such method, viral-mediated transgenesis, for stickleback fish. Threespine sticklebacks (*G. aculeatus*) are a classic system for the study of behavior, ecology, & evolution. Our recent studies have identified hundreds of differentially expressed genes in the

brain following social interactions. Viral-mediated transgenesis is an appealing method for directly manipulating gene expression in this system. The technique is flexible, as different promoters can alter the targeted location or timeframe. It's also fast, since stable transgenic lines are not required.
Based on work in zebrafish, we hypothesized that a herpes simplex 1 (HSV1) derived virus with the mCMV, hCMV or hEF1a promoters would drive continuous expression of a target gene. Adult fish were injected with 300nL of either a saline control or suspended virus causing expression of a fluorescent protein. Successful transgenesis was determined via widefield microscopy on whole mount brains. Behavioral recovery was also assessed based on stress levels and mating behaviors – female fecundity and male nesting.
We successfully altered expression in stickleback brains using both the short-term promoter mCMV, showing expression by four days post-injection, and the longer-term promoter hCMV, reaching peak expression at ten days post-injection with continued expression for several weeks afterward. No fluorescence was seen in saline-injected controls. Breathing rate returned to baseline within two hours. Behavioral recovery following the injection occurred within nine days for females and three days for males, prior to peak expression of the long-term construct.
Next, we will test the behavioral consequences of manipulating the expression of candidate genes. These genes (*prl*, *ajap1*, *npas4*, *nsmfb* and *trpc4*) were differentially expressed in male sticklebacks following a territorial challenge. Functionally altered expression will be confirmed by qPCR, comparing expression between injected and un-injected brain regions of an individual. Aggression will be assayed prior to transgenesis and at 14 and 16 days. This technique will enable us to demonstrate that gene expression is sufficient to induce behavioral change.

Disclosures: N. James: None. **A. Bell:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.03/JJJ70

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NRF Grant 2016M3C7A1905383

Title: Development of temporal sensitive lentiviral-based luciferase reporter for real-time monitoring of stress-induced GR activations in mouse infralimbic prefrontal cortex

Authors: *S. HER
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Abstract: Application of luciferase bioimaging has been extended to valuable means for real-time monitoring of biological processes in various organ of living animals including brain.

During longitudinal analysis of deep brain, however, technical limitations to monitor the biological processes exist such as weak signals as well as less dynamic monitoring of signals. To overcome these limitations, we designed and tested various combination of lentiviral-based luciferase glucocorticoid response element (GRE) reporters *in vitro* and *in vivo*. Replacing luciferase reporter gene *Luc* with *Luc2* showed a 142% brighter bioluminescent signals in H19-7 cells, whereas reduction of insulator size from 1.kb to 0.3kb had no significant improvement in bioluminescent brightness. Addition of destabilized sequences into the C-terminal of *Luc2* also reduced half-life of luciferase activity by 69% in H19-7 cells and 61% in right IL-PFC of CD-1 mouse. In the assessment of in vivo usefulness, the tagging destabilizing sequences allowed to sensitively monitor temporal variation in stress-induced GR activations as demonstrated by a 64% increase in intra-individual coefficients of variation (iCV) of GR activity compared to control group. Taken together, our results provide a useful tool for real-time monitoring of neurobiological processes in deep brain, opening a window into the new insight of neurobiological dynamics in brain.

Disclosures: S. Her: None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.04/LLL1

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: PET imaging of immature neural cells following human induced pluripotent stem-cell-derived neurospheres transplantation with TSPO ligand

Authors: *T. YUJI[1], N. NAGOSHI[2], O. TSUJI[2], I. AOKI[3], T. YAMASAKI[3], B. JI[3], M.-R. ZHANG[3], Y. FUJIBAYASHI[3], M. MATSUMOTO[2], M. ZINZAKI[4], H. OKANO[5], M. NAKAMURA[2]
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Abstract: [Introduction]We have previously reported the beneficial effects of transplanting human induced pluripotent neural stem progenitor cells (hiPSC-NS/PC) into the spinal cord of contusive injury model. However, transplanting certain hiPSC-NS/PC that are known to have tumorigenic properties resulted in the deterioration of motor function secondary to the oncogenic transformation. Tumors derived from these “bad clones” consisted of immature undifferentiated human-specific NESTIN positive cells. It is known from previous studies that NS/PC co-express 18kDa translocator protein (TSPO) with neural stem cell marker such as NESTIN. Therefore, the

purpose of this study is to develop a method that allows us to visualize the immature neural tissues using TSPO ligand PET. [Methods]253G1-NS/PC (oncogenic clones) or 414C2-NS/PC (benign clones), PBS was injected into the striata or intact cervical spinal cord of immunodeficient mice. These cells were cultured and labeled with firefly luciferase genes via lentiviral transduction. After transplantation, we monitored the growth of transplanted cells through weekly Bio-imaging. Four to eight weeks later, gadolinium enhanced MRI was performed followed by PET with ^{18}F-TSPO ligand (^{18}F-FEDAC). The mice were immediately sacrificed and the brain and spinal cord were dissected out for *ex vivo* autoradiography (ARG). [Results]Bio-imaging revealed that the cells had been successfully engrafted in all mice. Among them, the 253G1 group demonstrated rapid cell proliferation. MRI revealed a region with gadolinium enhancement and high intensity T2 weighed area at the transplanted site in the 253G1 group, whereas there were no specific findings in the 414C2 or PBS group. ^{18}F-FEDAC PET revealed a significant increase in tracer uptake at the transplanted site in the 253G1 group compared to the others . We found that there was a higher binding of ^{18}F-FEDAC at the transplanted site in the 253G1 group using ARG compared to the others ($p<0.05$). [Conclusion]We successfully detected the remnant immature neural tissues of hiPSC-NS/PC using ^{18}F-FEDAC PET.

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Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.05/LLL2

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: KAKENHI grant 16K08527
KAKENHI grant 16H01488
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a grant from Jichi Medical University

Title: Monomeric and dimeric RFP-dependent Cre and its application to detect glucocorticoid receptor activation

Authors: *A. INUTSUKA[1], H. MIZOGUCHI[2], R. KANEKO[3], R. NOMURA[4], K. TAKANAMI[4], H. SAKAMOTO[4], T. ONAKA[1]

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Abstract: Transgenic animals expressing fluorescent proteins are widely used to label specific cells and proteins. GFP-dependent gene regulation enabled us to utilize these transgenic animals for selective gene expression in GFP-expressing cells; however, application has been limited to fluorescent proteins derived from Aequorea jellyfish so far. In this study, we generated Cre dependent on RFP (Cre-DOR) by combining split-Cre with RFP-specific small binding proteins such as nanobodies and designed ankyrin-repeat proteins. We constructed both monomeric RFP-specific Cre and dimeric RFP-specific Cre. We confirmed target RFP-dependent gene expression in mouse brains using adeno-associated virus vectors. Selective expression by Cre-DOR in mRFP1-expressing neurons of transgenic rats was also confirmed. Translocation of target RFPs greatly affected the efficiency of Cre-DOR. Using a light-sensitive translocation domain, we achieved optical downregulation of Cre-DOR activity by inducing translocation of target RFPs from the nucleus to the cytosol. Using the glucocorticoid receptor, we achieved chemical upregulation of Cre-DOR activity by inducing translocation of target RFPs from the cytosol to the nucleus. Using dimer-dependent RFPs, we detected dimer formation of RFPs as an increment of recombinase activity of dimeric RFP-specific Cre. Taken together, our findings extend the potential use of RFP-expressing transgenic animals and provide unique methods to monitor or manipulate cellular signaling such as glucocorticoid receptor activation.

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Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.06/LLL3

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: East Carolina University Startup

Title: Targeting mesocortical and mesoaccumbens dopamine neurons with DREADDs using the combination of adeno associated viral vectors and retrograde transported herpes simplex viral vectors

Authors: *J. B. EELLS[1], J. M. NUTTER[2], H. S. PARTINGTON[2]
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Abstract: The mesencephalic dopamine neurons have various physiological roles depending on the areas of the brain they innervate. Dopamine neurons innervating the prefrontal cortex have been found to be critical for executive control and working memory while dopamine neurons innervating the nucleus accumbens signals salient or relevant environmental stimuli. Abnormalities in these pathways contribute to diseases including schizophrenia and addiction. Understanding how alterations in signaling to these neurons alters their function is critical to understanding how exposure to drugs of abuse or environmental risk factors for schizophrenia contribute to these diseases. This current work is focused on using viral vectors to specifically target these dopamine neuron populations based on the area of the brain they innervate and to activate or inhibit these neurons with DREADDs (Designer receptors exclusively activated by designer drugs). Preliminary studies tested independently the efficiency of retrograde transport of a herpes simplex viral (HSV) vector from the nucleus accumbens and dopamine neuron infection in the ventral tegmental area with an adeno associated viral (AAV) vector. Both independently labeled dopamine neurons in the ventral tegmental area. We next tested a combination of an HSV expressing Cre recombinase and yellow fluorescent protein (YFP) injected into either the nucleus accumbens or prefrontal cortex with an AAV vector with Cre dependent expression of the DREADD hM3Dq coupled with mCherry. Analysis found good retrograde transport of the HSV and expression of mCherry indicating co-infection of those neurons with AAV. Of interest was the observation that some mCherry labeled neurons did not show detectible YFP labeling, suggesting that only low levels of cre appear necessary for expression of mCherry. As expected a much larger dopamine neuron population was labeled with nucleus accumbens injection as compared to prefrontal cortex injection. Initial trials expressing hM3Dq+Cherry via the HSV in combination with an AAV expressing cre were not successful. Studies are ongoing to determine the specificity of this approach for targeting dopamine neurons. Future studies will also investigate molecular changes that result from altering activation of these dopamine neuron populations with the DREADD agonist clozapine to help understand how environmental stimuli and drugs of abuse alter dopamine function and contribute to disease.

Disclosures: J.B. Eells: None. **J.M. Nutter:** None. **H.S. Partington:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.07/LLL4

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH 2017 R21 MH

Title: Revealing the chromatin-bound long noncoding RNA landscape of the brain

Authors: *H. M. CATES[1,2], S. AKBARIAN[2]
[1]New York, NY; [2]Psychiatry, Icahn Sch. of Med. at Mt. Sinai, New York, NY

Abstract: Long noncoding RNAs (lncRNAs) are a diverse set of transcripts that bind the genome and regulate epigenetic states. They comprise approximately 58,000 genes in the human genome. Recently, there has been a growing interest in the role of lncRNAs in many psychiatric disorders, including drug addiction. While there have been many studies implicating histone-modifying enzymes and other epigenetic proteins, it has never been entirely clear what the mechanism is to bring an epigenetic modifier to a specific locus. Evidence has shown that lncRNAs can act as scaffolds to help recruit these complexes. To date, epigenetic regulation by lncRNAs has largely been studied on a single transcript basis. To better understand the global landscape of lncRNA in the brain, we are optimizing a recently published method which allows us to capture all chromatin-bound RNA and identify the RNAs as well as the loci to which they are bound. This will allow us to leverage available sequencing data from similar brain tissue-sets to determine how lncRNA are associated with histone marks, nuclear organization, expression levels, and more. This will lead to better understanding of how lncRNAs are regulating the epigenome in the brain both at baseline and in disease states.

Disclosures: H.M. Cates: None. **S. Akbarian:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.08/LLL5

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Roche RiSE program

Title: Mapping of schizophrenia risk genes and isoforms using *in situ* RNA sequencing

Authors: *M. M. HILSCHER[1,2], C. YOKOTA[1], D. MALHOTRA[2], M. NILSSON[1]
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Abstract: Schizophrenia (SZ) is a debilitating neuropsychiatric disorder with a strong genetic etiology. Major progress has been made in identifying genes/loci associated with risk of SZ. Recent advances in single-cell RNA sequencing allow investigating the cell type-specific expression of SZ risk genes and link genetic risks to individual cells. In single-cell RNA sequencing studies of dissociated brain tissues, however, the information on the spatial context is lost.
Here, we use *in situ* RNA sequencing with padlock probes to study the cell type- and cortical layer-specific expression of selected SZ risk genes. We profiled the cell type-specific expression

of Cacna1c (Cav1.2) isoforms that target brain and heart enriched exons. Cacna1c is a SZ risk gene and subjected to extensive alternative splicing. In order to study which isoforms are significantly enriched in the brain, we compared their spatial distribution on brain and heart tissues. We identified regions of interest and found certain exons to be highly expressed in heart samples while being almost absent in brain tissues. Moreover, we show that a pair of mutually exclusive Cacna1c exons displays an opposite enrichment in heart and brain and that specific cell types are implicated in SZ pathogenesis. For a subsequent set of experiments we designed our probes to promote comparisons between healthy control tissues with either postmortem human SZ tissues or genetic mouse models of SZ in order to allow further investigations on potential molecular targets for SZ.
Thus, by performing high resolution analysis on *in situ* RNA sequencing data we are able to map schizophrenia risk genes and isoform expression which is important to understand tissue functionality and pathological changes during schizophrenia.

Disclosures: M.M. Hilscher: None. **C. Yokota:** None. **D. Malhotra:** None. **M. Nilsson:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.09/LLL6

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: rsCaMPARI: An erasable marker of neuronal activity

Authors: *F. SHA, E. R. SCHREITER
Janelia Res. Campus, Howard Hughes Med. Inst., Ashburn, VA

Abstract: Identifying and comparing active neuron ensembles underlying different complex behaviors is a key challenge in neuroscience. Recent tools such as CaMPARI have enabled the optical marking and selection of active neuron populations.[1] However, CaMPARI is based on the activity of a photoconvertible fluorescent protein whereby the marking is permanent and irreversible. These properties limit the utility of CaMPARI in samples where multiple snapshots of activity are desirable or where different activity profiles must be compared within the same sample. We sought to overcome these limitations by developing an erasable neuronal activity marker based on a reversibly switchable fluorescent protein. Here we introduce a new tool named rsCaMPARI, a reversibly switchable calcium marker that enables spatiotemporal precise marking, erasing, and remarking of active neuron populations under widefield illumination. rsCaMPARI photoswitching kinetics are modulated by calcium concentration when illuminating with blue light, and the fluorescence can be reset with violet light. We demonstrate the utility of rsCaMPARI for repeated marking and erasing of calcium activity in cultured neurons.

1. Fosque, B. F. *et al.* Labeling of active neural circuits in vivo with designed calcium integrators. *Science (80-.).* **347,** 755-760 (2015).

Disclosures: E.R. Schreiter: None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

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Program #/Poster #: 610.10/LLL7

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NICHD F32 HD081835
HF Langbert Neuroimmunolgy Research Award

Title: Molecular profiling of reticular gigantocellularis neurons indicates that eNOS modulates environmentally-dependent levels of activity

Authors: *I. TABANSKY[1], Y. LIANG[1], M. FRANKFURT[2], M. DANIELS[1,3], M. HARRIGAN[1], S. A. STERN[4], T. A. MILNER[6], R. LESHAN[5], R. RAMA[5], T. MOLL[5], J. FRIEDMAN[7], D. W. PFAFF[5], J. N. STERN[8]
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Abstract: Neurons of the medullary reticular nucleus gigantocellularis (NGC) and their targets have recently been a focus of research on mechanisms supporting generalized CNS arousal (GA), required for proper cognitive functions. NGC neurons project to the spinal cord and to the midbrain, but little is known about the gene expression of NGC neurons with ascending projections. Using the retro-TRAP method, we characterized transcripts enriched in NGC neurons which have projections to the thalamus. We identified several hundred transcripts that are enriched in these neurons as compared to the surrounding tissue. Signaling pathway in these cells appeared to indicate that these neurons are involved in neurovascular coupling; a finding that was strengthened by our observation of their physical proximity to blood vessels. Pharmacological inhibition indicated that these pathways were involved in modifying behavioral arousal in response to environmental change.

Disclosures: I. Tabansky: None. **Y. Liang:** None. **M. Frankfurt:** None. **M. Daniels:** None. **M. Harrigan:** None. **S.A. Stern:** None. **T.A. Milner:** None. **R. Leshan:** None. **R. Rama:** None. **T. Moll:** None. **J. Friedman:** None. **D.W. Pfaff:** None. **J.N. Stern:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.11/LLL8

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Central Michigan University Department of Neuroscience
Central Michigan University College of Medicine Summer Scholars
Central Michigan University Department of Chemistry
Field Neuroscience Institute
John G. Kulhavi Professorship in Neuroscience at Central Michigan University

Title: *In vitro* delivery of large plasmids with temporal control of gene expression via polyamidoamine (PAMAM) dendrimer nanoparticles

Authors: *M. FLORENDO[1], B. SRINAGESHWAR[1,2,3], A. FIGACZ[1,2], R. KIM[1,2], C. THOMPSON[2,3], D. SWANSON[4], G. L. DUNBAR[2,3,5], A. SHARMA[4], J. ROSSIGNOL[1,2,3]
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Abstract: Polyamidoamine (PAMAM) dendrimers are nanoparticles composed of the following features: (1) diaminobutane (DAB) core, (2) variable branching which determines its generation (G)/size, and (3) a surface composed of terminal groups. Each feature of a PAMAM dendrimer can be modified to determine its structure and interaction with other molecules. Dendrimers are one of the smallest known nanoparticles, with its fourth generation (G4) measuring at a size of 4 nm. Features of dendrimers including its modularity, its similar size to biomolecules, and its ability to cross the blood-brain barrier (BBB) allow it to have great potential for therapeutic application. In particular, dendrimers can be used in gene therapy to deliver therapeutic DNA plasmids of 10kb or more to cells *in vitro* and *in vivo*. Terminal surface groups, such as positively charged -NH2, can allow dendrimers to be more positively charged. Not only are these positive charges necessary for crossing the BBB and the membrane of the cell, but is necessary for complex formation with DNA since it will allow the dendrimer to be attracted to the negatively charged phosphates of DNA. The following experiments used fluorescently labeled fourth generation dendrimers composed of 90% neutrally charged -OH and 10% positively charged -NH2 (G4-90/10). These slightly positively charged dendrimers facilitated complex formation with DNA plasmids of varying sizes without causing cytotoxicity. Our study compared delivery between two large sized plasmids (6 kb and 10 kb) having a fluorescent reporter gene (mCherry). Gel electrophoresis and fluorescence microscopy confirmed complex formation and delivery of DNA plasmids (6 kb and 10 kb) to cells *in vitro* via the G4-90/10

dendrimers. MTT Assay confirmed minimal cytotoxic effects to cells that received the dendrimer DNA complex. Further, we delivered Tetracycline-ON (TET-ON) system plasmid (gift from Dr. Breunig, Cedars-Sinai Medical Center) via G4-90/10 to cells *in vitro*. The TET-ON system plasmid allows for expression of gene of interest only upon administration of tetracycline or its equivalent, doxycycline (DOX), which was subsequently confirmed via fluorescence microscopy. Combining our results, we developed a system of delivering large DNA plasmids of various sizes, including TET-ON system plasmids which contained temporal control of gene expression, to cells *in vitro.* This technique can be applied to the development of non-invasive treatment for neurodegenerative diseases by delivering these DNA dendrimer complex via intravenous administration in order to cross the BBB and target diseased cells *in vivo.*

Disclosures: B. Srinageshwar: None. **A. Figacz:** None. **R. Kim:** None. **C. Thompson:** None. **D. Swanson:** None. **G.L. Dunbar:** None. **A. Sharma:** None. **J. Rossignol:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.12/LLL9

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: IARPA D16 PC00002
NIH R01AI087879
NIH F32CA220990

Title: Nanobody-assisted large volume immunostaining for ultrastructure-preserved clem

Authors: *X. LU[1], T. FANG[2], D. R. BERGER[1], R. L. SCHALEK[1], H. L. PLOEGH[2], J. W. LICHTMAN[1]
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Abstract: A shortcoming of serial electron microscopy is its inability to show the locations of multiple molecules within tissue volumes without compromising the quality of the underlying ultrastructure. We recently developed NATIVE (Nanobody-Assisted Tissue Immunostaining for Volumetric Electron microscopy), a correlated light and electron microscopy approach for thick-section tissue imaging that preserves ultrastructure. The success of NATIVE originates from the penetrating property of single domain antibodies (nanobodies) that are only several nanometers in their longest dimension. They stain both the intra- and extra-cellular epitopes in densely packed thick tissues, such as brain, with only mild paraformaldehyde fixation. We used this approach to label and image in a confocal microscope fluorescent nanobody-tagged microglial cells, astrocytes and vascular endothelial cells in mouse hippocampus. We then reconstructed the

same immunolabelled cells in a serial section electron microscopy volume of the same tissue (using the ATUM approach). We are extending this approach by generating many additional fluorescent nanobody markers to make identification of neuronal and glial cells types in electron microscopy data sets routine.

Disclosures: X. Lu: None. **T. Fang:** None. **D.R. Berger:** None. **R.L. Schalek:** None. **H.L. Ploegh:** None. **J.W. Lichtman:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.13/LLL10

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant F32 NS098809
HHMI Investigator Award

Title: Systems-level analysis of gene expression heterogeneity in the songbird song system

Authors: *B. COLQUITT[1,2], F. GREEN[3], M. S. BRAINARD[1,2]
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Abstract: Birdsong is a powerful framework for understanding how the brain, at multiple levels of analysis, subserves the performance and modification of learned behavior. At the molecular-genetic level, a key goal is to build models of how environmental and behavioral states influence gene expression in the song system - the multi-locus neural circuit dedicated to song - and how molecular features of the song system influence song behavior. To further this effort, we have developed a high-throughput and low-cost RNA-sequencing approach for laser capture microdissected (LCM) tissue. The protocol combines an optimized bead-based RNA purification protocol for LCM sections with Drop-seq style library construction for sub-nanogram amounts of total RNA. This protocol allows genome-wide expression estimates from single LCM sections, thus permitting the analysis of multiple anatomically defined regions within single animals and multiple subsamples per region. The protocol flexibly accommodates experimental designs that require large numbers of animals and an integrated analysis of expression across neural systems. Here, we used this approach to characterize the song system of the Bengalese finch (*Lonchura striata domesticus)* along three different axes of transcriptional heterogeneity: inter-region variation, cell type identity, and axial variation. First, we compared expression among the four major telencephalic song nuclei and adjacent non-song-associated regions, with a focus on defining differential gene regulatory networks. Correlation network analysis combined with random forest regression modeling incorporating transcription factor motifs in accessible

chromatin produced a collection of transcription factors that we predict drive song/non-song nuclei transcriptional differences. Second, we leveraged the high density collection and sequencing of LCM sections from one song motor pathway nucleus, the robust nucleus of the arcopallium (RA), to perform within-nucleus correlation network analysis, yielding cell type-associated sets of genes and leading to the identification and characterization of multiple neuronal subpopulations in RA. Finally, the ordered collection of sections along the dorsal-ventral axis of RA allowed us to identify axial-varying genes. This systems-level approach to transcriptional analysis will complement finer-scaled delineations of cellular heterogeneity using single-cell RNA-seq methods and serve as an important tool to characterize the complex changes to gene expression that occur during behavioral plasticity.

Disclosures: B. Colquitt: None. **F. Green:** None. **M.S. Brainard:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.14/LLL11

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Development of efficient autophagosome sensors by detecting endogenous LC3 using modified Legionella RavZ

Authors: Y.-W. JUN[1], S.-W. JUN[1], P. JEON[2], J.-A. LEE[2], *D.-J. JANG[1]
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Abstract: Autophagy is the intracellular bulky degradation pathway in lysosomes and its dysfunction is tightly associated with many human diseases including neurodegenerative diseases. RavZ is secreted from Legionella and inhibits host autophagy through irreversible Atg8 deconjugation in autophagosome membrane via LC3/GABARAP binding and membrane association. Based on its property on LC3/GABARAP binding, we have developed a new probe for autophagosomes that detects endogenous LC3 in the autophagosome by modifying RavZ. To do this, we generated RavZ(Δcat)-EGFP, a catalytic domain deletion mutant of RavZ. RavZ(Δcat)-GFP was efficiently localized to mRFP-LC3B- or mRFP-GABARAP-positive autophagosome in an autophagy-dependent manner in mammalian cell line and in post-mitotic neurons. We found that both the LIR motif within N- or C-terminus of RavZ and a PI3P binding motif are required for the stable targeting to autophagosome. Thus, our new developed autophagosome sensors are expected to be widely used in autophagy research in live cells in physiological or pathological conditions.

Disclosures: Y. Jun: None. **S. Jun:** None. **P. Jeon:** None. **J. Lee:** None. **D. Jang:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.15/LLL12

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: SUVN-I2004: In-vitro pharmacological profile of a novel muscarinic M1 positive allosteric modulator

Authors: R. SUBRAMANIAN, V. MEKALA, M. SRIRANGAVARAM, N. PRAVEENA, S. EDULA, S. PETLU, G. BHYRAPUNENI, S. GAGGINAPALLY, A. MOHAMMED, *S. M. IRAPPANAVAR, R. NIROGI
Suven Life Sci. LTD, Hyderabad, India

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by aberrant protein aggregation, including amyloid beta (Aβ) peptide accumulation. AD is the most common cause of dementia among older people. There are no effective medications currently available to prevent and treat AD and halt disease progression. Modulation of muscarinic M1 receptor by non-selective agonists improve cholinergic neurotransmission and reduced the symptoms of AD. However, undesired cholinergic side effects prevented their further development. Selective activation muscarinic M1 sub-type using positive allosteric modulators could be a useful approach to treat cognitive deficits associated with AD without any cholinergic side effects. SUVN-I2004 was evaluated for its ability to potentiate the effect of endogenous agonist acetylcholine and also it's binding potential towards orthosteric site of muscarinic receptors in binding and cell based assays.SUVN-I2004 was tested in assays employing G-protein dependent and independent signaling pathways of muscarinic M1 receptors. SUVN-I2004 was also tested in in-vivo IP1 assays for assessment of efficacy translation from in-vitro assays. Cardiovascular safety was assessed using the patch clamp technique. SUVN-I2004 demonstrated positive allosteric modulatory activity with no agonistic activity towards muscarinic M1 receptor sub-type. It also displayed selectivity over closely related muscarinic receptor subtypes M2 to M5 and tested panel of serotonin, adrenergic, cannabinoid, dopamine, histamine receptor sub-types and monoamine transporters. SUVN-I2004 did not show inhibitory potential when tested in hERG patch clamp assay. SUVN-I2004 has demonstrated selective modulation of muscarinic M1 receptor in in-vitro pharmacological characterization.

Disclosures: R. Subramanian: A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **V. Mekala:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **M. Srirangavaram:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **N. Praveena:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **S. Edula:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **S. Petlu:** A.

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Employment/Salary (full or part-time):; Suven Life Sciences Ltd.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.16/LLL13

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NHMRC 631057
NHMRC 1067137

Title: Characterising the schizophrenia-associated dysregulation of microRNA biogenesis

Authors: *M. GEAGHAN, M. J. CAIRNS
Univ. of Newcastle, Australia, Callaghan, Australia

Abstract: The canonical microRNA (miRNA) biogenesis pathway involves three major proteins - DGCR8, DROSHA, and DICER1. These proteins and their associated genes have been associated with psychiatric disease, including schizophrenia. The *DGCR8* gene is located within the 22q11.2 locus; hemizygous microdeletions in this region cause 22q11.2 deletion syndrome, which predisposes to a ~30% risk of developing schizophrenia. We have also previously identified elevated expression of *DGCR8*, as well as *DROSHA*, *DICER1*, and numerous miRNAs in the dorsolateral prefrontal cortex (DLPFC) and superior temporal gyrus (STG) of individuals with schizophrenia. More recently we observed reduced expression of the transcription factor YY1 in the same tissue, which has been shown to regulate *DGCR8* expression in some cell lines. To further explore these findings, *DGCR8* was overexpressed in an *in vitro* neuronal cell culture model to examine the changes to the mRNA and miRNA expression profiles using next-generation RNA sequencing. We also knocked down *YY1 via* RNA interference to determine if this transcription factor was capable of regulating *DGCR8*, *DROSHA*, and *DICER1* in neuronal cells. Following *DGCR8* overexpression, we observed 267 miRNAs and 396 genes which were significantly differentially expressed (FDR < 0.05, absolute $\log_2$ fold-change > 0.6). Several schizophrenia-associated miRNAs were dysregulated, such as miR-132-3p and various miR-181 and let-7 family members. Functional analysis of the differentially expressed mRNA revealed 43 schizophrenia-related genes enriched in the dataset, including glutamate receptor subunits *GRIK4* and *GRIN2C*, calcium channel subunits *CACNB2* and *CACNG8*, and the miR-137 host gene *MIR137HG*. Following knockdown of *YY1*, we observed the downregulation of *DGCR8*,

DROSHA, and *DICER1*, suggesting *YY1* may regulate miRNA biogenesis in neuronal cells. These results suggest that downregulation of YY1 in schizophrenia could be associated with the dysregulation of miRNA biogenesis also associated with this disorder. Our data also suggests that *DGCR8* elevation is sufficient to disrupt normal neuronal miRNA expression and has a significant impact on the expression of genes important for neuronal function and schizophrenia.

Disclosures: M. Geaghan: None. **M.J. Cairns:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.17/LLL14

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Rapid phenotyping of CNS development with single-cell mass cytometry

Authors: *A. VANDEUSEN[1], I. CHENG[2], A. KEELER[2], C. WILLIAMS[3], T. LARSON[2], K. FREAD[3], K. MCNEELY[4], N. DWYER[4], A. SPANO[2], C. DEPPMANN[2], E. ZUNDER[3]
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Abstract: Mass cytometry employs metal-conjugated antibodies and time-of-flight detection to quantify single-cell expression of up to 45 biomarkers, vastly improving our ability to phenotype changes occurring during development and disease. However, this emergent technology has not been applied to analyze neural cell subtypes. Using a curated library of 200+ antibodies targeting neural-related filaments, transcription factors, transmembrane receptors, adhesion molecules, and glycoproteins, we developed a modular system of interchangeable cassettes specific for neural stem cells and precursors, neuronal and macroglial progenitors, neurons, astrocytes, oligodendrocytes, microglia, and endothelial cells. Panels assembled from various cassettes were used to validate the ability of mass cytometry to recapitulate established developmental paradigms in the developing central nervous system (CNS) of mice aged embryonic day 10.5 to postnatal day 4. Our ability to enumerate the heterogeneity and relative abundances of neural cell types using high-dimensional clustering algorithms permits organization of cells present into taxonomic groups, similar to data presented in single-cell transcriptomic studies. However, in contrast to such analyses, our proteomic map of mouse CNS development can account for the signaling status of cells, thus enhancing interpretation of functional cell states. Moreover, by examining more than 3×10^7 cells representing daily time points for four neuroanatomical regions (i.e. telencephalon, diencephalon, mesencephalon, and rhombencephalon), our approach facilitates rapid discernment of discrete changes in the specification and fate of individual neural cell types, as well as a global overview of neural and non-neural components of the developing brain. In addition to gaining new insights into the spatiotemporal progression of nervous system development, this method has an unprecedented ability to elucidate potentially significant factors

related to disease. Thus, in addition to proof-of-principle studies characterizing early CNS development, we are currently focused on validating the robustness of this methodology for rapid high-resolution phenotyping of neurological disorders – a strategy that is certain to have broad applicability in the future.

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Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.18/LLL15

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: FRIPRO, TOPPFORSK

Title: Using enhancer-driven gene expression (EDGE) to generate viral vectors capable of driving transgene expression in particular cell types of targeted brain regions in any species

Authors: *R. R. NAIR, S. BLANKVOORT, C. KENTROS
The Kavli Inst. for Systems Neurosci. / CNC, Trondheim, Norway

Abstract: The past decade has seen the development of a variety of molecular tools capable of revolutionizing systems neuroscience. However, fully taking advantage of these tools requires their expression with a higher degree of anatomical specificity than commonly available in most model systems. Furthermore, the development of methods to express transgenes at the level of anatomical specificity at which circuits operate will not only enhance our understanding of normal and pathological brain functions: delivery of therapeutic transgenes to specific circuit elements associated with brain pathologies may ultimately provide novel avenues for therapy. Unfortunately, most native promoters lack the required specificity, as they express in multiple neuronal cell types. However, there are orders of magnitude more transcriptional cis-regulatory elements (i.e. enhancers) than promoters, raising the intriguing possibility that they may help solve this problem. Recently we identified region-specific enhancers via differential ChIP-Seq analyses of histone modifications from microdissected rodent brain tissues and combined them with a heterologous minimal promoter to generate transgenic mice targeting distinct cell-types of the targeted brain region, an approach we term ‘’Enhancer-Driven Gene Expression’’ (EDGE) (*Blankvoort et al, Curr Biology in press*). While transgenic mice are excellent research tools, some experiments are more appropriate in other species, and transgenics cannot provide avenues for direct therapeutic interventions. Towards these ends, we created anatomically-specific adeno-associated viruses regulated by a hybrid promoter consisting of relatively small enhancers

specific to mouse entorhinal cortex (EC) and an optimized minimal promoter. Stereotaxic injections of relatively large volumes of such EC-specific enhancer-driven viruses in wildtype mice showed restriction of gene expression to the same specific set of EC neurons as obtained in the EDGE transgenic lines based upon that enhancer. Interestingly, these viruses drove cell type-specific expression not only in mice, but in rats as well. Our results suggest that the region-specific transgene expression that we attained in EDGE-transgenics is achievable with EDGE-viral vectors as well in wildtype animals of multiple species. Morever, because one can perform differential enhancer ChIP-seq in any species with a well-annotated genome, EDGE-viruses may ultimately provide a means to introduce transgenes to any specific cell-type in the brain of any species.

Disclosures: S. Blankvoort: None. **C. Kentros:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.19/LLL16

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Karl Kirchgessner Foundation

Title: Biolistic gene transfer reveals diverse synaptic organization of retinal ganglion cells

Authors: *F. HASAN[1], B. G. BORGHUIS[2], A. LOVETT[2]
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Abstract: To process information, neurons of the sensory systems integrate excitatory and inhibitory input at synapses expressed on their dendritic arbors. How many excitatory and inhibitory synapses a particular neuron type expresses—and where on the dendrites they are located—is important because it determines how a neuron responds to sensory stimulation. Within the visual system, it is well established that retinal ganglion cells differ strongly in their light-evoked responses, but the extent to which these differences reflect differences in distribution density of their excitatory and inhibitory synaptic inputs remains unclear. Measuring the distribution density of synapses is a challenge. For example, the sheer number of synapses and high degree of overlap of dendritic arbors precludes the use of immunohistochemical labeling for efficiently resolving the excitatory and inhibitory synaptic organization of a particular neuron type. To address this, we developed a biolistic (gene gun) approach for labeling excitatory and inhibitory synapses in single retinal ganglion cells and used anatomical reconstruction to compare the distribution density of synapses across morphologically identified ganglion cell types.

Our results show that ganglion cell types differ in (1) the number of synapses expressed on their dendrites; (2) the density of synapses expressed on their dendrites, for example, a cell with a small arbor can receive as many inputs as a cell with a large arbor; and (3) the ratio of excitatory to inhibitory synapses, which ranged from predominantly excitatory to predominantly inhibitory. The demonstrated experimental paradigm efficiently resolves the synaptic organization of morphologically identified ganglion cell types. Because the same cell types can be subsequently targeted for electrophysiological whole-cell recording, to assess relative magnitude and signal-to-noise ration of excitatory vs. inhibitory synaptic inputs, we expect this paradigm to help explain the response properties of visual neurons by relating structure to function.

Disclosures: F. Hasan: None. **B.G. Borghuis:** None. **A. Lovett:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.20/LLL17

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant R21 DA039681

Title: Flexible and inducible BDNF gene knockdown in rat neurons

Authors: *M. T. WONG-RILEY[1], D. WANG[2], M. GRZYBOWSKI[3], L. MU[2], D. A. BAKER[4], S. CHOI[4], A. M. GEURTS[3]
[2]Cell Biology, Neurobio. and Anat., [3]Physiol., [1]Med. Col. of Wisconsin, Milwaukee, WI; [4]Dept Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: Brain-derived neurotrophic factor (BDNF) is known to be critical in neuronal development, survival, differentiation, synaptic transmission, and synaptic plasticity. Its presence is important throughout life, and its down-regulation is implicated in a host of neuropsychiatric disorders and neurodegenerative diseases. To tease out the exact relationship between BDNF and neuronal functioning during development and adulthood, appropriate animal models are necessary. BDNF-null mice suffer from severe developmental defects and sensory system degeneration, ending in death within days after birth. Knocking out the BDNF high-affinity TrkB receptors leads to even more severe phenotypes and death on postnatal day 1. Conditioned BDNF knockout in the postnatal brains of mice results in mature-onset obesity, hyperactivity to stressors, and a severe deficit in 5-HT_{2A}-mediated neurotransmission. To more closely mimic the natural state of adjusted neurotrophin levels in various neurological disorders, we generated an inducible BDNF knockdown rat model. These transgenic rats harbor an inducible, Synapsin 1 promoter-driven, Tet-On^{3G} for doxycycline-regulated expression of a second, responsive transgene harboring a fluorescence reporter and shRNA targeting the endogenous rat BDNF

mRNA. The responsive transgene contains sites for recombinase-mediated cassette exchange (RMCE), allowing swapping of the BDNF shRNA for other expression cassettes using the ΦC31/attP system. We tested the efficacy of doxycycline (Dox) in the drinking water for 2, 3, and 7 days and found that as brief as 2 days of Dox down-regulated the expression of BDNF and TrkB in both the prefrontal cortex and the visual cortex as compared to non-Dox littermate controls. Induced BDNF knockdown also reduced the expressions of a metabolic marker, cytochrome oxidase, and glutamatergic NMDA receptor subunit 1, but up-regulated the expressions of GABA and $GABA_A\alpha 1$ receptor in both the prefrontal and visual cortices as compared to non-Dox controls. Thus, our BDNF knockdown rats serve as a viable model for investigating the effects of BDNF down-regulation in a variety of systems and neurological disorders. (Supported by NIH grant R21 DA039681).

Disclosures: M.T. Wong-Riley: None. **D. Wang:** None. **M. Grzybowski:** None. **L. Mu:** None. **D.A. Baker:** None. **S. Choi:** None. **A.M. Geurts:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.21/LLL18

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant 1U01MH10903801
NARSAD Young Investigator Award
NIH Grant T32GM008347
UMN Medical School Innovation Grant

Title: Composite viral vectors for receptor-mediated gene delivery

Authors: A. ZDECHLIK[1], Y. HE[1], *D. SCHMIDT[2]
[1]Univ. of Minnesota, Minneapolis, MN; [2]Genetics, Cell Biol. & Develop., Univ. of Minnesota Twin Cities, Minneapolis, MN

Abstract: Viral vectors are a major means of gene delivery with the potential to impact both basic research as well as clinical applications. Naturally evolved properties of many viral vectors are, however, mismatched to the needs of either. In gene therapy, for example, cell type specificity is paramount, as ectopic expression in off-target tissues or cells is undesirable and poses a safety risk.
Using Adeno-associated virus (AAV) as a model, we remove these legacy constraints of natural evolution by functionally separating viral entry (host recognition) and viral replication (gene delivery). We achieve this by producing a tropism-null AAV and then, in a programmable fashion, ‘arm’ it with covalenty linked antibodies and other non-immunoglobulin scaffold. We

demonstrate that these virus composites infect cells in receptor-specific manner. We also demonstrate re-targeting to a different receptor only requires arming virus with a different antibody and no modification of the virus itself.
Viral vectors that use well-understood binding scaffolds (e.g., antibodies) for receptor-mediated infection have broad utility, ranging from cell-type specific manipulation of neural circuits to new kinds of gene therapy approaches could significantly impact the care of persons with neurological disorders.

Disclosures: A. Zdechlik: None. **Y. He:** None. **D. Schmidt:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.22/LLL19

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Wellcome Trust 205093

Title: Cell type classification by multiplexed *in situ* RNA sequencing

Authors: *T. HAULING[1], X. QIAN[2], R. K. RAGHUPATHY[3], C. REDDY[3], S. BUGEON[3], Y. ISOGAI[3], J. HJERLING LEFFLER, Dr[4], M. NILSSON[2], K. D. HARRIS[3]
[1]Wolfson Inst. for Biomed. Res., London, United Kingdom; [2]Stockholm University/ Sci. for Life Lab., Stockholm, Sweden; [3]Univ. Col. London, London, United Kingdom; [4]Karolinska Institutet, Stockholm, Sweden

Abstract: Single cell RNA sequencing (scRNA-seq) has revealed an enormous diversity of transcriptionally distinct cell types in mouse brain. However, single cell sequencing cannot identify the locations of the corresponding cell types, which is essential not only to understand the spatial architecture of brain circuits, but also to relate transcriptomic cell types to other experimental methods such as two-photon calcium imaging.
We describe an improved method for in situ RNA sequencing of hundreds of molecules simultaneously, and apply it to characterize the spatial organization of interneuron subtypes in hippocampal area CA1 of mouse. We used a scRNA-seq dataset to identify key genes required to distinguish CA1 interneuron subtypes, and designed a set of padlock probes to target these genes, with multiple probes per target gene. The laminar organization of CA1 allowed us to calibrate the method using known locations of particular genes. We found that signal density was greatly improved by using specific primers in the initial cDNA synthesis step. Furthermore, read accuracy improved substantially with longer barcodes, with redundant coding allowing for accurate RNA calling despite background autofluorescence and other sources of error.
To classify cells based on these gene detections we used a probabilistic model. The mean

expression level of the selected genes in each type was taken by scRNA-seq, and we modelled the spatial distribution of each RNA species as a spatial point process centered on the cell centroids as determined by DAPI staining. Using variational Bayesian inference, we obtained a probability for a cell to belong to each class, and a probability for an RNA detection to belong to each cell. This approach avoided the necessity of accurately delimiting cell borders histologically, which is difficult in cell-dense structures such as the CA1 pyramidal layer. We verified the technique by confirming that it placed classical cell types in their expected laminar locations, and also used the method to identify the laminar locations of novel interneuron types identified by scRNA-seq.

Disclosures: T. Hauling: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CartaNA AB. **X. Qian:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CartaNA AB. **R.K. Raghupathy:** None. **C. Reddy:** None. **S. Bugeon:** None. **Y. Isogai:** None. **J. Hjerling Leffler:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CartaNA AB. **M. Nilsson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CartaNA AB. **K.D. Harris:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.23/LLL20

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: CBET-1606882

Title: A real time screening assay for cannabinoid cb1 receptor-mediated signaling

Authors: *H. K. ANDERSEN, K. B. WALSH
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Abstract: Cannabinoid-type 1 receptors (CB1) are highly expressed in the central nervous system where they modulate neurotransmitter release and synaptic plasticity through intracellular signaling. Specifically, CB1 activates the $G_i\beta\gamma$ subunit, which binds to G protein-coupled inwardly rectifying potassium (GIRK) channels and initiates an efflux of K^+ ions. GIRK channels suppress neuronal excitation through driving the neuronal membrane potential to more negative potentials, or hyperpolarizing the neuron. We developed a real-time, membrane-potential fluorescent assay for cannabinoids using pituitary AtT20 cells that endogenously express GIRK channels and were stably transduced with the human CB1 receptor using a

recombinant lentivirus (AtT20/CB1). In whole-cell, patch-clamp experiments, application of the cannabinoid agonist WIN 55, 212-2, to the AtT20/CB1 cells, activated GIRK currents that were sensitive to block by $BaCl_2$. WIN 55,212-2 activation of the GIRK channels was associated with a time- and concentration-dependent (EC_{50} = 309 nM) hyperpolarization of the membrane potential in the AtT20/CB1 cells when monitored using the fluorescent assay. The WIN 55,212-2-induced fluorescent signal was inhibited by pretreatment of the cells with the GIRK channel blocker, tertiapin-Q, or the CB1 receptor antagonist, SR141716. Using this assay, we determined the efficacies of four common cannabinoids tested at maximal concentrations: WIN 55,212-2 ≈ anandamide (AEA) > CP 55,940 > Δ9-tetrahydrocannabinol (THC). Additional testing identified cannabinoid compounds displaying unique efficacies and kinetics. In conclusion, this method provides a reliable and efficient screen for cannabinoid compounds that signal through $G_i\beta\gamma$, with possible future applications for the treatment of neuropathic pain.

Disclosures: H.K. Andersen: None. **K.B. Walsh:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.24/LLL21

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Brain Initiative Grant 1U01NS090600

Title: Improving genetically encoded voltage indicators with a novel screening system

Authors: *S. W. EVANS[1], D. SHI[2], M. CHAVARHA[3], L. PRADHAN[4], I. DIMOV[4], R. YANG[4], J. B. DING[6], M. J. SCHNITZER[7], M. Z. LIN[5]
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Abstract: Understanding how the brain works at the circuit level requires sensors that can track various types of neuronal activity with sufficient resolution in space and time. Genetically-encoded voltage indicators (GEVIs) offer great potential in achieving this goal, but still require a lot of optimization to be routinely used in vivo. Development of GEVIs has been hampered by a lack of a high-throughput screening methods, which have been instrumental for rapid improvement of calcium indicators. GEVI screening is challenging because one must induce a change in membrane potential while accurately monitoring fluorescence output from the sensor on a fast timescale. Here we report the development of a fast screening platform for improving GEVI variants based on a novel concept for inducing rapid membrane voltage changes. Using

this approach, we obtained an improved ASAP-family GEVI, ASAP3, with a response amplitude of 50% to steady state voltage changes from -70 to +30 mV, and 20% to individual neuronal action potentials. We also report in vivo data using these same indicators.

Disclosures: S.W. Evans: None. **D. Shi:** None. **M. Chavarha:** None. **L. Pradhan:** None. **I. Dimov:** None. **R. Yang:** None. **J.B. Ding:** None. **M.J. Schnitzer:** None. **M.Z. Lin:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.25/LLL22

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: IARPA MICrONS (D16PC0008 to AMZ)
BRAIN Initiative (1U19MH114821 to AMZ)
SCGB Postdoctoral Fellowship (350789 to XC)
CZI SVCF (2017-174399, subaward 2017-0530)

Title: Combinatorial cadherin expressions in the mouse visual cortex detected by targeted *in situ* sequencing

Authors: *Y.-C. SUN, X. CHEN, A. M. ZADOR
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Cell adhesion molecules are required to establish the highly diverse neuronal projections in the vertebrate brain. Individual cadherins have been shown to specify fiber tracts and target areas, but it is unclear to what extent the expressions of multiple cadherins within a single neuron contribute to the complexity of neuronal projections. Testing this hypothesis requires detection of many cadherins within the same neuron for a comprehensive combinatorial cadherin expression profiling. Here, we adapt a targeted *in situ* sequencing technology to detect multiple endogenous mRNA transcripts simultaneously. We characterize the combinatorial neuronal expressions of over 60 cadherin superfamily members in the visual cortex of juvenile and adult mice. Because targeted *in situ* sequencing is also compatible with BARseq (Chen et al, 2018), a high-throughput technique for mapping long-range axonal projections based on *in situ* sequencing of RNA barcodes, this approach can be combined with *in situ* sequencing of endogenous mRNAs and barcodes to correlate gene expression and projections at cellular resolution. Such an approach would uncover the degree to which combinatorial cadherin expressions can predict neuronal projection patterns.Reference:
Chen, X., Kebschull, J.M., Zhan, H., Sun, Y., and Zador, A.M. (2018). Spatial organization of projection neurons in the mouse auditory cortex identified by *in situ* barcode sequencing. Biorxiv, doi: 10.1101/294637.

Disclosures: Y. Sun: None. **X. Chen:** None. **A.M. Zador:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); owner and founder of MapNeuro.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.26/LLL23

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: HHMI
NINDS (NS079419)
NIMH (MH105949)

Title: A single spectrum of neuronal identities across thalamus

Authors: J. PHILLIPS[1], *A. SCHULMANN[1], E. HARA[2], C. LIU[3], L. WANG[1], B. SHIELDS[4], W. KORFF[1], A. LEMIRE[1], J. T. DUDMAN[1], S. NELSON[3], A. HANTMAN[1]
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Abstract: Uncovering common principles by which diverse modalities of information are processed is a fundamental goal in neuroscience. In mammalian brain, thalamus is the central processing station for inputs from sensory systems, subcortical motor systems, and cortex; a function subserved by over 30 defined nuclei. Multiple thalamic nuclei send convergent information to each region of the forebrain, but whether there is a conserved architecture across the set of thalamic pathways projecting to each forebrain area has remained unresolved. To uncover organizational principles of thalamic pathways, we produced a near-comprehensive transcriptomic atlas of thalamus. This revealed a common logic for thalamic nuclei serving all major cortical modalities. We found that almost all nuclei belong to one of three major profiles, with a given cortical area getting input from each of these profiles. These profiles lie on a single axis of variance aligned with the mediolateral axis of thalamus, and this axis is strongly enriched in genes encoding receptors and ion channels. We further show that each projection profile exhibits different electrophysiological signatures. Single-cell profiling revealed that rather than forming discrete classes, thalamic neurons lie on a spectrum, with intermediate cells existing between profiles. Thus, in contrast to canonical models of thalamus that suggest it is a switchboard primarily concerned with routing distinct modalities of information to distinct cortical regions, we show that the thalamocortical system is more akin to a molecularly-defined ‘filter bank’ repeatedly applied across modality. Together, we reveal striking covariation in the organization of thalamic pathways serving all input modalities and output targets, establishing a simple and comprehensive thalamic functional architecture.

Disclosures: J. Phillips: None. **A. Schulmann:** None. **E. Hara:** None. **C. Liu:** None. **L. Wang:** None. **B. Shields:** None. **W. Korff:** None. **A. Lemire:** None. **J.T. Dudman:** None. **S. Nelson:** None. **A. Hantman:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.27/LLL24

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Biodistribution of AAVHSCs in the central nervous system of non-human primates

Authors: *J. GINGRAS[1], K. OLIVIERI[2], N. ZAPATA[2], L. SMITH[2], H. RUBIN[2], P. MORALES[3], J. ELLSWORTH[2], A. SEYMOUR[2]
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Abstract: Adeno-associated viruses (AAVs) have emerged as key viral-based delivery vehicles for gene therapy in the nervous system due to their stable transgene expression in post-mitotic cells, neuronal tropism, lower risk of insertional mutagenesis and diminished immune response. We have recently reported the identification of novel AAVs derived from human hematopoietic stem cells (AAVHSCs). These novel AAVHSCs map to AAV Clade F alongside AAV9, which has been demonstrated to successfully cross the blood-brain-barrier (BBB) following systemic administrations. We set out to characterize the AAVHSCs to: 1) determine whether crossing the BBB was a generalized trait of Clade F AAVs and 2) assess whether the AAVHSCs could be attractive viral-vehicle candidates for gene therapy applications in the central nervous system (CNS). Herein, we report the biodistribution of AAVHSC7, AAVHSC15 and AAVHSC17 compared to that of AAV9 in the nervous system of 3- to 4-month old male cynomolgus macaques (*Macaca fascicularis*). Animals pre-screened for anti-AAVHSC neutralizing antibodies received a single intravenous (IV; 0.7-1E14 vg/kg) injection of recombinant AAVs packaging a self-complementary enhanced green fluorescent protein (sc-eGFP) transgene driven by the chicken beta actin (CBA) promoter. Biodistribution of AAVHSCs and AAV9 was assessed by anti-eGFP immunohistochemistry (IHC). All three AAVHSCs showed anti-eGFP immunoreactivity in the brain following IV administration. Furthermore, the three AAVHSCs displayed a distinct rostro-caudal distribution of anti-eGFP expression with the highest levels seen in the mesencephalon and myelencephalon. The largest cellular population displaying anti-eGFP were of glial origin, but anti-eGFP-positive neurons were also observed throughout different regions of the brain. Both neuronal cell bodies, dendrites and axons /axonal tracts were detected. These data demonstrate that, like AAV9, AAVHSCs effectively cross the BBB

following intravenous delivery in non-human primates, creating the potential for therapeutic applications in treating human genetic diseases of the CNS.

Disclosures: J. Gingras: A. Employment/Salary (full or part-time):; Homology Medicines Inc. **K. Olivieri:** A. Employment/Salary (full or part-time):; Homology Medicines. **N. Zapata:** A. Employment/Salary (full or part-time):; Homology Medicines. **L. Smith:** A. Employment/Salary (full or part-time):; Homology Medicines. **H. Rubin:** A. Employment/Salary (full or part-time):; Homology Medicines. **P. Morales:** A. Employment/Salary (full or part-time):; The Mannheimer Foundation, Inc. **J. Ellsworth:** A. Employment/Salary (full or part-time):; Homology Medicines. **A. Seymour:** A. Employment/Salary (full or part-time):; Homology Medicines.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.28/LLL25

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: DA037161
DA043829
NARSAD, CA TRDRP23XT-0007
TRDRP27IP-0057

Title: Fluorescent biosensors for the “inside-out pharmacology” of nicotinic and opioid drugs

Authors: *A. K. MUTHUSAMY[1], A. V. SHIVANGE[2], P. M. BORDEN[3], A. L. NICHOLS[2], A. KAMAJAYA[2], J. JEON[2], J. S. MARVIN[3], E. K. UNGER[4], B. N. COHEN[2], H. BAO[5], E. R. CHAPMAN[6], L. TIAN[4], L. LOOGER[3], H. A. LESTER[2]
[1]Chem., [2]Biol. and Biol. Engin., Caltech, Pasadena, CA; [3]Janelia Res. Campus, Ashburn, VA; [4]Biochem. and Mol. Med., Univ. of California, Davis, Davis, CA; [5]Neurosci., Univ. of Wisconsin-Madison, Madison, WI; [6]Neurosci., Howard Hughes Med. Inst., Madison, WI

Abstract: Some chronic effects of nicotine begin when the drug enters cells and then, in the endoplasmic reticulum and *cis*-Golgi, binds to nascent nicotinic receptors (nAChRs). To quantify the extent and dynamics of this permeation with imaging in live cells, we employ genetically encoded fluorescent biosensors, targeted to various organelles. Our biosensors are built on a OpuBC-GFP platform. OpuBC is a bacterial periplasmic binding protein (PBP) with a cation-π box, favorable for binding amines common in neural drugs. Ligand binding induces a “venus fly-trap” conformational change. We connect hinge sequences to a circularly permuted “superfolder” GFP (cpGFP). With the aid of structural data, we used directed evolution to create a family of “intensity-based nicotine-sensing fluorescent reporters” (iNicSnFRs) meeting the criterion of

ΔF/F0 > 1 at 1 μM. Complementing previous studies, we directly show that nicotine itself enters the ER in clonal cell lines and in primary hippocampal cells, within 10 s of application of the sub-micromolar CSF concentrations of a cigarette smoker or vaper. When nicotine is removed from the external solution, it leaves the ER just as rapidly. Moreover, the nAChR ligand varenicline, a smoking cessation drug, shows only slightly slower kinetics, with implications for both the successes and weakness of varenicline. We now extend to other neural iDrugSnFRs, because most orally available and inhaled neural drugs have pK_a and logP metrics consistent with an "inside-out" pathway. We have curated a "panel" of biosensor mutants with varying binding pockets and linkers. Our drug "library" includes molecules with indications for analgesia, nicotine addiction control, schizophrenia, bipolar disorder, major depressive disorder, anxiety, and epilepsy. We screen "biosensors x neural drugs". This work also tests the first step in the hypothesis that μ, δ, and κ-opioid opiate drugs also exert some chronic effects via an "inside-out" pathway, beginning in the ER. We are optimizing iOpioidSnFRs for methadone and morphine, hoping to achieve sub-100 nM detection. This work complements other efforts to more completely understand the mechanism of tolerance to opioids.

Disclosures: A.K. Muthusamy: None. **A.V. Shivange:** None. **P.M. Borden:** None. **A.L. Nichols:** None. **A. Kamajaya:** None. **J. Jeon:** None. **J.S. Marvin:** None. **E.K. Unger:** None. **B.N. Cohen:** None. **H. Bao:** None. **E.R. Chapman:** None. **L. Tian:** None. **L. Looger:** None. **H.A. Lester:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.29/LLL26

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: U01NS013522

Title: Engineering a fluorescent serotonin sensor using machine learning

Authors: E. K. UNGER[1], R. LIANG[1], C. E. DONG[1], J. SUN[1], D. A. JAFFE[1], G. J. BROUSSARD, JR[2], G. O. MIZUNO[1], P. M. BORDEN[3], A. L. NICHOLS[4], A. K. MUTHUSAMY[5], *L. UNGER[1], H. A. LESTER[6], S. HARTANTO[1], A. J. FISHER[1], V. YAROV-YAROVOY[1], J. S. MARVIN[3], L. L. LOOGER[3], L. TIAN[1]
[1]UC Davis, Davis, CA; [2]Univ. of California at Davis, Davis, CA; [3]Janelia Res. Campus, Ashburn, VA; [5]Chem., [6]Biol. and Biol. Engin., [4]Caltech, Pasadena, CA

Abstract: In order to enable high spatial and temporal resolution optical interrogation of neuromodulatory circuits, we set out to generate a genetically encoded fluorescent sensor for serotonin. To do this we chose to use the bacterial periplasmic binding protein (PBP)

superfamily as a source of scaffolds. This has three major benefits: soluble PBPs are amenable to high-throughput bacterial screening and easy crystallization, they have no known endogenous activity in eukaryotic cells, and most importantly, they have the potential for very large dynamic range, due to large ligand binding-dependent conformational changes. One disadvantage is that there are no known PBPs that bind serotonin; thus we opted to redesign the binding pocket of an existing acetylcholine-binding protein to recognize serotonin. In addition to using traditional methods for protein engineering, we developed a machine learning based approach to directed evolution that has the potential to produce very large improvements in performance with only low- to medium-throughput screening burden. Our method first employs a random forest model to identify which positions will be most efficient to mutate, then an absorbing Markov chain Monte Carlo simulation to calculate the minimum library size to be screened, and finally, once screening is underway, a generalized linear model to determine the contribution of individual mutations to the overall improvement in performance of the sensor, which can also predict the best amino acid combinations. This method is straightforward, easy and free to implement (requiring only rudimentary knowledge of statistics and coding), and can be used in conjunction with other methods, thus adding a powerful new tool to the protein engineering toolbox. Using this method, we were able to redesign the binding pocket to generate a fluorescent serotonin sensor whose affinity for serotonin is four orders of magnitude greater than the parent sensor, and which has three times the dynamic range. Furthermore, this sensor is sensitive enough to detect endogenous serotonin release.

Disclosures: E.K. Unger: None. **R. Liang:** None. **C.E. Dong:** None. **J. Sun:** None. **D.A. Jaffe:** None. **G.J. Broussard:** None. **G.O. Mizuno:** None. **P.M. Borden:** None. **A.L. Nichols:** None. **A.K. Muthusamy:** None. **L. Unger:** None. **H.A. Lester:** None. **S. Hartanto:** None. **A.J. Fisher:** None. **V. Yarov-Yarovoy:** None. **J.S. Marvin:** None. **L.L. Looger:** None. **L. Tian:** None.

Poster

610. Molecular, Biochemical, and Genetic Techniques: Molecular Techniques II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 610.30/LLL27

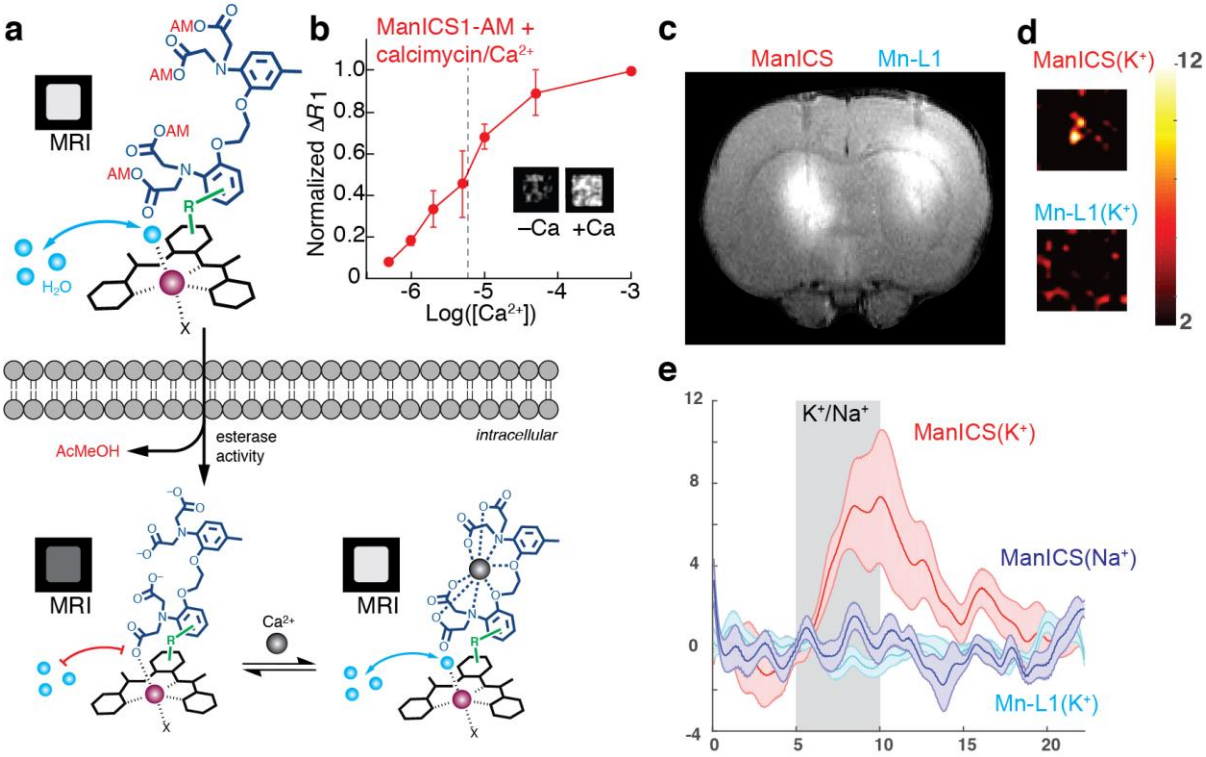
Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: U01-NS090451
R01-DA038642
DP2-OD2114

Title: Molecular-fMRI of intracellular calcium using a novel, small molecule sensor

Authors: *B. B. BARTELLE[1], A. BARANDOV[2], C. G. WILLIAMSON[2], A. JASANOFF[1]
[1]Biol. Engin., [2]MIT, Cambridge, MA

Abstract: Developing a whole brain, noninvasive readout of intracellular calcium signaling is one of the greatest challenges in chemical neuroscience. Here we present a new calcium responsive MRI contrast agent (ManICS1) and its cell trappable variant (ManICS1-AM) that can report MRI signal changes in response to stimuli that elevate intracellular calcium. We demonstrate the utility of ManICS in cells and present the first *in vivo* neuroimaging of intracellular calcium activity using KCl stimulation seizure model in the striatum of an adult rat. The ManICS sensor consists of a cell permeable contrast agent and a BAPTA-based calcium chelator(**a**). Prior to cell entry BAPTA carboxylates are protected with AM esters, allowing water exchange at the metal center and T_1-weighted MRI contrast. Once in cells, AM esters are cleaved, and sensor enters the calcium-free "off" state (exchange blocked), with low MRI contrast. When calcium binds, BAPTA and paramagnetic center disengage, activating the sensor. ManICS1 shows significant relaxivity (r1) changes over physiologically relevant levels of calcium ManICS1-AM-loaded cells permeablized with a calcium ionophore showed calcium-induced changes over physiologically relevant concentrations of calcium with a midpoint at $[Ca^{2+}] = 5$ µM (**b**). Infusing ManICS-AM into the brain of an adult rat shows a broad distribution of the sensor across the striatum, similar to a non-functionalized cell permeable contrast agent (**c**), however an infusion of KCl causes T1 contrast changes only in the ManICS infused regions with a dynamic response over time (**d,e**). These results demonstrate Calcium-fMRI as a breakthrough technique for analysis of neural circuits in animals, with the short term potential for brain wide distribution and imaging and longer term applications in humans.



Disclosures: B.B. Bartelle: None. **A. Barandov:** None. **C.G. Williamson:** None. **A. Jasanoff:** None.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.01/LLL28

Topic: I.03. Anatomical Methods

Title: Multimodal microscopic imaging of atherosclerosis plaque multi-composition for cerebrovascular events study

Authors: H. HUI[1], *X. YANG[1], J. TIAN[2]
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Abstract: Atherosclerotic plaques are the main cause of cerebrovascular events such as ischemic heart disease and stroke. The cell components and structure of atherosclerotic plaques are the basis of plaque stage, events prediction and intervention evaluation. There is a high temporal and spatial heterogeneity in cell components and structure of atherosclerotic plaques. The current microscopic evaluation methods of plaques are evaluating a single sample in cross-sectional and single-component way. They cannot meet the needs of basic research in atherosclerotic plaques. Here we present a multimodal microscopic imaging system which integrating the technologies of two-photon excitation fluorescence imaging, nonlinear optical microscopy and photoacoustic microscopy imaging. It consists of two-photon microscopy imaging module, second harmonic imaging module, third harmonic imaging module, photoacoustic microscopic imaging module, dual-frequency laser light source module and image acquisition module. The equipment provides label-free, quantitative, sub-micron imaging of the different components and overall structure of the atherosclerotic plaque. The multimodal images are registered, three-dimensional and visualized within a unique software platform. This imaging device can be used to investigate the temporal and spatial heterogeneity of plaque stage and components and provides accurate and convenient image for basic research in atherosclerotic plaque and cerebrovascular diseases.

Disclosures: H. Hui: None. **X. Yang:** None. **J. Tian:** None.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.02/LLL29

Topic: I.03. Anatomical Methods

Support: ERC grant 682426 - VISONby3DSTIM
EFOP-3.6.3-VEKOP-16-2017-00009

Title: Fast 3D imaging method for long-term recording neuronal activity and plasticity by acousto-optical two-photon microscopy

Authors: *D. PINKE[1], M. MAROSI[1], G. DOBOS[2], G. SZALAY[1], D. NAGY[1], C. CSUPERNYÁK[1], A. PLAUSKA[1], G. KATONA[1], B. RÓZSA[1,3]
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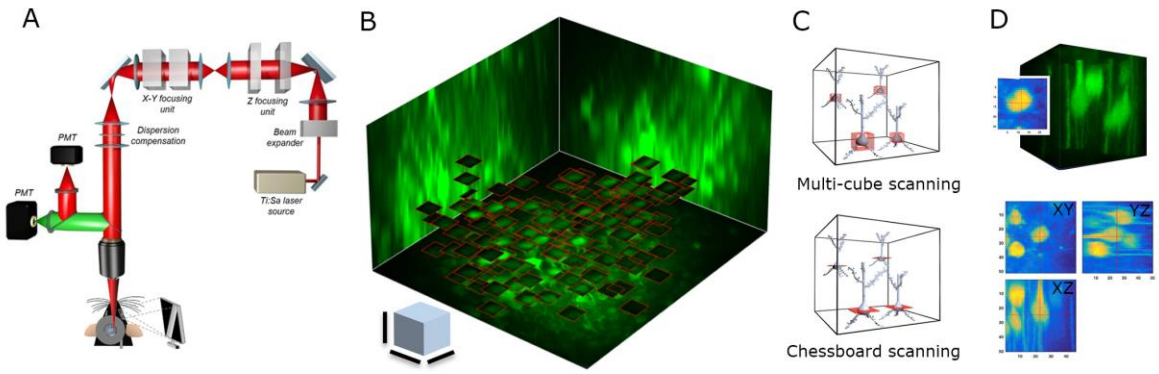
Abstract: Introduction: The systematic understanding of brain function requires methods that allow neuronal activity to be recorded at different spatial scales in 3D at a high temporal resolution. Recording techniques are required that collect information from a neuronal population situated in an extensive volume of tissue.
Aims: Technical challenge of two-photon imaging in long-term is the stability of cell recordings. Lateral and Z displacement can be managed with 2D shift scanning and Z-focusing, however, effects of XYZ rotation and tissue deformation remain unsolved.
Methods: Here we present a novel fast 3D volumetric imaging method, suitable for long-term in vivo tracking of neuronal activity in mouse cortex by acousto-optical two-photon microscopy. We could precisely identify and record from the center plane of cell bodies and track the visually evoked neuronal activity. On the first day of the experiment after a control Z-stack acquisition in near-cubic-millimeter scan range, 3D drifting acousto-optical volumetric imaging (50x50x50µm Multi-cube scans) were recorded around the selected cell bodies. On the next imaging sessions the recording coordinates were re-loaded to the acquisition software and the recording sites were roughly identified by the vascular architecture. Then new Multi-cube scans were acquired and used for fine alignment in a 3D volume.
Results: With the Multi-cube scanning method we are able to locate and record Ca2+ activity (with GCaMP6f) of the same neuronal ensemble during our 10-20 days long protocol including baseline, training and post learning imaging sessions of up to 200 cells. Our results show this method is significantly more accurate than a conventional Z-stack ROI selection.
Conclusions: We demonstrate a method, that allows us to record long term neuronal plasticity from the same cell population in a near-cubic millimeter 3D volume, up to 2 months. For this reason, the Multi-cube scanning method is suitable for long term imaging behavior protocols.
Grant: EFOP-3.6.3-VEKOP-16-2017-00009



(A) Acousto-optical 2p microscope schematic. (B) 3D Z-stack with fast drifting somatic chessboard scanning, scale bars: 50 um (C) Multi-cube and chessboard scanning schematics. (D) Multi-cube scanning with maximal-intensity projections

Disclosures: D. Pinke: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; ERC grant 682426, EFOP-3.6.3-VEKOP-16-2017-00009. **M. Marosi:** None. **G. Dobos:** None. **G. Szalay:** None. **D. Nagy:** None. **C. Csupernyák:** None. **A. Plauska:** None. **G. Katona:** None. **B. Rózsa:** None.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.03/LLL30

Topic: I.03. Anatomical Methods

Support: We wish to thank the Allen Institute for Brain Science founders, Paul G. Allen and Jody Allen, for their vision, encouragement and support.
This work was supported also by the National Institutes of Health (R01NS092474 and R01MH104227).

Title: Machine learning for conjugate light-electron array tomography

Authors: *O. GLIKO, S. SESHAMANI, F. COLLMAN, L. ELABBADY, M. KARLSSON, M. NAUGLE, R. SERAFIN, J. SCHARDT, S. J. SMITH
Allen Inst. For Brain Sci., Seattle, WA

Abstract: Conjugate array tomography (AT) integrates immunofluorescence (IF) and scanning electron microscopy (SEM) imaging of arrays of serial ultrathin sections with volume reconstruction. IF imaging using multiple molecular markers enables localization of presynaptic and postsynaptic proteins. The pipeline for generating such datasets involves several complex

steps that can be enhanced with the use of machine learning. Here, we highlight 3 such applications: focus classification, cross modal registration and synapse detection and show how the results can be integrated back into the AT pipeline.
Fully automatic identification of focus quality of a single image cannot be achieved using standard algorithmic approaches. Deep neural networks have been shown to classify out-of-focus microscope images with higher accuracy [1]. We use this approach to assess absolute defocus levels of image sub-regions. This information can then be fed back into the pipeline for automated quality control during image acquisition where images would either pass quality control, be restorable by deconvolution using an adjusted point spread function or require a retake.
Registration of IF and SEM images is a challenging problem since the appearance of structures can vary widely between these modalities. In initial work [2] we used deep learning for the prediction of myelin basic protein (MBP) IF images from SEM images. This enabled us to perform simple cross correlation based registration to register these two modalities in areas where sufficient MBP is present. Here, we generalize the application by using deep learning to estimate multiple IF markers which enhance registration by reducing dependency on a single channel and provide varied densities of features for global and local registration.
Currently used manual synapse detection methods are very tedious and time consuming for analysis of large volume datasets. We use deep networks to learn to localize synapses from multi-channel IF data. Ground truth information is collected from SEM data that is manually annotated and we show that we are able to perform pixel based detections at a rate of 85% which conforms with the current state of the art synapse detection rate in high resolution EM.
1. Yang SJ *et al.* Assessing microscope image focus quality with deep learning. *BMC Bioinformatics* (2018) 19:77.
2. Ounkomol C *et al*. Label-free prediction of three-dimensional fluorescence images from transmitted light microscopy. *bioRxiv doi: https://doi.org/10.1101/289504*

Disclosures: O. Gliko: None. **S. Seshamani:** None. **F. Collman:** None. **L. Elabbady:** None. **M. Karlsson:** None. **M. Naugle:** None. **R. Serafin:** None. **J. Schardt:** None. **S.J. Smith:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aratome LLC.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.04/LLL31

Topic: I.03. Anatomical Methods

Support: U01MH114824 to P.O.

Title: Beam shaping oblique light sheet tomography

Authors: *X. QI, A. NARASIMHAN, K. U. VENKATARAJU, D. F. ALBEANU, P. OSTEN
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Brain functions involve neural circuits with long-range projections, such as corticospinal layer 5B neurons of the motor cortex neurons mediating movement control and motor learning.
Furthermore, neurodevelopmental and psychiatric disorders have been speculated to include deficits of long-range neural circuits connecting multiple brain regions. Thus, mapping whole-brain neuron morphology at submicron resolution has the potential to reveal novel circuit organizations in normal brain and shed light on circuit pathologies in mouse models of human brain disorders.
Oblique Light Sheet Tomography (OLST) is a whole-brain volumetric imaging platform operating on the principle of light-sheet microscopy combined with serial tissue sectioning. The light-sheet illumination is based on a Gaussian beam with z thickness varying between ~ 5~7 μm. To further improve the z resolution of OLST for imaging fine details of neuronal morphology, we developed an advanced beam-shaping OLST instrument that employs beam shaping methods (Bessel, Airy and lattice beams) to achieve high axial resolution with a more uniform illumination field. Additional instrument improvements include algorithms for 3D stitching of the large whole-brain datasets and the integration of 2 cameras for two channel imaging. The 2nd generation OLST instrument promises to open new avenues to single neuron reconstructions across the whole mouse brain, improving our understanding related to the relationships between long-range neuronal circuits and brain functions as well as brain disorders.
Funding: U01MH114824 to P.O.

Disclosures: X. Qi: None. **A. Narasimhan:** None. **K.U. Venkataraju:** None. **D.F. Albeanu:** None. **P. Osten:** None.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.05/LLL32

Topic: I.03. Anatomical Methods

Support: U01MH114824

Title: Somato-dendritic morphological analysis using oblique light sheet tomography

Authors: *A. NARASIMHAN[1], U. SÜMBÜL[2], K. UMADEVI VENKATARAJU[1], R. PALANISWAMY[1], D. ALBEANU[1], P. OSTEN[1]
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Abstract: The somato-dendritic morphology is a classic feature in defining neuronal types. While morphologies of smaller subsets of anatomical regions have been studied in more detail, we aim to study the morphology and axonal projections of the pyramidal cells of the entire mouse neocortex using a custom-built Oblique Light Sheet Tomography (OLST). OLST is a whole-brain volumetric imaging platform operating on the principle of light sheet fluorescence microscopy. Briefly, the illumination/detection paths in OLST are oriented obliquely (45°) with respect to the tissue surface, allowing for imaging up to ~400 μm depth from the surface in an XY raster pattern. An integrated vibratome sections the imaged portion of the tissue once the raster scan is completed. The raster scan and automated sectioning are repeated iteratively to obtain whole brain coverage. We report that the current instrument configuration allows us to image an adult mouse brain within ~14 hrs at 0.4 x 0.4 x 2.5 μm voxel resolution with overlapping regions for image registration and reconstruction.
To identify individual neurites in our images, we obtain a sparse labeling of the pyramidal cells in Emx1-Cre mice using intravenous injections of Cre-dependent reporter AAV viruses. We also report an optimized CUBIC tissue clearing protocol to improve image quality, especially deeper into the tissue. Clearing, however, removes the lipids in the tissue, and makes the brain structurally soft. Therefore, we developed a novel gelatin-based embedding to improve the rigidity of the brain for sectioning. Finally, we developed a supervised machine learning-based approach to overcome the limitations of manual annotation and to process large volumes of data that our OLST platform generates: deep neural networks were trained to binarize the raw images and subsequently obtain the skeletons of neuronal arbors automatically. This framework enables us to study fine morphology and the spatial patterns of cortical neurons at a high-throughput, a prerequisite for identification of cell types.
Funding: U01MH114824 to P.O.

Disclosures: A. Narasimhan: None. **U. Sümbül:** None. **K. Umadevi Venkataraju:** None. **R. Palaniswamy:** None. **D. Albeanu:** None. **P. Osten:** None.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.06/LLL33

Topic: I.03. Anatomical Methods

Title: Light sheet fluorescence expansion microscopy: Fast mapping of neuronal connectivity at super resolution

Authors: *J. E. RODRIGUEZ GATICA[1], I. PAVLOVA[2], J. BÜRGERS[1], M. K. SCHWARZ[2], U. KUBITSCHECK[1]
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Abstract: The goal of understanding the architecture of neural circuits at the synapse level with a brain wide perspective (connectome) has powered the interest in high-speed and large field of view volumetric imaging at subcellular resolution. The critical details of neuronal connectivity, e.g. synapses, occur on length scales of about 100 nm. Structures small like this can optically be resolved using super resolution light microscopy. Unfortunately, this is not feasible for the reconstruction of extended neuronal networks, because all available super resolution approaches are restricted to thin samples of about 20 µm in depth, and synaptically connected neurons can be spatially separated from each other by hundreds of micrometers.
Here we combined tissue expansion and light sheet fluorescence microscopy to allow volumetric super resolution high-speed imaging of large mouse brain samples. These two methods are an ideal match to obtain super-resolved images of extended neuronal circuits with three distinctive features, namely (i) high imaging rates up to 50 Hz, (ii) high contrast and (iii) low photobleaching.
We achieve a virtual lateral and axial optical resolution of 80 and 250 nm, respectively.
We demonstrate the capabilities of this method by performing fast volumetric super resolution imaging of mouse dentate gyrus. Additionally, our approach allows us to observe eGFP-labeled proteins, thus avoiding antibody staining. In this manner neural connections can be mapped throughout all the acquired/imaged sample, allowing a better segmentation of dentate granule cell neurites for further morphology analysis, e.g. a three-dimensional Sholl analysis within the context of large cell ensembles spanning several orders of magnitude.

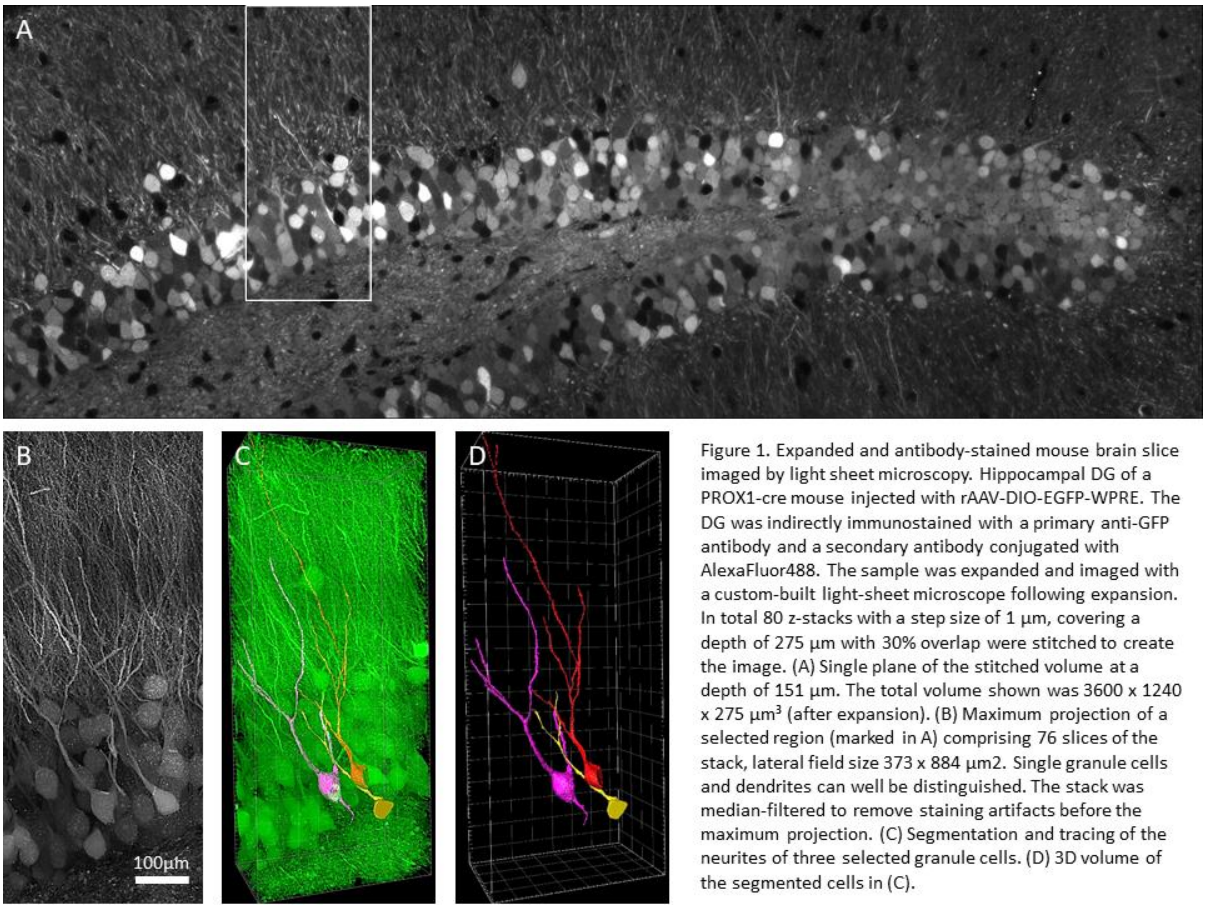

Figure 1. Expanded and antibody-stained mouse brain slice imaged by light sheet microscopy. Hippocampal DG of a PROX1-cre mouse injected with rAAV-DIO-EGFP-WPRE. The DG was indirectly immunostained with a primary anti-GFP antibody and a secondary antibody conjugated with AlexaFluor488. The sample was expanded and imaged with a custom-built light-sheet microscope following expansion. In total 80 z-stacks with a step size of 1 µm, covering a depth of 275 µm with 30% overlap were stitched to create the image. (A) Single plane of the stitched volume at a depth of 151 µm. The total volume shown was 3600 x 1240 x 275 μm^3 (after expansion). (B) Maximum projection of a selected region (marked in A) comprising 76 slices of the stack, lateral field size 373 x 884 µm2. Single granule cells and dendrites can well be distinguished. The stack was median-filtered to remove staining artifacts before the maximum projection. (C) Segmentation and tracing of the neurites of three selected granule cells. (D) 3D volume of the segmented cells in (C).

Disclosures: J.E. Rodriguez Gatica: None. **I. Pavlova:** None. **J. Bürgers:** None. **M.K. Schwarz:** None. **U. Kubitscheck:** None.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.07/LLL34

Topic: I.03. Anatomical Methods

Title: Generalized registration of multiple views in light sheet microscopy

Authors: N. PAPP, *K. KILBORN
3i, Denver, CO

Abstract: Many approaches to light sheet imaging involve acquiring 3D volumes from multiple directions in order to achieve higher resolution, reduce artifacts, and overcome limited light sheet penetration. However, proper registration of these views often requires either embedding

fluorescent beads as fiducial markers ('interest points') or locating punctate structures in the specimen itself that can serve as substitutes for deliberate fiducial markers. Either way, these approaches typically require significant user interaction and parameter selection, making registration a labor-intensive process. We propose a method which does not rely on the detection of interest points, but uses localized cross-correlation to determine shifts between corresponding small sub-volumes of the component views. Our only assumptions are that registration between views can be approximated with an affine transformation and that there is non-periodic high-frequency content in the component views.
We assume that registration error can be bounded by the physical geometry or motion control precision that has produced the individual views. The overall volume of each view is divided into sub-volumes that are large enough to span this error bound. From these sub-volumes we can construct corresponding points between views to be solved as a system of linear equations, producing a 4 x 4 matrix that describes an affine transformation from one view to another or from one view to a canonical orientation. Multiple iterations will improve the accuracy of the transformations.
Examples drawn from data collected using dual inverted selective plane illumination microscopy (diSPIM) and other light sheet architectures are presented and performance is compared to other registration approaches.

Disclosures: N. Papp: None. **K. Kilborn:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); K. Kilborn is part owner of 3i..

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.08/LLL35

Topic: I.03. Anatomical Methods

Title: Advanced Light sheet Imaging Center (ALICE): Development of a full service imaging platform - from sample clarification to 3D VR visualization

Authors: *S. PAGÈS[1,2], F. F. VOIGT[3,4], G. REYMOND[1], L. BATTI[1], C. BRANA[1], A. TISSOT[1], R. CHEREAU[2], Q. BARRAUD[5], N. CHO[5], J. SQUAIR[5], F. MOREILLON[6], P. PASSERAUB[6], F. HELMCHEN[3,4], M. GOUBRAN[7], M. ZENEIH[7], R. TOMER[8], K. DEISSEROTH[7], A. HOLTMAAT[2], G. COURTINE[5], C. LÜSCHER[2], J. DONOGHUE[1]
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Western Switzerland (HES-SO), Geneva, Switzerland; [7]Stanford Univ., Stanford, CA; [8]Columbia Univ., New York, NY

Abstract: Light sheet microscopy is a fluorescence imaging technique that allows visualization of whole organs or small organisms while preserving their physical integrity i.e. without the need to slice them prior imaging. Although the principles of this technology were developed more than 100 years ago, it is only in the last fifteen years that researchers have started to routinely apply it to biological specimen and that it has developed into a field of research of its own. At the Wyss Center for Bio and Neuroengineering in Geneva, Switzerland, we have created an imaging center which integrates a series of cutting edge and custom-tailored tools into a single working pipeline aimed at imaging whole organs at high temporal or spatial resolution. The center includes a customized version of the COLM/SPED (Tomer *et al.*, Cell 163, 2015; Tomer *et al.*, Nat. Protoc. 9, 2014) microscope for near diffraction-limited resolution imaging of large clarified samples (cm range). We have optimized the design of this microscope to allow a quick exchange of different objectives and to enable the imaging of very large samples (> 10 cm) at a sub micronic spatial resolution. Recently we expanded the capabilities of light sheet microscopy, setting up a large-scale imaging system: mesoSPIM (see poster by Fabian F. Voigt). This customized system enables whole brain imaging at cellular resolution, in a few minutes with no need for further image-stitching processes. Finally, we are collaborating with research groups setting up innovative analysis tools (e.g. MIRACL pipeline now available for automated segmentation of clarity-optimized data sets and registration in the Allen Brain Atlas) and developing in house innovative 3D exploration that will enable researchers to navigate and segment their own light sheet data in a Virtual Reality environment. This pipeline offers to the researcher the possibility for large scale screening, high-resolution imaging and data visualization and analysis. We will show, as an example, how using this unique pipeline, it is possible to map anatomical projections emerging from and targeting the posterior medial nucleus of the thalamus in mouse brain. We will also show how this unique technical approach may help to understand the organization of the ascending motor pathways from the spinal cord to different brain regions involved in the control of voluntary movement.

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Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.09/DP15/LLL36

Topic: I.03. Anatomical Methods

Support: ERC ID 670757: BRAINCOMPATH

Title: The mesoSPIM initiative - open-source light-sheet microscopes for imaging in cleared tissue

Authors: *F. F. VOIGT[1,3], D. KIRSCHENBAUM[4], S. PAGES[5], L. EGOLF[6,3], R. KASTLI[6,3], A. VAN DER BOURG[2,3], K. LE CORF[7], K. HAENRAETS[8,9], N. FREZEL[10], F. MOREILLON[11], E. PLATONOVA[12], A. IQBAL[6,3], T. TOPILKO[13], N. RENIER[13], H. U. ZEILHOFER[14,9], T. KARAYANNIS[6,3], A. A. FRICK[15], U. ZIEGLER[12], L. BATTI[16], A. HOLTMAAT[17], C. LUSCHER[18], A. AGUZZI[4], F. HELMCHEN[19,3]
[1]Brain Res. Inst., Zurich, Switzerland; [2]Brain Res. Inst., Zürich, Switzerland; [3]Neurosci. Ctr. Zurich, Zurich, Switzerland; [4]Univ. Hosp. Zurich, Zurich, Switzerland; [5]Wyss Ctr. For Bio and Neuroengineering, Geneve, Switzerland; [6]Brain Res. Institute, Univ. of Zurich, Zurich, Switzerland; [7]Neurocenter Magendie, Bordeaux, France; [8]Univ. of Zurich, Inst. of Pharmacol. and Toxicology, Zurich, Switzerland; [9]Inst. of Pharmaceut. Sciences, ETH Zurich, Zurich, Switzerland; [10]Inst. for Pharmacol. and Toxicology, Univ. of Zurich, Zurich, Switzerland; [11]Univ. of Applied Sciences, Western Switzerland, Geneva, Switzerland; [12]Ctr. for Microscopy and Image Analysis, Univ. of Zurich, Zurich, Switzerland; [13]ICM - Brain & Spine Inst., Paris, France; [14]Inst. of Pharmacology, Univ. of Zurich, Zurich, Switzerland; [15]INSERM U1215, Bordeaux cedex, France; [16]Wyss Ctr., Geneva, Switzerland; [17]Neurosci., Univ. of Geneva, Geneva, Switzerland; [18]Univ. Geneva, Geneva, Switzerland; [19]Brain Res. Inst. / Univ. of Zurich, Zurich, Switzerland

Abstract: Tissue clearing methods have recently seen a renaissance with a wide variety of clearing approaches now available. In neuroscience, the combination of tissue clearing with light-sheet microscopy is ideal to bridge scales from the µm to cm-level, thus providing a link on the mesoscale for detailed 3D anatomical investigations. To optimally image cleared samples, we set out to design a modular light-sheet microscope that combines extremely simple sample mounting and exchange with large field-of-views (FOV) of 2-22 mm to provide users with overview datasets within minutes. Especially for such large FOVs, common light-sheet microscopes suffer from non-uniform axial resolution due to the varying thickness of the light-sheet which in turn drastically reduces data quality. To circumvent this problem, we are using tuneable lenses to shift the excitation beam waist through the sample in synchrony with the rolling shutter of the camera. For whole mouse brains, typical datasets are isotropic (5 µm sampling), small (12-16 GB/colour), and generated quickly (7-8 minutes). Together with standardized quick-exchange sample holders, these features allow fast screening of samples for clearing, imaging, and labelling quality and thus speed up research projects tackling questions involving cell distributions and projection patterns. After creating overview datasets, users can zoom in and acquire high-resolution data. The microscope has been tested and validated in combination with common clearing methods ranging from hydrogel-based techniques such as CLARITY to organic solvent approaches such as iDISCO – by using a modular design of the imaging chambers, switching between different imaging media can be done in less than a minute. Recently, we have realized four such microscopes at various institutions across Switzerland as

part of the mesoSPIM initiative (mesospim.org) – a project aimed at creating a community to accelerate the exchange of tissue clearing and mesoscale imaging expertise. Microscope hard- and software are open-source and we welcome suggestions and improvements.

Disclosures: F.F. Voigt: None. **D. Kirschenbaum:** None. **S. Pages:** None. **L. Egolf:** None. **R. Kastli:** None. **A. Van Der Bourg:** None. **K. Le Corf:** None. **K. Haenraets:** None. **N. Frezel:** None. **F. Moreillon:** None. **E. Platonova:** None. **A. Iqbal:** None. **T. Topilko:** None. **N. Renier:** None. **H.U. Zeilhofer:** None. **T. Karayannis:** None. **A.A. Frick:** None. **U. Ziegler:** None. **L. Batti:** None. **A. Holtmaat:** None. **C. Luscher:** None. **A. Aguzzi:** None. **F. Helmchen:** None.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.10/LLL37

Topic: I.03. Anatomical Methods

Title: SPED microscopy with GPU accelerated deconvolution

Authors: *P. SCHWARZ, M. HIRTE, C. R. YU
Stowers Inst. for Med. Res., Kansas City, MO

Abstract: The advent and evolution of tissue clearing methods has increased demand for microscopy methods capable of acquiring images with a high speed and a large field of view. One such method, Spherical-aberration-assisted Extended Depth-of-field light sheet microscopy (SPED-LSM) generates and scans a light sheet along a depth of field that has been elongated by induced spherical aberration. SPED-LSM can theoretically acquire data as fast as modern sCMOS cameras can operate, but it suffers from computationally expensive deconvolution after the acquisition. Here we discuss the construction of a SPED-LSM using salvaged components from a decommissioned microscope including lasers, acousto-optic tunable filters, and galvanometric scanning mirrors. Together with an improved embedding method and modified software, we performed imaging of entire mouse brains cleared with CUBIC and SCALE. We also implemented a modified algorithm to accelerate the deconvolution by moving the computation from the CPU to a GPU. The deconvolution approach improved speed performance by two to four times when compared with the algorithm used in the original demonstration of SPED microscopy, making SPED microscopy more practical for large datasets. These improvements enable us to analyze the structure and function of interconnected neurons throughout the brain.

Disclosures: P. Schwarz: None. **M. Hirte:** None. **C.R. Yu:** None.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

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Program #/Poster #: 611.11/LLL38

Topic: I.03. Anatomical Methods

Support: Agence Nationale de la Recherche under contracts ANR-11-EQPX-0029 (Equipex Morphoscope2)
ANR-10-INBS-04 (France BioImaging)
ANR-10-LABX-65 (LabEx LifeSenses)
Université Paris-Saclay (Initiatives Doctorales Interdisciplinaires)
Fondation pour la Recherche Médicale
European Research Council (ERC-CoG 649117)

Title: Multicolor large volume imaging using chromatic serial multiphoton microscopy

Authors: *L. ABDELADIM[1,2], K. S. MATHO[3,2,4], S. CLAVREUL[3], P. MAHOU[2], J.-M. SINTES[2], X. SOLINAS[2], I. ARGANDA-CARRERAS[5], S. TURNEY[6], J. W. LICHTMAN[6], A.-P. BEMELMANS[7], K. LOULIER[3], W. SUPATTO[2], J. LIVET[3], E. BEAUREPAIRE[2]
[1]Ecole Polytechnique, Paris, France; [2]Lab. of Optics and Biosci., Ecole Polytechnique, CNRS, INSERM, Palaiseau, France; [3]Inst. de la Vision, Sorbonne Universités, INSERM, CNRS, Paris, France; [4]CSHL, Cold Spring Harbor, NY; [5]Univ. of the Basque Country (Ikerbasque) and Donostia Intl. Physics Ctr., San Sebastian, Spain; [6]Ctr. for Brain Sci. and Dept. of Mol. and Cell. Biol., Harvard Univ., Cambridge, MA; [7]Mol. Imaging Res. Cnter, CEA, Fontenay-aux-roses, France

Abstract: Recent strategies for large-scale microscopy enable micron-resolution imaging over cubic millimeters of tissue, hence transforming brain imaging. These approaches however currently lack efficient multicolor contrast modalities. We present chromatic serial multiphoton (Chrom-SMP) microscopy, a novel method combining multicolor two-photon excitation through wavelength mixing (Mahou et al. Nature Methods 2012) and serial block-face acquisition. This approach enables organ-scale imaging of spectrally distinct fluorescent proteins with intrinsic submicron channel registration and constant diffraction-limited resolution over the entire imaged volume. This technology also permits whole-brain label-free imaging based on third harmonic generation (THG) and coherent anti-Stokes Raman scattering (CARS) contrast which provide detailed morphological context. We combine Chrom-SMP with Brainbow transgenic markers and viral or electroporation-based multicolor labeling strategies in the mouse brain and demonstrate continuous 3D multicolor imaging over cubic millimeters of neural tissue as well as brain-wide serial 2D multichannel imaging. We illustrate the potential of this method through color-based analysis of astrocyte morphology and spatial interactions in the cerebral cortex, and

multiplexed whole-brain mapping of axonal projections labeled with distinct tracers. Chrom-SMP is therefore a robust and broadly applicable scheme for high resolution multicolor imaging over large tissue volumes, enabling to upscale color-based approaches for analysis of neural cell morphology, connectivity and lineage to the whole brain.

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Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.12/LLL39

Topic: I.03. Anatomical Methods

Support: Kavli Neuroscience Discovery Institute (JHU) Postdoctoral Fellowship Grant

Title: Registration methodology for cleared rodent brain tissue

Authors: *A. E. BRANCH[1], D. J. TWARD[2], V. CHANDRASHEKHAR[3], M. MILLER[3], J. T. VOGELSTEIN[3], M. GALLAGHER[4]
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Abstract: Anatomical mapping of brain imaging data is essential for assessing disease-relevant alterations in brain structure and analyzing neural circuit connectivity. Numerous protocols were recently developed to render brain tissue optically clear, facilitating intact whole-brain lightsheet microscopy. However, methods for mapping the resulting cleared-brains to reference atlases are both ineffective and computationally burdensome. We are building a computational ecosystem to register cleared brains to atlases which is reliable and compatible with multiple clearing protocols, lightsheet imaging modalities, and species. Existing nonrigid registration methodologies were largely developed for human MRI. Application of such algorithms to lightsheet generated whole brain image sets requires overcoming challenges not present in human brain MRI. The primary challenge is missing data, caused by limited field of view acquisitions or removed tissue, resulting in a mismatch between the atlas and data set. We address this by registration of observed data only, employing binary masks in the case of limited field of view, or non-binary weights when the location of missing tissue must be estimated. The second challenge is inconsistent image intensities between available atlases and observed images. When intensities of anatomical structures are ordered consistently locally, for example a structure in the atlas is brighter than its surroundings whenever the same is true for the observed

image, a local rank transformation is sufficient to bring the intensities into agreement, and estimating mappings by minimizing a mean square error objective function is appropriate. When this is violated, we use an objective function based on mutual information. The final challenge is modality specific artifacts that arise in lightsheet imaging. Shadowing artifacts that occur when tissue interfaces are tangent to the direction of an illuminating laser are removed with a notch filter to remove spatial frequencies normal to each laser. Grid artifacts from stitching together small volumes of nonuniform illumination are removed by estimating the nonuniformity using its consistency from one slice to the next. These strategies have enabled accurate registration between well characterized atlases and small animal images, allowing us to quantify the distribution of cells and their connections by anatomical region. These approaches are being combined into a open source package called ndreg, available from neurodata.io, which can be used to align CLARITY and iDISCO cleared brains generated from either mouse or rat brains.

Disclosures: A.E. Branch: None. **D.J. Tward:** None. **V. Chandrashekhar:** None. **M. Miller:** None. **J.T. Vogelstein:** None. **M. Gallagher:** None.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.13/LLL40

Topic: I.03. Anatomical Methods

Support: NSF EAGER: ACI-16449916

Title: Whole brain cellular resolution imaging reveals layer-specific neuronal deficits upon cortical topoisomerase I deletion

Authors: *O. KRUPA[1], Q. WANG[2], G. FRAGOLA[3], P. ARIEL[4], E. HADDEN-FORD[3], Z. HUMPHREY[3], T. LIU[3], S. FRIDAY[3], S. WANG[2], M. J. ZYLKA[3], G. WU[2], J. L. STEIN[5]
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Abstract: Topoisomerase I (Top1) is a transcriptional regulator that is broadly expressed in postmitotic neurons of the developing and adult brain. Inhibition of topoisomerase activity dose-dependently downregulates transcription of long neuronal genes typically involved in synaptic function, neurotransmission, and axonogenesis. Mutations in topoisomerases have also been linked to autism, intellectual disability, and neurodegeneration, yet little is known about the functional or structural brain deficits caused by these mutations. To address this question, we generated a conditional knockout mouse (cKO) model using a Nex1-CRE driver to specifically delete Top1 in excitatory neurons. By performing whole mount immunostaining of P15 mice using iDISCO+ along with rapid imaging by light-sheet microscopy, we observe 1) a dramatic

reduction of cortical thickness including a complete loss of layer V throughout the cortex and 2) degeneration of pyramidal layers within the hippocampus. This suggests Top1 preferentially influences the survival of only certain neuronal populations. To automatically localize and quantify cell-types within these structures, we implement a novel cascading convolutional neural network (CCNN) to accurately segment cell nuclei in densely labeled regions and random forest regression of image landmarks to map anatomical correspondence in the Top1 phenotype. Currently we are upgrading our CCNN to whole brain 3D nuclear segmentation scenario while also acquiring cellular resolution images of multiple whole brain samples. Using this approach, we will quantify whole brain structural impacts of Top1-associated neuropathology. Furthermore, the tools developed here will be useful in quantifying cellular level structural deficits in other animal models of neuropsychiatric disease or human samples.

Disclosures: O. Krupa: None. **Q. Wang:** None. **G. Fragola:** None. **P. Ariel:** None. **E. Hadden-Ford:** None. **Z. Humphrey:** None. **T. Liu:** None. **S. Friday:** None. **S. Wang:** None. **M.J. Zylka:** None. **G. Wu:** None. **J.L. Stein:** None.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.14/LLL41

Topic: I.03. Anatomical Methods

Support: Korea Research Foundation

Title: Optimizing tissue clearing method for human brain tissue: Preparing methods, clearing efficiency, staining methods, and imaging strategy

Authors: *K. MIN SUN[1], J. AHN[2], J. MO[2], H. SONG[2], H. CHOI[2]
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Abstract: Currently, the tissue clearing method is actively used in neuroscience research. Recent advances in tissue clearing method allow visualization of neural networks inside of unsectioned whole brain tissues. However, a protocol applicable for human brain tissue has not yet been optimized because of difficulty to gain fresh human samples. We aimed to optimize human brain clearing and imaging methods using fresh human samples. Fresh human brain samples obtained at autopsy and cadavers donated for medical school practice were used. The cadaver sample was fixed for more than one year, and the autopsy sample was fixed for about one week. We applied active electrophoretic clearing for human brain tissue. Every hour after clearing, we quantified the degree of transparency using Image J. DAPI(Nucleus, 5days dye incubation) and GFAP(Astrocyte, 5 days primary antibody incubation) was stained. Confocal microscope was

used to investigated staining depth. Fresh human brain samples showed higher clearing efficiency compared with cadaver samples (30% transparent; 1hr vs. 30hr). Fresh human brain samples showed fewer autofluorescence compared with cadaver sample. DAPI was stained to over 300μm for fresh human brain samples. However, there was no DAPI staining in cadaver sample. GFAP staining was vivid until the staining depth of 20-50um, on the other hand, DAPI showed higher penetration pattern 300um. For GFAP staining, fresh human brain samples were stained denser than cadaver samples. For NeuN, no staining was shown. We have shown successful human brain 3D imaging results using fresh human samples. Fresher samples have better clearing efficiency, fewer autofluorescence, high number of stained antigen. Fresh human brain samples are prerequisite for 3D visualization of human brain.

Disclosures: J. Ahn: None. **J. Mo:** None. **H. Song:** None. **H. Choi:** None.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.15/LLL42

Topic: I.03. Anatomical Methods

Support: U01MH105971

Title: Creation and anatomically based registration of 3D histological brain atlas in the prairie vole

Authors: *R. MUÑOZ CASTAÑEDA[1], K. UMADEVI VENKATARAJU[1], T. BURKHARD[2], S. M. PHELPS[2], P. OSTEN[1]
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Univ. of Texas at Austin, Austin, TX

Abstract: Complex behavior requires the coordinated function of a complex network of different types of neurons and glia. To understand how behavior emerges from these many interacting cell types, it is essential to study the three-dimensional brain at cellular resolution. New imaging techniques, such as serial two-photon tomography (STPT) and light-sheet fluorescence microscopy (LSFM), allow us to generate whole-brain datasets at a cellular resolution. To acquire and make sense of this enormous amount of information, however, it is also essential to automate acquisition and analysis of such data. In order to better understand the neurobiology of bonding, we are developing an automated pipeline for the acquisition and analysis of cellular resolution data from the brains of the socially monogamous prairie vole. Such data can be compared to similar data from traditional model species like the laboratory mouse. The first step in creation of an automated computational pipeline for each new species is the creation of a 3D reference brain whose regional volumes are co-registered to an anatomical atlas. Since Nissl and related stains are the standard methods to define anatomical brain structures, we used

NeuroTrace fluorescent Nissl-staining on intact prairie vole and mouse brains to perform 3D imaging with STPT and LSFM. We use genetic labels such as Cre-based reporter mouse lines (eg ChAT-IRES-Cre) to provide additional delineations of neuroanatomical structures. We demonstrate the validity of the present pipeline by creating mouse and prairie vole 3D brain atlases with cellular resolution. The resulting atlases allow us to analyze whole brain anatomy and cell distribution in the two rodent species and suggest a general framework for the creation and analysis of such maps in other useful species.

Disclosures: R. Muñoz Castañeda: None. **K. Umadevi Venkataraju:** None. **T. Burkhard:** None. **S.M. Phelps:** None. **P. Osten:** None.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.16/LLL43

Topic: I.03. Anatomical Methods

Support: CONACYT 253631, Fronteras 374
DGAPA IN210215.

Title: Identification in male rats by manganese enhanced magnetic resonance, of the neural circuits controlling sexually motivated behaviors

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[1]Intituto de Neurobiología, UNAM, Querétaro, Mexico; [2]Neurobiología, UNAM, Juriquilla, Mexico

Abstract: Two motivated behaviors that are crucial for the expression of sexual behavior are sexual incentive motivation (SIM) and partner preference (PP). In the SIM test no physical contact is possible while In the PP test the subjects can interact with the stimulus animals quantifying the sexual interaction and the time spent in each compartment. The possible circuits controlling these behaviors have not been studied using magnetic resonance imaging. The aim of the present study is to determine by manganese enhance magnetic resonance imaging (MEMRI) the different neural circuits, activated in SIM and PP**.** The use of MEMRI allows mapping the brain of the animal in vivo where manganese ions (Mn2+) pass through the blood brain barrier and enter into excited cells via voltage-gated calcium channels identifying brain regions activated by a particular behavior (Takeda et al., 2003). In the present experiments, MnCl2 (16 mg/kg) was administered 24 h before the behavioral tests and immediately thereafter the subjects were placed in a Bruker 7T MR scanner. Sexual behavior, PP and SIM were not affected by the administration of manganese at 16 mg/kg. With this dose, we obtained a good contrast for MRI analysis. The image analysis revealed an activation of the medial preoptic area, anterior

hypothalamus, amygdala, nucleus accumbens and hippocampus after the SIM and PP tests. These regions were activated in the females when tested in week 5 and 10. The same regions were activated in males in week 10 suggesting that experience in males and females induces a differential activation of circuits controlling motivated behaviors such as SIM and PP. **Technical assistance F. Camacho, supported by CONACYT 253631, Fronteras 374 and DGAPA IN203518.**

Disclosures: R. Paredes: None.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.17/LLL44

Topic: I.03. Anatomical Methods

Title: Identification of brain regions by hyperspectral imaging without staining

Authors: *S. INOUE[1], K. HOTTA[2], K. OKA[2]
[1]Keio Univ., Yokohama, Japan; [2]Keio Univ., Yokohama, Kanagawa, Japan

Abstract: Brain has many anatomically identified regions with specific functions. For the comparative studies of brains from different animals, brain map plays an important role. However, in previous studies, specific brain regions are generally identified by the cytoarchitectures visualized by several staining methods. Furthermore, their validity and reproducibility as brain maps have not been adequately studied, so brain maps have been conventionally prepared empirically. In this study, we propose a new method for identifying specific brain regions by hyperspectral imaging of brain tissues. The transmitted light of tissues reflects anatomical structure and compositions as spectral intensity. We detect difference of the hyperspectral information and rationally identify specific brain regions. This method requires non-staining of tissues, so we expect the results different from preceding study. It is difficult to identify specific anatomical information from the spectrum, *a priori*. Therefore, we analyzed

broad-band spectrum with several information technique and tried imaging without staining or specific targets. We applied this method to the brain of zebra finch (*Taeniopygia guttata*). The brain sections were irradiated with white light and transmitted light was acquired with a hyperspectral camera. The spectral resolution was 5 nm in the observable wavelength range (380-1000 nm). Each band provided different images from the same sample. We applied principal component analysis to the spectrum, and the image of the second principal component showed a specific structure that cannot be seen obviously in the original image.

Disclosures: S. Inoue: None. **K. Hotta:** None. **K. Oka:** None.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.18/LLL45

Topic: I.03. Anatomical Methods

Title: 3D brain structure in zebra finch by CUBIC and voronoi tessellation

Authors: *M. ENDO, S. INOUE, M. INDA, K. HOTTA, K. OKA
Keio-Univ, Yokohama-Shi, Japan

Abstract: The zebra finch (*Taeniopygia guttata*) is one of the species that acquires song, and communicates each other by songs. The male zebra finch leans tutor's song and acquires his own song with individually difference. While the female zebra finch discerns male's songs and shows preference for specific songs. From these sexually different functions in vocal communication, the brains of male and female finches have different brain structures and functions. Recently, sexual differences of the brains have been studied, but it was mainly to focus on limited auditory areas such as LMAN and HVC (Long *et al*., 2009). We, therefore, comprehensively investigate the whole brain structure by using transparent technique. We focused on the female brains because there is no detailed brain map for female zebra finches. Also, even in the male zebra finch, a 2D brain map has been produced, but a 3D brain map has not been created. Therefore, in this research, we attempt to create 3D brain maps of male and female finches to compare between their brain structures. We succeeded in clearing the female brain using the clearing method, CUBIC (Susaki *et al*., 2014) with a little modification. In addition, we succeeded to detect the nucleus by propidium iodide (PI) staining with light sheet microscopy (Alpha ^{3}, PhaseView) and we obtained about 50,000 positions of the nuclei in part of the midbrain and cerebellum. Based on these nuclear positions, Voronoi tessellation is performed to create a draft map of the brain. By comprehensively observing, we explored sexual differences that have not been characterized before.

Disclosures: S. Inoue: None. **M. Inda:** None. **K. Hotta:** None. **K. Oka:** None.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

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Program #/Poster #: 611.19/LLL46

Topic: I.03. Anatomical Methods

Support: The Swedish Research Council (grant 2017-00815)
The Swedish Brain Foundation (grant FO2017-0107)

Title: Precise calculations of Iba-1 positive cells in cerebral hemispheres of mice

Authors: *D. E. KACZYNSKA[1], S. KANATANI[1], N. TANAKA[1,2], P. UHLÉN[1]
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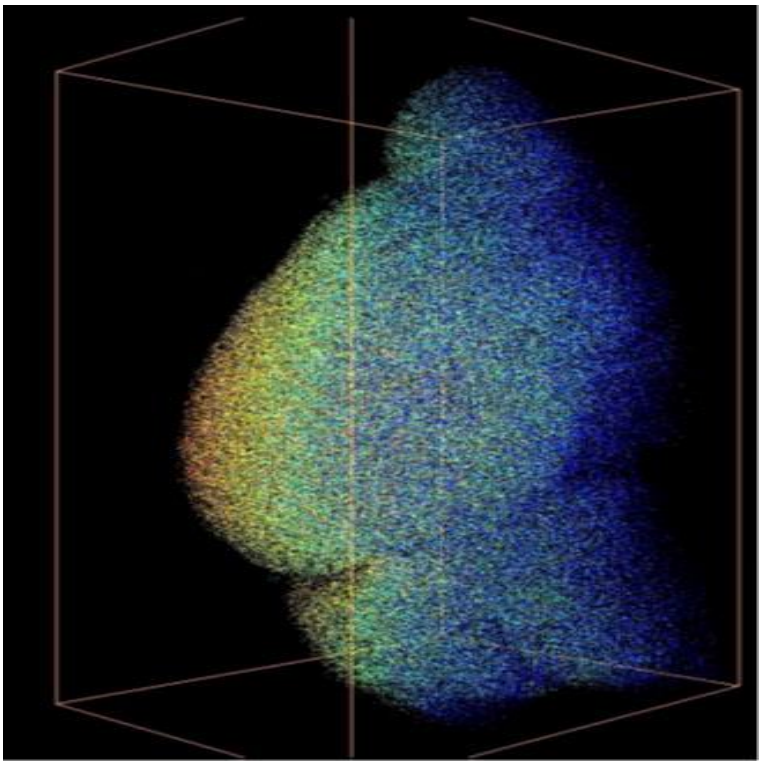
Abstract: Cutting tissues into thin slices has been the standard practice for many years in scientific research. This method provides two-dimensional information about the tissue. However, life occurs in three dimensions (3D); for this reason, scientists have always tried to extend tissue imaging to thick specimens. In neuroscience, visualization of intact brains in 3D is of intense focus. Previously, we developed a new imaging platform termed DIPCO (Diagnosing Immunolabeled Paraffin-Embedded Cleared Organs) that uses 3D light-sheet microscopy and whole-mount immunolabelling of cleared samples to study proteins and micro-anatomies deep inside of tumors (Tanaka et al., Nature Biomedical Engineering, 2017). Here, we have further optimized this method for whole-mount immunostaining of mice brains to calculate the number of cells in the intact tissue. We were able to accurately calculate the number of microglial cells to 1.599.622 in the cerebral hemisphere of a P28 mouse using the Iba-1 marker. To our knowledge this is the first time that the exact number of cells has been determined in a mouse brain. We believe that this pipeline can be applied for precise calculations of the cellular composition of various brain regions to help us understand the structure and function of adult brain.



Disclosures: D.E. Kaczynska: None. **S. Kanatani:** None. **N. Tanaka:** None. **P. Uhlén:** None.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.20/LLL47

Topic: I.03. Anatomical Methods

Title: Volumetric analysis of hexachlorophene-treated rats by MRM and stereology for neuropathology

Authors: *M. A. STAUP[1], D. BROWN[1], C. JOHNSON[1], P. LITTLE[2], R. SILLS[3], G. A. JOHNSON[4]
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Abstract: Standard neuropathological assessment of the effect of toxic compounds on supraspinal areas of the central nervous system (CNS) involves a routine examination of seven coronal brain sections. A microscopic evaluation of prescribed 2-dimensional fields of view from

these sections is the traditional approach for identifying suspect lesions and other abnormalities. This method draws conclusions about whole structures in the brain based upon abnormal histological findings and, occasionally, length and area measurements. Two methods that complement our standard neuropathological assessments include magnetic resonance microscopy (MRM) and stereology, which offer the advantage of absolute and estimated (respectively) volumetry of lesions and other regions of interest. In the present study, myelinated tracts of adult male Sprague-Dawley rats were targeted with oral-administration of hexachlorophene. Hexachlorophene is a well-characterized organochlorine compound that causes vacuolation within the intraperiod line of central and peripheral myelin sheaths. Volume differences of discrete fiber bundles within the CNS were measured in hexachlorophene-treated (n=7) and vehicle-treated (n=10) animals using the stereology-based Cavalieri method of volume estimation from systematic uniform random sections. Volume analysis was also performed on the same animals using diffusion-weighted, 3-dimensional renderings reconstructed from MRM sectioning of the entire brain. The areas analyzed were the anterior part of the anterior commissure (aca), the longitudinal fasciculus of the pons (lfp), and the pyramidal tracts (py). Sampling for the Cavalieri method was adjusted to obtain a suitable coefficient of error (CE), which contributed less to the overall variance than the biological variability between animals. The standard deviations generated by both techniques were tested for statistical power. Our results lead us to the conclusion that volumetric analysis by MRM and stereology add significant value to our standard 2-dimensional microscopic evaluations.

Disclosures: M.A. Staup: A. Employment/Salary (full or part-time):; Charles River Labs, Inc. **D. Brown:** A. Employment/Salary (full or part-time):; Charles River Labs, Inc. **C. Johnson:** A. Employment/Salary (full or part-time):; Charles River Labs, Inc.. **P. Little:** None. **R. Sills:** None. **G.A. Johnson:** None.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.21/LLL48

Topic: I.03. Anatomical Methods

Title: Optogenetic blood oxygenation level dependent (BOLD) and cerebral blood volume (CBV) fMRI

Authors: *F. SCHMID[1], M. CHOY[1], A. J. WEITZ[2], J. H. LEE[1,2]
[1]Neurol., [2]Bioengineering, Stanford Univ., Stanford, CA

Abstract: fMRI is an important technique that offers non-invasive assessment of activity across the whole brain. It provides crucial information about network activity, complimenting electrical and optical neurophysiological methods that offer high specificity and cellular resolution. fMRI

can detect signal changes due to the inherent blood oxygen-level dependent effect (BOLD), or due to mediated signal changes using iron oxide nanoparticles as a vascular contrast agent reflecting changes in cerebral blood volume (CBV). Both techniques can indirectly measure changes in brain activity with good spatial and temporal resolution, revealing information about changes in network activity related to external stimulations such as sensory stimulation or selective direct activation of cells in the brain through neuromodulation technologies such as optogenetics. However, fMRI detected signal changes are usually small and limited by the contrast to noise ratio (CNR). Especially at high magnetic fields strengths and in the presence of implants, images are prone to artifacts. This calls for careful optimization of MRI methods to find the optimal balance between CNR, image quality and spatial and temporal resolution.
Here, we optimize both BOLD and CBV fMRI. We compare gradient echo MRI using echo-planar (EPI) or Spiral readouts, assess choice of acquisition bandwidth and echo time related to the T_2^* relaxation time and discuss shimming and use of saturation bands to remove unwanted signal. Experiments were performed on rats expressing ChR2 virally transduced under the CamKII promotor. MRI was performed on a Bruker Biospec at 7 T. Optogenetic stimulations were performed under medetomidine sedation for BOLD and CBV fMRI in subsequent experiments in the same animals, and imaging sequence parameters were varied to assess signal-to-noise and CNR dependence. For CBV fMRI, ferumoxytol was injected into the tail vain prior to imaging.
Results show larger areas of activation from CBV scans compared to BOLD fMRI. Variation of echo times (TE) showed the highest CBV contrast with a signal change of approximately 10%. Highest BOLD contrast was achieved with a signal change of 3.5%. Repeated measurements of T_2^* in CBV experiments showed a continuous increase in T_2^* of approximately 1 ms/h, hinting that for long scan sessions and quantitative analyses of CBV signal amplitudes, adjustment of TE could be beneficial.

Disclosures: F. Schmid: None. **M. Choy:** None. **A.J. Weitz:** None. **J.H. Lee:** None.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 611.22/LLL49

Topic: I.03. Anatomical Methods

Title: Accurate and rapid estimation of cell culture confluency, transfection efficiency and total cell number

Authors: *L. ANTANAVICIUTE[1], S. DUBACQ[2], O. VARET[2]
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Abstract: Accuracy and efficiency in cell culture quality checks are extremely important in cell biology based studies in order to avoid any potential complications in the downstream analysis. Usually, key cell parameters such as total cell counting, confluency and cell size measurement are assessed and estimated visually in a subjective way. However, visual assessments are unreliable, time consuming and often yielding inaccurate results which lead to incorrect conclusions and recommendations. A novel approach was employed and investigated for rapid and accurate estimation of cell confluency, transfection efficiency and total number of cell counting in an automated manner using a smart cell imager (InCellis®, Bertin Technologies) in this study. An appropriate application embedded on the imager was used to estimate cell confluency and a total cell number using breast cancer cell line (MCF-7) cultures, whereas HeLa cell line culture was used for transfection efficiency estimation. Images of both cell lines were captured in series using smart cell imager in phase contrast mode using 20x magnification across the three-day experiment. The results obtained showed the MCF-7 cell culture confluency increased over the period as expected, and ranged from 14% on day-1 to 77% on day-3. Total cell counting was performed on two MCF-7 culture dilutions (1/5 and 1/10) to investigate the accuracy of the application capabilities. A total of 6.3M cells were observed in the culture petri dish with a 3% standard deviation for 1/5 dilution and a 3.5% for 1/10 dilution. The cell counting results obtained using InCellis® were further compared to the results obtained from the manual cell counting (Malassez cell) method, which was also used a reference. No significant difference was observed in a total cell number between the two methods. HeLa cell culture line and GFP fluorescence label were used for validation of an automated transfection efficiency application. A user-friendly transfection application that requires only a 3-step workflow allows for easy and spontaneous observation of transfected cells. All three applications (confluency, transfection and total cell counting) tested here, yielded robust results in a significantly less “hands-on” time compared to the traditional methods, and is a reliable new tool for all cell-based assays.

Disclosures: L. Antanaviciute: A. Employment/Salary (full or part-time):; Bertin Corp. **S. Dubacq:** A. Employment/Salary (full or part-time):; Bertin Technologies. **O. Varet:** A. Employment/Salary (full or part-time):; Bertin Technologies.

Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

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Program #/Poster #: 611.23/LLL50

Topic: I.03. Anatomical Methods

Support: NIDA R01 DA042057
VA I01 RX001511
Wayne State MD/PhD Program

Title: Optimizing photoacoustic imaging of lacZ cleavage products

Authors: *J. I. MATCHYNSKI[1], R. MANWAR[2], K. KRATKIEWICZ[2], S. A. PERRINE[3], A. C. CONTI[3], M. R. N. AVANAKI[2]
[1]TNP, Wayne State Univ. Sch. of Med., Detroit, MI; [2]Biomed. Engin., [3]Wayne State Univ., Detroit, MI

Abstract: PA imaging can provide high spatial and temporal resolution images based on a technique that can distinguish the location and quantify the amount of light absorption from chromophores in tissue. This has wide applicability, such as in PA functional imaging, a technique based on endogenous chromophores. However, PA imaging is not limited to endogenous compounds, it is also inclusive of exogenously-added contrast agents. Selective organic dyes and nanoparticles with high optical absorption at wavelengths where endogenous chromophores absorb weakly can be valuable for generating a targeted signal with a high contrast to noise ratio. For example, virally-infected tumor cells can be made to heavily express enzymes capable of cleaving colorless substrates into colored products, a process observed in the lacZ gene enzyme system. This system is ideal for contrast-enhanced PA as a vast array of available substrates can be used to create dyed products that absorb light strongly in the near infrared window (650-900 nm), where many endogenous chromophores have lower absorptivity. Both X-gal (absorption peak = 615 nm) and Green-gal (absorption peak = 665 nm), have the potential to be effective contrast agents. We used a PA phantom setup to observe where PA signal differences between the exogenous dyes and blood, the strongest endogenous signal, are maximized. Our phantom consisted of a tube filled with the colored product of interest submerged in an acrylic box filled with milk to simulate brain tissue's optical scattering. We used an 18.5 MHz, 128 element L-22 Ultrasound transducer to record PA signal produced by pulsed laser illumination and compared it to blood and saline at 550-1100 nm. We report PA signal, as a function of wavelength, for X-gal and Green-gal products, and blood. We then found wavelengths on the scanned electromagnetic spectrum with the highest difference in PA signal between blood and colored products to be maximized for Green-gal and X-gal product at 700 nm and 725 nm, respectively. At these wavelengths, we tested substrates to optimize the concentrations needed to create a PA signal that was significantly higher than blood's background intensity. Overall, our findings support use of Green-gal as a potential agent for contrast-enhanced PA based on our acquired signal differences.

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Poster

611. Anatomical Methods: Staining, Tracing, and Imaging Techniques

Location: SDCC Halls B-H

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Program #/Poster #: 611.24/LLL51

Topic: I.03. Anatomical Methods

Support: JSPS KAKENHI Grant Number 16J05041
JSPS KAKENHI Grant Number 25221004
World Premier International Research Center Initiative
JSPS KAKENHI Grant Number 23115006
AMED Brain/MINDS

Title: Three-dimensional single-cell-resolution whole-brain atlas using CUBIC-X expansion microscopy and tissue clearing

Authors: *T. MURAKAMI, T. MANO, H. UEDA
The Univ. of Tokyo, Tokyo, Japan

Abstract: A three-dimensional single-cell-resolution mammalian brain atlas will accelerate systems-level identification and analysis of cellular circuits underlying various brain functions. However, its construction requires efficient subcellular resolution imaging throughout the entire brain. To address this challenge, we developed a fluorescent-protein-compatible, whole-organ clearing and homogeneous expansion protocol based on aqueous chemical solution (CUBIC-X). The expanded highly-cleared brain enabled us to construct a mouse brain atlas with single-cell annotation (CUBIC-Atlas). The CUBIC-Atlas demonstrated inhomogeneous entire-brain development, revealing a significant decrease in the cerebral visual and somatosensory cortical areas during post-natal development. Probabilistic activity mapping of pharmacologically stimulated Arc-dVenus reporter mouse brains onto CUBIC-Atlas revealed the existence of distinct functional structures in the hippocampal dentate gyrus. Since the CUBIC-Atlas is shareable by an open-source web-based viewer (CATMAID), this pointillistic brain atlas provides a new platform for whole-brain cell profiling.

Disclosures: **T. Murakami:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Olympus. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Tokyo Chemical Industry. **T. Mano:** None. **H. Ueda:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Olympus. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Tokyo Chemical Industry.

Poster

612. Physiological Methods: Optical Methodology: Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 612.01/LLL52

Topic: I.04. Physiological Methods

Support: NIH MH084315
NIH NS106969
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NIH MH113071

Title: Temporal dynamics of spatial information encoding within retrosplenial cortex

Authors: *M. SEHGAL[1], S. MARTIN[1], A. PEKCAN[1], D. AHARONI[2], A. LAVI[1], D. J. CAI[3], M. R. MEHTA[4], A. J. SILVA[5]
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Abstract: Memories are dynamic in nature and 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. 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*). Retrosplenial cortex or RSC is another brain structure that is also critical for contextual learning and memory. It is unclear whether memory linking is 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 RSC. Using head-mounted miniature microscopes, we imaged GCaMP6f-meadiated calcium dynamics in retrosplenial cortical neurons while the mice 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 the RSC can mediate temporal memory linking by recruiting a shared neuronal ensemble for memories encoded within a day. To understand whether such ensemble overlap was driven by neurons encoding spatial information, we performed linear track experiments where RSC calcium transients were imaged using miniaturized microscopes. We found that ~10% of RSC cells displayed place cell like dynamics. Furthermore, the same cells could be tracked over repeated linear track sessions and displayed stable firing patterns. We are currently investigating whether neuronal overlap is changed as a function of information content. Our data indicate that co-allocation of neuronal ensembles encoding temporally proximate contextual memories may be a general mechanism of memory linking across the brain regions that process spatial and contextual information.

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Poster

612. Physiological Methods: Optical Methodology: Development II

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Program #/Poster #: 612.02/LLL53

Topic: I.04. Physiological Methods

Support: U01 NS094286-01
1700408 Neurotech Hub

Title: Miniaturized open source devices for calcium imaging, electrophysiology, and real-time control of neural activity

Authors: *D. AHARONI[1], M. SEHGAL[6], Z. CHEN[2], L. YANG[2], O. SKOCEK[7], A. J. SILVA[8], A. VAZIRI[7], H. T. BLAIR, IV[3], J. CONG[4], S. C. MASMANIDIS[5], P. GOLSHANI[9]
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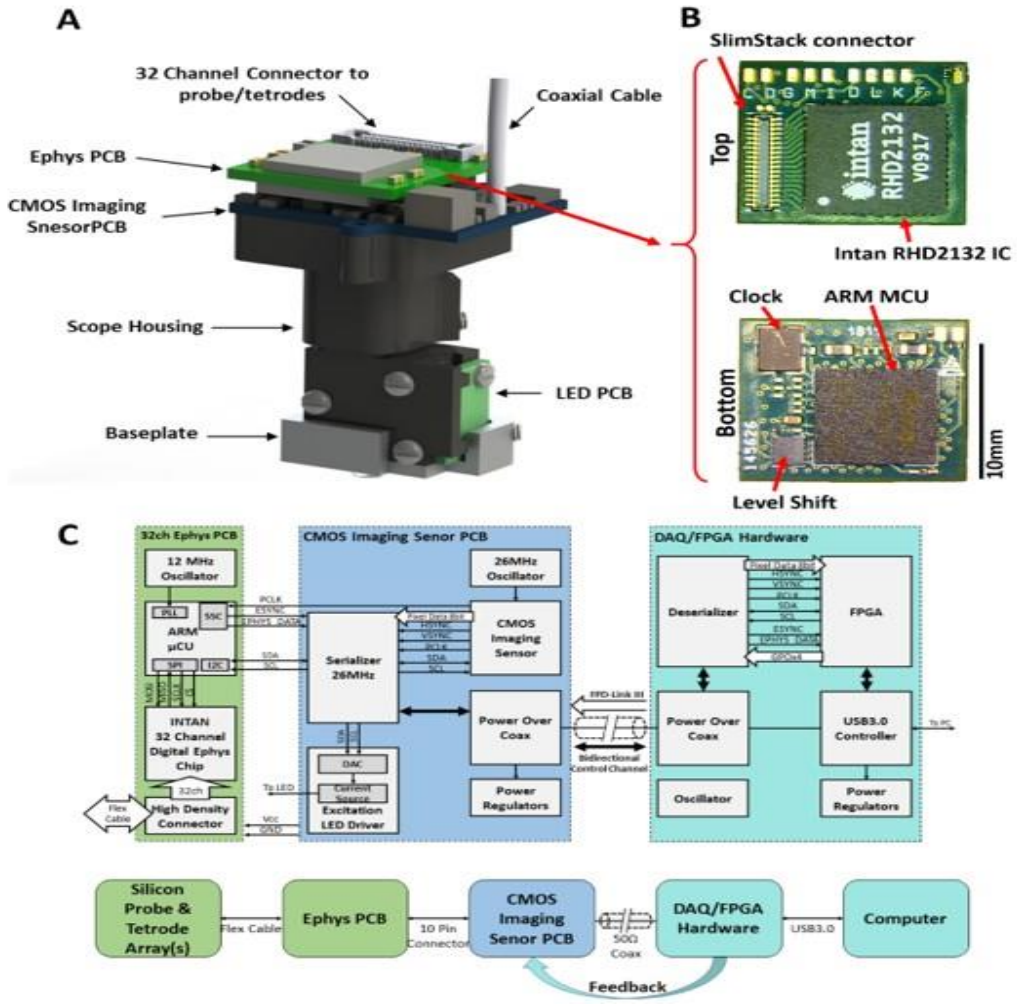
Abstract: The goal of this NSF Neurotechnology Hub is to develop and broadly share hardware that integrates optical and electrophysiological sensing of large-scale neural dynamics, as well as real-time signal processing and feedback capabilities. Our team has developed miniaturized microscopes (Miniscopes), which, in combination with genetically encoded calcium indicators, allow recordings from 100s of genetically identified neurons over weeks in freely moving animals. Additionally, our team has developed silicon-based microelectrode arrays and tetrodes that allow recording of local field potential (LFP) and units from up to a hundred cells. These approaches (calcium imaging and ephys) have distinct advantages and disadvantages, and thus are highly complementary.
Here we will present our progress towards developing a variety of tools that combine calcium imaging and electrophysiology, enabling multimodal measurements of neural dynamics: 1) Miniaturized microscopes with integrated circuitry for calcium imaging and electrophysiology in a single device, 2) Manufacture high density silicon microprobes or tetrode drives which can be implanted and connected to a single device for simultaneous cellular-resolution or bulk photometric calcium imaging and electrophysiological recordings, and 3) Develop a new generation of light field miniaturized microscopes to facilitate volumetric imaging. A further hardware challenge we will address is that analysis of imaging and electrophysiological recordings is usually done offline and can take days to weeks; but to understand how defined neurons drive specific networks during behavior it is essential to integrate real-time feedback capabilities into recording devices. To address this we will develop FPGA-based tools for real-time alignment, segmentation of calcium imaging movies, as well as real-time spike sorting and

LFP phase detection for on-the-fly optogenetic feedback. Building off the already existing online open-source resource, miniscope.org, we will share all these devices with the neuroscience community.



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Poster

612. Physiological Methods: Optical Methodology: Development II

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Program #/Poster #: 612.03/LLL54

Topic: I.04. Physiological Methods

Support: NSF NeuroNex Neurotechnology Hub 1707408

Title: Open-source silicon microprobes for large-scale neural recordings

Authors: *L. YANG, K. LEE, S. C. MASMANIDIS
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Abstract: As part of an NSF-funded NeuroNex Neurotechnology Hub, we have produced a silicon-based multielectrode technology aimed at addressing the growing need to inexpensively scale up electrophysiological recordings in vivo. These probes contain up to 256 independently addressable electrodes for extracellular single-unit and local field potential measurements, with over a dozen user-inspired electrode array designs. Moreover, they were explicitly developed for the purpose of being widely and openly distributed to the community, and we present a straightforward procedure for sharing these tools with other labs at a cost of about $300 per probe. To enable widespread dissemination the silicon devices are mass produced at a commercial microelectronics foundry, and all other components such as printed circuit boards and electrical connectors, are also readily available from third party manufacturers. Furthermore, the probes are fully compatible with commercially available head stage amplifiers, data acquisition, and impedance testing systems. The devices are primarily aimed at recording from behaving head-restrained rodents. In addition to using individual microprobes to record from up to a few hundred units in parallel, we show how probes can be combined together to simultaneously record from multiple brain areas. We also show how the devices can be readily paired with optical fibers for optogenetic tagging and perturbation studies. We demonstrate the recording capabilities of the tools in head-restrained mice performing a reward-guided task. Together, this technology provides an inexpensive, plug-and-play approach to measuring large-scale neural dynamics. Information on obtaining probes is found at: masmanidislab.neurobio.ucla.edu/technology.html.

Disclosures: L. Yang: None. **K. Lee:** None. **S.C. Masmanidis:** None.

Poster

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Topic: I.04. Physiological Methods

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Title: CCR5 closes the window for contextual memory linking by regulating neuronal ensemble overlap

Authors: *M. ZHOU[1], Y. SHEN[1], D. CAI[1,2], Y. CAI[1], A. LAVI[1], S. HUANG[1], T. SILVA[1], A. J. SILVA[1]
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Abstract: Although the mechanisms involved in the encoding, consolidation and retrieval of memory have been widely studied, little is known about the mechanisms that link multiple memories across time. Previous studies in our laboratory showed that a temporary increase in neuronal excitability biases the representation of a subsequent memory to the neuronal ensemble encoding the initial memory. As a result, the recall of one memory increases the likelihood of recalling the other memory, thus linking the two memories. Understanding the mechanisms that regulate the temporal window of memory linking is critical for understanding how different memories are either linked or separated across time. Here, we show that CCR5, a G-protein coupled receptor, plays a key role in closing the temporal window for memory linking. Our studies showed that following contextual learning the hippocampal mRNA levels for Ccr5 and its ligand Ccl5 increase over time in a pattern consistent with the hypothesis that these increases close the window for memory linking. Experiments with head-mounted fluorescent miniscopes showed that a Ccr5 null mutation extended the temporal window for the overlap between CA1 neural ensembles encoding two separate contextual memories. Accordingly, the Ccr5 null mutation also results in an extension of the window for contextual memory linking. Importantly, aging increases the levels of Ccl5 and Ccr5, and the Ccr5 mutation reverses the age-related decline both in neural ensemble overlap and in contextual memory linking. These results demonstrate that delayed increases in CCR5 levels following memory formation decrease the overlap between memory ensembles, and therefore close the window for memory linking. Additionally, our results also showed that age-related increases in CCL5/CCR5 signaling contribute to age-related deficits in memory ensemble overlap and memory linking, and indicate that these deficits can be reversed by manipulations that target this signaling pathway.

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Poster

612. Physiological Methods: Optical Methodology: Development II

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Program #/Poster #: 612.05/LLL56

Topic: I.04. Physiological Methods

Support: R01 MH084315

Title: Deficits in shared neuronal ensemble between multiple contextual exposures in mouse model of Noonan Syndrome

Authors: *Y. CAI[1], A. J. MACALINO[2], L. CHIU[2], M. MAYASHIRO[4], Y. SHEN[2], M. ZHOU[5], M. SEHGAL[5], A. LAVI[2], D. AHARONI[3], S. K. CHEUNG[2], Y.-S. LEE[6], A. J. SILVA[7]
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Abstract: Noonan Syndrome (NS) is an autosomal genetic disorder that affects 1 in 2,500 live births. Clinical studies have reported that 30-50% of patients with NS display cognitive deficits. Mutations in *Ptpn11* are responsible for most cases of NS. Here, we report neuronal circuit studies in mice of a *Ptpn11* mutation described in humans: *Ptpn11*^{D61G} heterozygous germ line mutation. We have previously demonstrated that this gain-of-function mutation causes deficits in spatial learning in the hippocampus-dependent water maze task, ERK signaling, AMPAR function, and hippocampal CA1 long-term potentiation (*Lee et al. Nature Neuroscience, 2014*). Since water maze learning involves the integration of information gathered in multiple trials across days, we investigated whether the *Ptpn11*^{D61G} mice show deficits in a circuit process known to be critical for integrating or linking spatial/contextual information across time. Our previous studies showed that mice link memories encoded close in time (e.g. within a day), but not memories encoded across a week. Furthermore, we demonstrated that the overlap between the CA1 neuronal ensembles encoding each contextual memory is critical for the animal's ability to link contextual memories close in time (*Cai et al. Nature, 2016*). Here, we studied calcium transients in the hippocampal CA1 region, with GCAMP6f and head mounted fluorescent miniscopes in *Ptpn11*^{D61G} mice (and their wild type controls). We found that there was significantly lower overlap in the neuronal ensemble activated by two different contexts separated by 5 or 24 hour intervals in *Ptpn11*^{D61G} mice relative to their WT littermates. These results demonstrate that *Ptpn11*^{D61G} mice have deficits in circuit processes that integrate information across time and suggest that these circuit deficits contribute to their impairments in spatial learning.

Disclosures: Y. Cai: None. **A.J. Macalino:** None. **L. Chiu:** None. **M. Mayashiro:** None. **Y. Shen:** None. **M. Zhou:** None. **M. Sehgal:** None. **A. Lavi:** None. **D. Aharoni:** None. **S.K. Cheung:** None. **Y. Lee:** None. **A.J. Silva:** None.

Poster

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Topic: I.04. Physiological Methods

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Beckman Institute
Heritage Medical Research Institute
NSF NeuroNex Technology Hub 1707316
Defense Advanced Research Projects Agency (DARPA) Biological Technologies Office

Title: Engineering designer AAVs for non-invasive systemic delivery to specific cell-types or organs using CREATE 2.0

Authors: *S. RAVINDRA KUMAR, Q. HUANG, X. CHEN, X. DING, E. MACKEY, N. FLYTZANIS, N. GOEDEN, D. BROWN, Y. LUO, T. DOBREVA, K. CHAN, B. DEVERMAN, V. GRADINARU
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Abstract: With increased use of recombinant adeno-associated viruses (rAAVs) as gene delivery vehicles in research and for gene therapy, there is a need for rAAVs with enhanced transduction for specific brain cell-types and regions with minimal off-target expression in other organs. In 2016 we reported a Cre recombination-based AAV targeted evolution (CREATE) method and looked for positively enriched variants with better transduction capabilities across the central and peripheral nervous systems (CNS and PNS). We identified a few highly efficient CNS transducing variants AAV-PHP.B (Deverman BE, et al, Nat. Biotech., 2016) and AAV-PHP.eB (enhanced PHP.B); and a PNS transducing variant, AAV-PHP.S (Chan K, et al, Nat. Neurosci., 2017). However, CREATE's positive selection strategy is limited to selection of capsids with enhanced transduction. To select for capsids with cell-type or tissue specificity, we designed a new library recovery method, CREATE 2.0, that enables us to perform both positive and negative selections across multiple Cre transgenic lines *in vivo*. CREATE 2.0 uses next generation sequencing (NGS) to obtain a complete recovery of viral capsid libraries across multiple Cre transgenic mouse lines. NGS can facilitate positive and negative selections across cell-types or organs in a high-throughput parallelized manner and this selection strategy can increase the efficiency of finding variants with desired specificity and enhanced transduction properties. Access to this depth of sequencing information also facilitated the investigation of selected variants for their unique tropisms using the amino acid characteristics of the peptide insertions/substitutions, Rosetta modeling and advanced machine learning algorithms. As a proof-of-concept, we built a rAAV capsid library by mutating the exposed surface of the capsid and performed two rounds of in vivo selections across different brain cell-types using the following mouse Cre lines: Tek-cre for endothelial cells, SNAP25-cre or Syn-cre for neurons, and GFAP-cre for astrocytes; and extracted the transduced viral libraries from brain and liver. After one round of selection, we identified a novel variant with biased transduction for brain endothelial cells, and another variant that preferentially transduced the liver. After two rounds of selection, we identified a few novel variants with biased transduction towards neurons while being de-targeted from liver. Collectively, CREATE 2.0 is a powerful selection strategy that

multiplexes engineered viral library selections, accelerating experimental outcomes while also providing mechanistic insight into viral tropism.

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Poster

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Topic: I.04. Physiological Methods

Support: U01NS090604
U01NS013522
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R01NS085938
P30CA014195
U01NS094247

Title: Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors

Authors: *L. TIAN[1], T. PATRIARCHI[1], J. CHO[2], K. MERTEN[3], M. HOWE[4], A. MARLEY[6], W. XIONG[7], G. J. BROUSSARD, JR[8], R. LIANG[8], H. ZHONG[7], D. A. DOMBECK[5], M. VON ZASTROW[6], A. NIMMERJAHN[9], V. GRADINARU[2], J. T. WILLIAMS[7]
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Abstract: Neuromodulatory systems exert profound influences on brain function. Understanding how these systems modify the operating mode of target circuits requires measuring spatiotemporally precise neuromodulator release. We developed dLight1, an intensity-based genetically encoded dopamine indicator, to enable optical recording of dopamine dynamics with high spatiotemporal resolution in behaving mice. We demonstrated the utility of dLight1 by imaging dopamine dynamics simultaneously with pharmacological manipulation, electrophysiological or optogenetic stimulation, and calcium imaging of local neuronal activity. dLight1 enabled chronic tracking of learning-induced changes in millisecond dopamine

transients in striatum. Further, we used dLight1 to image spatially distinct, functionally heterogeneous dopamine transients relevant to learning and motor control in cortex. We also validated our sensor design platform for developing norepinephrine, serotonin, melatonin, and opioid neuropeptide indicators.

Disclosures: L. Tian: None. **T. Patriarchi:** None. **J. Cho:** None. **K. Merten:** None. **M. Howe:** None. **A. Marley:** None. **W. xiong:** None. **G.J. Broussard:** None. **R. Liang:** None. **H. Zhong:** None. **D.A. Dombeck:** None. **M. Von Zastrow:** None. **A. Nimmerjahn:** None. **V. Gradinaru:** None. **J.T. Williams:** None.

Poster

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Support: Howard Hughes Medical Institute (AEC)
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Title: Wide-area all-optical neurophysiology mapping using hadamard microscopy

Authors: *V. J. PAROT[1], S. L. FARHI[1], A. GRAMA[2], M. YAMAGATA[5], A. S. ABDELFATTAH[6], Y. ADAM[1], S. LOU[7], J. KIM[1], R. E. CAMPBELL[8], D. D. COX[3], A. E. COHEN[4]
[2]Mol. & Cell. Biol., [3]Mol. and Cell. Biol., [4]Chem. and Chem. Biol., [1]Harvard Univ., Cambridge, MA; [5]Mol Cell Biol & Ctr. Brain Sci., Harvard Univ. Ctr. for Brain Sci., Cambridge, MA; [6]Janelia Res. Campus, Ashburn, VA; [7]Cygnal Therapeut., Cambridge, MA; [8]Dept. of Chem., Univ. of Alberta, Alberta, AB, Canada

Abstract: A longstanding challenge in neuroscience is to stimulate and record simultaneously from many genetically defined neurons in intact tissue. Achieving all-optical neurophysiology requires selection of a spectrally orthogonal actuator/reporter pair, as well as development of an optical system capable of targeted stimulation and optically sectioned imaging in a highly scattering, three dimensional tissue. Optical tools can in principle access $>10^5$ neurons in acute brain slices, but two-photon (2P) all-optical neurophysiology in tissue has been limited to ensembles of ~50 neurons due to the high optical power requirements of 2P stimulation. To record and stimulate neuronal activity simultaneously, we paired a trafficking-optimized variant of the most blue-shifted channelrhodopsin, TsChR, with a nuclear-localized red-shifted calcium (Ca^{2+}) indicator, H2B-jRGECO1a. This combination allowed optical induction and detection of

single action potentials in cultured neurons with negligible optical crosstalk or photoartifacts. To apply these tools in tissue, we devised a computational structured illumination method using a digital micromirror device (DMD) to illuminate neighboring sample locations with orthogonal functions of time (Hadamard codes). A demodulation algorithm rejected scattered and out-of-focus light from large field of view (4.6 x 4.6 mm^2) series of images. This approach enabled large area optical sectioning in acute brain slice, yielding >6,000 simultaneous single-cell recordings. We developed a protocol to quantify neuronal excitability via the relative change in Ca^{2+} response as a function of optogenetic stimulation strength. To map effect of antiepileptic drugs, we compared responses before and after drug applications: Carbamazepine, phenytoin, and retigabine produced qualitatively distinct inhibition patterns across layers of the cortex. We further developed a technique to map synaptic transmission by expressing the optogenetic actuator and the Ca^{2+} reporter in disjoint subpopulations. These results demonstrate the combination of spectrally orthogonal TsChR and jRGECO with Hadamard optical sectioning to obtain wide-area maps of neuronal function.

Disclosures: **V.J. Parot:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on Hadamard microscopy patent. **S.L. Farhi:** None. **A. Grama:** None. **M. Yamagata:** None. **A.S. Abdelfattah:** None. **Y. Adam:** None. **S. Lou:** None. **J. Kim:** None. **R.E. Campbell:** None. **D.D. Cox:** None. **A.E. Cohen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on Hadamard microscopy patent.

Poster

612. Physiological Methods: Optical Methodology: Development II

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Topic: I.04. Physiological Methods

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Children's Tumor Foundation Young Investigator Award 2016-01-006 to JER
NIMH National Research Service Award F31MH102913 to CNB

Title: Minimally-invasive optogenetic circuit modulation with designer channelrhodopsin variants and systemic AAVs

Authors: C. N. BEDBROOK[1], *J. E. ROBINSON[2], K. K. YANG[3], F. H. ARNOLD[3], V. GRADINARU[1]
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Abstract: In mammalian systems, current optogenetic tools based on the light-gated ion channel channelrhodopsin (ChR) require approximately 1-15 mW light delivered near the target cell population to reliably elicit cell firing, which confines light-dependent activation to a small volume of brain tissue [approximately 1 mm^3]. It would be desirable to have optogenetic access to large brain volumes and/or non-invasive optogenetic access to the brain without stereotaxic injections for opsin delivery or implantation of invasive fiberoptics for light delivery. We have recently reported on engineered AAV capsids that can efficiently deliver transgenes to the central and peripheral nervous systems via systemic injection [AAV-PHP.eB and AAV-PHP.S]. However, relative to direct injections, systemic delivery has a low multiplicity of infection. Therefore, systemic delivery of ChR2 results in modest light-induced currents that are at times insufficient for neuronal activation. In order to overcome these limitations, we leveraged the significant collection of published ChR variants to train statistical models that enable the design of new high-conductance ChR variants with strong currents under low intensity light ($1x10^{-3}$ mW mm^{-2}) and reaching ≥1 nA currents with $5x10^{-2}$ mW mm^{-2}. These ChRs display significantly larger light-evoked currents than ChR2(H134R) across all light powers tested in cultured neurons and in acute brain slices after direct injection [$p = 2x10^{-5}$ and $8x10^{-5}$, respectively at $8x10^{-3}$ mW mm^{-2} light intensity] and exhibit 100% spike fidelity with 1-2 orders of magnitude lower light intensity than ChR2(H134R). After systemic delivery ($1x10^{11}$ vg/mouse), our high-conductance ChRs exhibit 100% spike fidelity in acute cortical slices at 0.7 mW mm^{-2} light intensity, while ChR2(H134R) expressing cells do not reliably produce light-evoked firing with any light intensity tested. Preliminary testing *in vivo* revealed that the sensitivity of systemically delivered high-conductance opsins to low light intensities allows for optogenetic behavioral control with the light source placed on the skull surface, enabling neuronal modulation with high temporal precision without invasive intracranial surgery for virus delivery or fiberoptic implantation. Additionally, these high-conductance tools could be particularly useful when activating large brain nuclei in mice or in model systems with larger brains (e.g. rats or non-human primates). Ongoing studies seek to characterize these variants across the central and peripheral nervous system and extend the application of minimally invasive optogenetic manipulations past superficial brain structures.

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Poster

612. Physiological Methods: Optical Methodology: Development II

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G.M.C. is supported by a PGS-D3 fellowship from the Natural Sciences and Engineering Research Council of Canada

Title: Scalable single-cell profiling by systemic AAVs for sparse stochastic labeling compatible with tissue clearing and multiplexed RNA labeling, applied to mouse GnRH neurons

Authors: G. M. COUGHLIN, *A. KAHAN, M. JANG, V. GRADINARU
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Abstract: Amongst standing challenges for neuroscience are the efficient phenotyping and reconstruction of neurons with complex patterns (whether in distribution or morphologies). Gonadotropin-releasing hormone (GnRH) neurons represent one particularly challenging population for reconstruction. These neurons play key roles in the entry to puberty and in the normal functioning of the mature hypothalamic-pituitary-gonadal axis. The challenge inherent in labeling and reconstructing this population arises during their unique developmental trajectory; these neurons are sparsely distributed through an expansive spatial continuum from the olfactory bulb into the anterior hypothalamus (Wray), and they send long-range projections (up to 3 mm) to divergent brain regions. Thus, the efficient and accurate reconstruction of GnRH neurons requires molecular-phenotyping and tract-tracing through whole brain regions. We have approached this problem using a toolbox comprised of: (1) systemically delivered adeno-associated virus (AAV) vectors such as AAV-PHP.eB (Chan et al.), which achieve sparse, stochastic, and brain-wide multicolor labeling of genetically specified cell types (akin to a "Genetic Golgi stain"); (2) tissue clearing by PACT (Passive CLARITY) and RIMS (Refractive Index Matching Solution) (Yang et al.); and (3) multiplexed RNA labeling by hybridization chain reaction (HCR)-based fluorescence in situ hybridization (FISH). Tracing GnRH neurites over long distances in the brain of GnRH1-cre mice was facilitated by the delivery of multiple spectrally distinguishable fluorescent proteins, resulting in combinatorial labeling of the target population (VAST, for vector-assisted spectral tracing; see Chan et al.). These multicolor-labeled thick (<0.5 mm) sections were optically cleared through incubation in RIMS, and then imaged with confocal microscopy. Molecular identification and phenotyping of GnRH neurons in thick tissue was performed using HCR-based FISH (Greenbaum et al.), using probes directed against *GnRH1* and other transcripts of interest. Compared to antibody staining, this RNA-based labeling approach is advantageous as it enables high signal amplification, easy multiplexing, and efficient penetration of the probes into thick tissue. Individual GnRH neurons were identified and reconstructed, yielding important morphological parameters, such as cell body position and projection targets. This work demonstrates the utility of the VAST system in combination with multiplexed HCR-based FISH and tissue clearing, for high-throughput labeling, phenotyping and reconstruction of broadly distributed neuronal populations.

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Poster

612. Physiological Methods: Optical Methodology: Development II

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Topic: I.04. Physiological Methods

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Title: Tissue clearing and optogenetics help reveal pathological effects of seeding alpha synuclein fibrils in enteric and olfactory systems

Authors: *C. CHALLIS[1], T. R. SAMPSON[1], B. B. YOO[1], S. K. MAZMANIAN[1], L. A. VOLPICELLI-DALEY[2], V. GRADINARU[1]
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Abstract: Aggregation and accumulation of alpha synuclein (aSyn) is the defining feature of synucleinopathies. aSyn pathology has been well studied in Parkinson's disease (PD), where pathological hallmarks include loss of dopamine neurons in the substantia nigra pars compacta and motor impairment. Though 10% of PD cases are genetic, there is no consensus on the origin of the 90% of idiopathic diagnoses. Reports have identified a prodromal phase of PD where patients exhibit non-motor symptoms prior to motor dysfunction. Postmortem biopsies have revealed Lewy pathology in associated peripheral tissues such as olfactory mucosa and gastrointestinal (GI) lining. Pathologic staging studies suggest that formation of aSyn-containing inclusions originates in the periphery before appearing in the brain[1-3]. However, the direct impact of pathologic aSyn on the peripheral nervous system has not been thoroughly investigated. Here, we introduced aSyn preformed fibrils (PFF) into duodenal lining of the GI tract or nasal cavity of adult, wild type C57Bl/6N mice and assessed functional and physiological adaptations. We used tissue clearing (e.g. PACT, Bone CLARITY)[4,5], optogenetics, and behavioral paradigms to pursue this question in higher resolution than previous work. After inoculation of aSyn PFF in the gut, we observed reductions in fecal pellet weight and water content 21 days post inoculation (1WA, $F = 6.044$, $p < 0.001$). This was accompanied by decreased enteric nervous system (ENS) network connectivity (1WA, $F = 39.99$, $p < 0.001$), which we evaluated by systemically delivering jRGECO1a packaged in AAV-PHP.S, a novel capsid with preferential tropism for peripheral cells[6]. Morphological and histological evaluation of the ENS showed persistent gliosis

and accumulation of phospho-aSyn (S129P), and further analysis revealed that aSyn PFF impaired lysosomal mechanisms (i.e. decreased glucocerebrocidase) and inflammation (i.e. increased IL6). Irrigation of aSyn PFF in the nasal cavity caused immediate olfactory-dependent behavioral deficits, which was accompanied by S129P signal in the olfactory epithelium and olfactory bulb. Ongoing work is evaluating the pathological mechanisms that underlie olfactory symptoms associated with aSyn pathology. Together, our data strengthens our knowledge of pathological aSyn and provides the framework to study peripheral synucleinopathy.

References

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3. Holmqvist et al. *Acta neuropathologica* (2014)
4. Yang et al. *Cell* (2014)
5. Greenbaum et al. *Science translational medicine* (2017)
6. Chan et al. *Nature neuroscience* (2017)

Disclosures: C. Challis: None. **T.R. Sampson:** None. **B.B. Yoo:** None. **S.K. Mazmanian:** None. **L.A. Volpicelli-Daley:** None. **V. Gradinaru:** None.

Poster

612. Physiological Methods: Optical Methodology: Development II

Location: SDCC Halls B-H

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Program #/Poster #: 612.12/MMM2

Topic: I.04. Physiological Methods

Support: Department of Biongineering Start-up

Title: Multiplexing imaging development using phase light interference and fluorescence microscopy to bridge the scales from single molecule to whole organ

Authors: *J. A. MALDONADO[1], C. BEST[2], A. SMITH[3], G. POPESCU[4]
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Abstract: Microscopy techniques are allowing the neuroscience field to better understand single cell dynamics, cellular and whole organ architectures through super high resolution features and novel imaging technologies. Most of these super resolution microscopy techniques require invasive and targeted labeling through staining or fluorescent methods that require expensive, lengthy and time consuming protocols. Our objective is to explore and investigate a label-free approach which identifies cellular structures based on their intrinsic physical and mechanical properties such as dry mass, refraction index, and elastic modulus in living cells and *ex vivo*

brains. We also couple quantum dots with SLIM to probe the single molecule dynamics associated with AMPA receptor trafficking.To achieve our goal, we used both 4 micron slices of murine hippocampi, and primary neuron cells in culture, Alexa 488 labeled AMPA receptors, DAPI nuclear stain, Mito-tracker, and quantum dots, coupled with Spatial Light Interference Microscopy (SLIM). We collected information about subcellular molecules and tissue properties that provide unique indicators for synaptic receptors, cell nucleus and mitochondria. These properties were then used to identify and differentiate these structures from surrounding cellular structures independent of extrinsically added labels.We provide a powerful tool to augment fluorescence imaging studies which can be used for long duration, live cell imaging. Subcellular structures including vesicles, mitochondria and the post synaptic density are easily identified with SLIM following fluorescence correspondence confirmation. Furthermore, this technique may lead to identification of clinically relevant biomarkers as well as pathological receptor trafficking mechanisms. We show that following validation with fluorescence, SLIM can be utilized for identification of intracellular structures at the cell and across the whole organ level. Our technique avoids the invasive and extensive protocols with inherent processing artifacts, and preserves the natural environment of the tissue. This will provide access to enhanced and dynamic data from cellular and tissue properties in future studies.

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Poster

612. Physiological Methods: Optical Methodology: Development II

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Title: Optical activity readout and modulation of serotonergic neurons in the dorsal raphe show frequency-dependent, bidirectional effects on sleep

Authors: *M. ALTERMATT, J. CHO, G. OIKONOMOU, D. PROBER, V. GRADINARU
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Abstract: A role for serotonin (5-HT) in sleep has first been reported 60 decades ago (Bradley, 1958). Early discoveries from pharmacological inhibition of 5-HT synthesis and lesion experiments led to a hypothesis that 5-HT is a sleep-promoting neurotransmitter (Delorme et al., 1966; Jouvet, 1968). However, single-unit recordings showed that 5-HT neurons are mostly active during the wake state, followed by the non-rapid eye movement (NREM) sleep and were almost silent during the rapid-eye movement sleep (REM) (McGinty & Harper, 1976). This influenced the current view of a wake-promoting and REM-inhibiting action of 5-HT (Scammell et al., 2017). Although 5-HT neurons exhibit both phasic and tonic firing patterns, causal investigations of frequency-dependent effects on sleep-wake regulation are lacking. We first measured population activity levels of dorsal raphe 5-HT neurons of serotonin transporter-cre mice across behavioral states, using fiber photometry in combination with EEG/EMG recordings, which confirmed earlier observations that 5-HT neurons display the highest activity during wake state and a decreasing activity from NREM to REM state. Fluorescence changes of GCaMP6s at behavioral state transitions were significant for all transitions ($n = 4$, $p<0.001$). Next, we used optogenetics to induce phasic and tonic firing modes in 5-HT neurons, which revealed opposite effects on regulating sleep-wake states. Phasic stimulation (25 Hz; 3 s of stimulation with 7 s break; Duration: 5 min) during the light phase caused a transient >70% increase in time spent in wake state ($n = 9$, $p<0.001$) and a persistent inhibition of REM state ($n = 9$, $p<0.01$). Conversely, tonic activation (3 Hz; Duration: 12.5 min) during the dark phase decreased the time spent in wake state by >20% ($n = 9$, $p<0.05$). These results show a complex role for 5-HT neurons in modulating sleep and provide evidence that distinct firing modes of 5-HT neurons play distinct roles in controlling arousal states.

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Poster

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Topic: I.04. Physiological Methods

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Title: Engineering better, brighter bioluminescent light sources for neuronal imaging

Authors: L. M. BARNETT, G. G. LAMBERT, *N. C. SHANER
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Abstract: To observe authentic biological processes in living cells, it is critical to avoid damaging or perturbing them. Fluorescence microscopy suffers from the need to illuminate biological samples with extremely bright light to collect sufficient signal. A majority of this incident light is not absorbed by the fluorophores being imaged and is a source of collateral damage to many other components of the cell. Bioluminescent proteins, luciferases, produce light via enzymatic oxidation of a small molecule substrate, a luciferin. These substrates, such as coelenterazine, are essentially biologically inert in cells, and because the light they produce does not require exogenous excitation, imaging with bioluminescent probes does not lead to phototoxicity or other undesirable perturbations. In theory, bioluminescence is ideal for live cell imaging. Unfortunately, all of the available bioluminescent probes are too dim for use in most imaging experiments. Increasing the photon output is difficult because luciferases are fundamentally limited by the need to balance catalytic rate and luminescence quantum yield. In other words, to achieve a high quantum yield, the luciferase must stabilize the excited oxyluciferin in a protected binding pocket that discourages non-radiative relaxation to the ground state. This requirement places major constraint on the dissociation constant of the ground state oxidized substrate, slowing down substrate turnover and reducing the number of photons emitted per second. Here, we use a novel approach leveraging Förster resonance energy transfer (FRET) to eliminate this trade off. First, we optimize FRET efficiency between our highest-activity luciferases and our brightest fluorescent protein variants to increase total light output. We then use structure-guided design and directed evolution to improve the turnover number of the luciferase component. This combination design process circumvents the critical 'catalytic rate versus quantum yield' barrier, giving way to the next generation of bright bioluminescent light sources.

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Poster

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Title: Bioluminescence driven optogenetics for investigating functional synaptic communication across co-cultured neuronal networks on multi-electrode arrays

Authors: *M. PRAKASH[1], R. S. LAURENT[2], A. PAL[1], A. BJOREFELDT[1], B. W. CONNORS[2], D. LIPSCOMBE[2], J. A. KAUER[3], C. I. MOORE[2], U. H. HOCHGESCHWENDER, 48859[1]
[1]Neurosci., Central Michigan Univ., Mount Pleasant, MI; [2]Dept. of Neurosci., [3]Dept. of Neurosci. & Dept. of Mol. Pharmacology, Physiology, and Biotech., Brown Univ., Providence, RI

Abstract: In BioLuminescent driven OptoGenetics (BL-OG) a genetically encoded light source, a luciferase, activates a light-sensing optogenetic element, a channelrhodopsin or a pump. When light emitter and light sensor are tethered, as in luciferase-opsin fusion proteins (luminopsins, LMO), application of luciferase substrate coelenterazine (CTZ) and subsequent light production will change the membrane potential of the cell expressing the LMO. Here we co-cultured cortical neurons expressing the luciferase with hippocampal neurons expressing the opsin, with the goal of investigating BL-OG effects across synapses between these two neuronal populations. Neurons isolated from E18 rat cortex and hippocampus were nucleofected with a pre-synaptically targeted luciferase construct and either an excitatory or inhibitory opsin construct, respectively, and were plated on multi-electrode array (MEA) dishes using a two-chamber silicon insert to separate the two populations. Neuronal processes originating from both populations crossed the gap separating them, forming synaptic contacts between cortical and hippocampal neurons. Recordings were carried out between DIVs 14-28. External blue light from an LED source was used to modulate opsin expressing hippocampal neurons directly, while bioluminescence emission by cortical neurons generated with application of CTZ was used to drive hippocampal neurons across synapses. Responses from hippocampal neurons elicited with CTZ were likely due to trans-synaptic communication. Electrical stimulations of cortical neurons by individual electrodes were carried out in parallel to confirm the inter-population connectivity. The overall effect of CTZ application on activity of opsin-expressing hippocampal neurons in the co-cultures was significantly higher compared to that of non-expressing hippocampal neurons and of the cortical neurons in the co-cultures. Such biological light activation, across synaptic partners originating from brain regions known to be synaptically connected, offers the potential to optogenetically dissect synaptic communication non-invasively.

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Poster

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Title: A multifunctional bioluminescent calcium indicator

Authors: *A. PAL[1], W. E. MEDENDORP[1], S. DASH[1], T. BROWN[1], Z. ZALDI[1], M. PRAKASH[1], D. LIPSCOMBE[3], C. I. MOORE[4], U. HOCHGESCHWENDER[2]
[1]Central Michigan Univ., MT Pleasant, MI; [2]Neurosci., Central Michigan Univ., Mt Pleasant, MI; [4]Neurosci., [3]Brown Univ., Providence, RI

Abstract: Bioluminescent Ca^{2+} sensors have distinct advantages over fluorescent Ca^{2+} indicators; first and foremost they function without external illumination. Rather, they produce light in the presence of Ca^{2+} and of a luciferase substrate, a feature that can be exploited for combining Ca^{2+} sensing with optogenetic applications. We developed a bioluminescent Ca^{2+} indicator, Lumicampsin (LMC) that employs a split, mutated Gaussia luciferase (sbGluc) with a CaM-M13 calcium sensing moiety introduced between the two split halves. To date we have several versions of LMC with varying sensitivities to Ca^{2+} by using different, pre-established CaM-M13s (from GCaMP6f, GCaMP6m, GCaMP6s, etc.). In order to investigate subcellular Ca^{2+} dynamics, we have fitted the LMC with various organelle localizing sequences that effectively shuttles it to the organelles of interest (ER, Mitochondria and Golgi apparatus). The superior light emission from LMC, capable of producing a delta RLU/RLU_o of around 200% *in vitro*, has motivated us to explore activity dependent neuronal modulation by co-expressing LMCs with various optogenetic elements in primary neuronal cultures. We are currently optimizing conditions to achieve reliable and efficient coupling of Ca^{2+}-induced light production and optogenetic effector activation.

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Poster

612. Physiological Methods: Optical Methodology: Development II

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Title: Bioluminescent Optogenetics produces fewer nonspecific effects compared to DREADDs

Authors: *M. L. WADDELL[1], W. E. MEDENDORP[2], U. HOCHGESCHWENDER[3]
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Abstract: Neuroscience offers many tools for manipulating neural activity in genetically targeted neuronal circuits during specific time windows, with tools varying in modes of activation and types of actuator molecules. Chemogenetic approaches, such as Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), and bimodal chemogenetic and optogenetic approaches, such as Bioluminescent Optogenetics (BL-OG), provide control of neuronal firing by a systemically applied small molecule, clozapine N-oxide (CNO) and coelenterazine (CTZ), respectively. Recent evidence suggested that the ligand for the DREADDs, CNO, is converted to clozapine before crossing the blood brain barrier. This has raised questions of nonspecific effects from the chemogenetic effector molecules. Here we compare the BL-OG and DREADD models in a variety of experimental settings with respect to nonspecific effects in control animals. Heterozygous knock-in mice with Lox-Stop-Lox (LSL)-LMO3 and LSL- hM3Dq transgenes were bred with heterozygous Emx1-Cre mice. Experiments then were carried out applying CTZ and CNO, respectively, during early postnatal development and acutely. When testing control animals not expressing the respective actuators on the rotarod and in open field, application of CNO produced reduced time to fall on the rotarod and reduced exploration in open field; application of CTZ had no effect in control groups. This research will assist investigators in choosing a suitable model for their specific research question with appropriate controls.

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Poster

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Title: Tracking neocortical dynamics using genetically-encoded bioluminescent molecules *in vivo*

Authors: *M. GOMEZ-RAMIREZ[1], J. W. MURPHY[1], A. I. MORE[2], A. PAL[4], D. LIPSCOMBE[3], U. HOCHGESCHWENDER[5], C. I. MOORE[1]
[1]Neurosci., [2]Brown Univ. Neurosci., [3]Brown Univ., Providence, RI; [4]Central Michigan Univ., Mount Pleasant, MI; [5]Neurosci., Central Michigan Univ., Mt Pleasant, MI

Abstract: Fluorescence imaging with genetically-encoded calcium indicators, such as GCaMP, has provided fundamental insights into the role of cell-specific populations in neural coding and perception. Recent advances and pragmatic advantages have made 1-photon imaging a viable strategy for interrogating activity within and across large-scale neural populations. Yet, a limitation of fluorescence imaging is that the technique requires a light source to excite fluorescent proteins. This light creates artifacts that may lead to a reduction in the signal to noise ratio (SNR) of the image. Noise artifacts from the excitation light include: (1) Autofluorescence, (2) Photon scattering from the incoming light, and (3) Photobleaching. As an alternative to fluorescent imaging, we are developing calcium-dependent genetically-encoded molecules using bioluminescent probes. Bioluminescence is chemically generated light that occurs when a photon-containing molecule (luciferin) is catalyzed by a photoenzyme (luciferase). Bioluminescence does not require light excitation and creates very little thermal reaction, thus substantially reducing noise related to autofluoresence, photon-scattering, and photobleaching. Our calcium-dependent bioluminescent molecule, Lumicampsin-4 (LMC4), is a split variant of the slow-burn Gaussia luciferase (sb-GLuc). The two elements are joined by the Ca2+-sensing peptide CaM-M13, thereby providing activity detection. Here, we tested the efficacy of LMC4 to track neural dynamics in mouse somatosensory cortex. To date, we have found that CTZ and a Ca+2 driver (NMDA or L-Glutamic Acid) generate robust bioluminescence signals restricted to the area neighboring the injection pipette. In addition, pilot experiments show that vibrissae deflection generates LMC4-mediated bioluminescence in primary somatosensory neocortex. A key future direction will be to assay the reliability of imaging neural activity across multiple areas using LMC4 while animals engage in perceptual tasks.

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Poster

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Topic: I.04. Physiological Methods

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Title: Radiationless bioluminescence resonance energy transfer from luciferase to opsin in the luminopsin fusion protein

Authors: *K. BERGLUND[1], U. HOCHGESCHWENDER[2], R. E. GROSS[1]
[1]Neurosurg., Emory Univ., Atlanta, GA; [2]Neurosci., Central Michigan Univ., Mt Pleasant, MI

Abstract: Although molecular tools for controlling neuronal activity by light have vastly expanded, there are still unmet needs which require development and refinement. For example, light delivery into the brain is still a major practical challenge that hinders potential translation of optogenetics in human patients. In addition, it would be advantageous to manipulate neuronal activity acutely and precisely as well as chronically and non-invasively, using the same genetic construct in animal models. We have previously addressed these challenges by employing bioluminescence and have created a new line of opto-chemogenetic probes termed luminopsins by fusing light-sensing opsins with light-emitting luciferases. Bioluminescence is inherently dim light, yet it is bright enough to activate nearby opsins and change rodent behaviors as we have shown in several iterations of luminopsins. Although the utility of luminopsins has been firmly established, the nature and mechanisms of this efficient energy transfer from luciferases to opsins are thus far not known. Specifically, the energy to activate opsins may be transmitted via radiationless bioluminescence resonance energy transfer (BRET) due to proximity of the two molecules in the fusion protein. Alternatively, luciferases may activate opsins simply through bioluminescent radiation similar to physical light sources, such as LED and laser. In this study, we tested these two opposing hypotheses by conducting a series of systematic examination of BRET within the luminopsin molecules. We compared luminopsin fusion proteins with co-expression of opsins and luciferases in bioluminescence measurements and electrophysiological recordings *in vitro*. Our results indicate that BRET is the dominant form of energy transfer in activating opsins, supporting the hypothesis of radiationless energy transfer in the luminopsin fusion protein. These results may be useful in rationally designing and developing new luminopsins in the future.

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Poster

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Topic: I.04. Physiological Methods

Title: Effect of chemogenetic inhibition of mammillary bodies in a rat pentylenetetrazole seizure model

Authors: *A. FERNANDEZ[1], K. BERGLUND[2], F. SHIU[1], C.-A. GUTEKUNST[1], R. E. GROSS[1]
[1]Neurosurg., [2]Neurosurg. and Anesthesiol., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Epilepsy affects 1% of the world population with approximately one third of patients being resistant to conventional pharmacotherapy. Thus, there is a need for alternative modes of treatments for seizures. Optogenetics has proven to be a useful tool to understand network dynamics, but it has translational challenges. We have developed a chemogenetic tool, called luminopsins, that consists of a light-sensitive channel fused with a luciferase enzyme that bioluminesces in the presence of its substrate coelenterazine (CTZ), eliminating the need for hardware implantation. Previous studies in our laboratory have shown that simultaneous inhibition of glutamatergic cells in the dentate gyrus (DG) and anterior nucleus of thalamus (ANT), with an inhibitory luminopsin, lead to a reduction in seizure severity and duration (Tung et al., 2018). The present study aimed at exploring the effect of modulating neuronal activity in the mammillary bodies (MB), another structure within the Papez circuit. We hypothesized that inhibition of this structure would suppress seizures in the pentylenetetrazole (PTZ) model. To test this, rats were injected with a recombinant adeno-associated viral vector carrying the inhibitory luminopsin gene into the medial mammillary nucleus and subjected to a PTZ seizure test two weeks after virus injection. Rats were pre-treated with either vehicle or CTZ (intravenously) five minutes before intraperitoneal PTZ injection and monitored for 40 minutes. Three days later, this procedure was repeated but rats were pre-treated with the opposite treatment (vehicle or CTZ). Thus, rats served as their own control. Seizure latency, duration, and severity were calculated and compared. Compared to controls, a decrease in seizure duration was observed following inhibition of the MB with CTZ injections. Postmortem histology confirmed adequate targeting of the MB with inhibitory luminopsins. These results support our hypothesis of seizure suppression in the PTZ model due to inhibition of the MB. Similar to our previous study of simultaneous inhibition of two targets, DG and ANT, we expect that this anticonvulsive effect of MB inhibition can be further augmented by simultaneous neuromodulation by luminopsin in other targets in the brain.

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Poster

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Title: Imaging and control of neurons in mice expressing luminopsins

Authors: D. K. JOHNSTON, J. R. ZENCHAK, W. E. MEDENDORP, A. BJOREFELDT, *U. HOCHGESCHWENDER
Neurosci., Central Michigan Univ., Mt Pleasant, MI

Abstract: Luminopsins (LMOs) are fusion proteins of a light emitting luciferase and a light-sensing opsin. Application of the luciferase substrate coelenterazine (CTZ) leads to emission of bioluminescence and subsequent activation of the fused optogenetic element. Depending on the biophysical nature of the opsin, this will result in hyper- or hypo-polarization of membrane potential of cells expressing LMOs. At the same time, emission of bioluminescence allows activated neurons to be imaged in vivo.
Here we compared efficiencies of LMOs delivered to mice through viral transduction and transgenic expression, and after applying CTZ through various routes. Readouts are in vivo bioluminescence imaging for real-time monitoring of light emission and c-fos staining to determine activation of neurons upon light emission.

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Poster

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Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 612.22/MMM12

Topic: I.04. Physiological Methods

Title: Chemogenetic modulation with luminopsin in rat septo-hippocampal pathway

Authors: *S.-E. PARK[1], A. FERNANDEZ[2], K. BERGLUND[2], C.-A. N. GUTEKUNST[2], R. E. GROSS[2]
[1]Georgia Inst. of Technol., Atlanta, GA; [2]Dept Neurosurg., Emory Univ. Sch. Med., Atlanta, GA

Abstract: The medial septum (MS) provides an advantageous upstream target to modulate hippocampal activity. Three distinctive neuronal subpopulations in MS form complex local connections as well as distant connections with hippocampal neurons. Chemogenetics can help to understand the effect of neuromodulation in the septo-hippocampal pathway with cell type specific excitation and inhibition. Luminopsin, a fusion protein consisting of marine luciferase fused to light sensitive opsins activated in the presence of coelenterazine (CTZ), has the advantage of homogeneously activating or inhibiting a larger region of the brain compared to conventional optogenetics. Two different adeno-associated viral vectors were used, carrying two different luminopsins: an excitatory channelrhodopsin (LMO3) with a hSynapsin promoter transfecting neurons non-selectively and an inhibitory halorhodopsin (iLMO2) with a CamKIIa promoter targeting glutamatergic neurons. Virus (LMO3 or iLMO2) was injected into the rat medial septum, and after 10-14 days a 16 channel multi-electrode-array was driven to the hippocampus: a row of 8 electrodes targeted CA1, and the other row targeted CA3. Experiments were performed under anesthesia with 2% isoflurane. A baseline local field potential (LFP) recording was followed by a modulation recording period after administration of CTZ through tail vein. All recordings were performed for 10-15 minutes. All data analysis including the statistical test was performed in MATLAB. Power spectral density (PSD) reflects the neural activity of the recording region, and it has been reported that PSD is related to a specific brain state or behavior. Clear differences were observed in the low frequency band between iLMO2 and LMO3 modulations. First, LMO3 increased theta band (4-12Hz) power in CA3 whereas iLMO2 decreased theta band power in the same region. Delta band (0.1-3Hz) power was increased in CA1 region only when iLMO2 modulation was applied. However, both luminopsin modulations induced a similar pattern in higher frequency bands with beta (13-30Hz) and low gamma (30-50Hz) band power increased in CA1 and CA3. These results indicate that the chemogenetic modulation with different luminopsins induce different, and not predictable, effects in the septo-hippocampal pathway. Relating these results well-known biomarkers of hippocampal activity (e.g. theta and gamma band power) can shed light about the appropriate neuronal subpopulation that should be targeted.

Disclosures: S. Park: None. **A. Fernandez:** None. **K. Berglund:** None. **C.N. Gutekunst:** None. **R.E. Gross:** None.

Poster

612. Physiological Methods: Optical Methodology: Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 612.23/MMM13

Topic: I.04. Physiological Methods

Title: Dual-plane two-photon mesoscopy: Multi-column calcium imaging of mouse visual cortex

Authors: *N. ORLOVA, D. TSYBOULSKI, F. GRIFFIN, J. LECOQ, P. SAGGAU
Allen Inst., Seattle, WA

Abstract: Several canonical cortical circuit models propose interaction between two full cortical columns as one possible elementary unit of sensory processing. In particular, the dynamic interplay of bottom-up and top-down circuits across two connected cortical columns plays a key role in how sensory information is processed. Testing these models has been limited by the inability to measure activity across multiple layers and multiple columns simultaneously. Two-photon laser scanning microscopy (2P-LSM) allows for recording of neural activity in the mammalian brain using fluorescent Ca^{2+} indicators of neuronal activity. Recent advances in 2P-LSM have increased the imaging field-of-view (FoV) from ~ 0.4 x 0.4 mm^2 to ~ 5 x 5 mm^2 and now support random positioning of multiple regions-of-interest (RoI) within this large FoV [1]. However, even in such a mesoscope, simultaneous recording of the spread of neuronal activity across two interconnected cortical columns has been limited to a small subset of pairs of cortical layers. We have developed an advanced system that combines two-photon random-access mesoscopy (2P-RAM) with dual-plane remote focusing, increasing the number of simultaneously recorded RoIs and achieving imaging of multiple layers of two cortical columns at frame rates of up to ~11 Hz. We compare signal-to-noise (SNR) in *in vivo* data recorded with this system to conventional 2P-LSM and discuss inter-plane cross talk as well as post-processing methods of de-mixing calcium signals from two planes. We demonstrate *in vivo* imaging at two cortical columns located in mouse primary visual cortex (V1) and other higher visual areas with image planes located at different cortical layers.
1. N. J. Sofroniew, et al., “A large field of view two-photon mesoscope with subcellular resolution for in vivo imaging,” eLife 5, e14472 (2016).

Disclosures: N. Orlova: None. **D. Tsyboulski:** None. **F. Griffin:** None. **J. Lecoq:** None. **P. Saggau:** None.

Poster

612. Physiological Methods: Optical Methodology: Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 612.24/MMM14

Topic: I.04. Physiological Methods

Title: Dual-plane two-photon mesoscopy: System design and characterization

Authors: *D. TSYBOULSKI, N. ORLOVA, F. GRIFFIN, J. LECOQ, P. SAGGAU
Allen Inst., Seattle, WA

Abstract: Multiphoton microscopy has become a standard tool for morphological and functional imaging of neuronal structures given its ability to achieve increased imaging depths without

significant loss of resolution. At present, the technique achieves data acquisition rates of about 10 Mpixels/s and allows for observation of fluorescently labeled neurons within ~ 1x1 mm area at 10-40 frames per second. Nevertheless, functional recordings from large ensembles of neurons labeled with Ca^{+}- or voltage-sensitive indicators within optically accessible volume remain challenging due to limitations in scanning speed and data acquisition rates. Recently introduced Two-Photon Random Access Mesoscope (2P-RAM) [1] features ultra-large field of view (FoV) of ~ 5 mm in diameter while maintaining high excitation and collection aperture, and enables access to ~25× larger imaging volume as compared to conventional multiphoton microscopy systems. The system utilizes principles of remote focusing [2] for fast imaging depth adjustment and galvo-galvo lateral positioning of the scanning beam to enable rapid transition between regions of interest in 3D in less than 10 ms. Nevertheless, limited data acquisition rate requires compromises between the size of an imaging area, the number of laterally positioned regions of interest (ROIs), and the number of axial planes within ROIs which can be imaged sequentially with a satisfactory temporal resolution.
Here, we introduce a modification to the 2P-RAM system that enables simultaneous imaging with two focal planes independently positioned in axial direction. We have introduced a secondary excitation beam that is orthogonally polarized relative to the original beam. These two beams utilize different remote focusing units, but share the rest of the scanning and imaging optics. Femtosecond laser pulses in each beam path are delayed relative to each other by ~ 6.25 ns to create temporally interleaved fluorescence signals from each channel, which are detected by a single photomultiplier and then de-multiplexed into separate channels with custom electronics based on fast analog multiplication. Our de-multiplexing scheme features full synchronization with a dithered laser pulse rate and provides adjustable duty cycle of gating signals, resulting in a reduced cross-talk between imaging channels. The upgraded system features similar signal-to-noise ratio and the same dynamic range of recorded fluorescence signals as the original design.
1. N. J. Sofroniew, *et al*., eLife **5**, e14472 (2016).
2. E. J. Botcherby, *et al*., Optics Communications **281**, 880-887 (2008).

Disclosures: D. Tsyboulski: None. **N. Orlova:** None. **F. Griffin:** None. **J. Lecoq:** None. **P. Saggau:** None.

Poster

612. Physiological Methods: Optical Methodology: Development II

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 612.25/MMM15

Topic: I.06. Computation, Modeling, and Simulation

Support: National Research Foundation of Korea(NRF) Grant 2017M3C7A1048092
National Research Foundation of Korea(NRF) Grant 2015M3C7A1029037

KBRI basic research Grant 18-BR-02-02

Title: Development of 3D imaging processing system for neural network analysis

Authors: *N. KIM[1], J. CHOI[1], B. KANG[2], S. JEONG[1]
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Abstract: The massive data are being generated by new technologies in neuroscience such as monitoring tools for neural activity and imaging tools for circuit formation. High resolution images, especially, are produced by the techniques of tissue processing and optical equipment so that the data processes are essential in this field.
Confocal and light sheet microscope are widely used for neural network and generates high-resolution images. However, it is still necessary to develop an efficient 3D rendering and visualizing system because it is nonexchangeable imaging file format in huge volume affecting data analysis.
In this study, we propose the web-based 3D visualization and analysis system that supports the entire process of storage, extraction, analysis, visualization of neural network information. To visualize 3D objects converted from 2D images, the overlapping feature points of x and y axis were crossed between the input images of A and B matched through the Fourier transform. The images coordinated the feature of first and second brain images at z-axis side utilizing Rigid transformation technique. The aligned 3D data were applied in Marching Cubes algorithm following transformation to volume geometry and expansion into web. Total 5440 images produced by Lavision light sheet microscope were aligned at 2x2 matrix [0.0],[0.1],[1.0],[1.1] points of each 2D images and matched to generate 3D data based on real used 8GB RAM.This performance enabled a set of n images tiles configuration to create 3D image data in approximately 45GB, which make the reduction of the memory load for data analysis process. This strategy showed that 2D slice images were rapidly converted and visualized to 3D data with rendering approach based on web service allowing us to image process faster than possible with other current methods.

Disclosures: N. Kim: None. **J. choi:** None. **B. kang:** None. **S. jeong:** None.

Poster

612. Physiological Methods: Optical Methodology: Development II

Location: SDCC Halls B-H

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Program #/Poster #: 612.26/MMM16

Topic: I.06. Computation, Modeling, and Simulation

Support: DARPA N66001-17-C-4015
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NIH Grant NIH U01NS090501-03
Getty Foundation

Title: Thermal model for *in vivo* temporally focused light-shaped optogenetics

Authors: V. EMILIANI[1], A. PICOT[1], C. LIU[1], P. BERTO[1], N. ACCANTO[1], D. TANESE[1], C. MOLINIER[1,2], E. RONZITTI[1,2], D. SOLEDAD[1], I.-W. CHEN[1], G. TESSIER[2], B. C. FORGET[1], E. PAPAGIAKOUMOU[1,3]
[1]Neurophotonics Lab., Cnrs-University Paris Descartes, Paris, France; [2]Sorbonne Université, Inserm, CNRS, The Vision Inst., Paris, France; [3]Inst. Natl. de la Santé et de la Recherche Médicale, Paris, France

Abstract: Over the past decades, optogenetics has been transforming neuroscience research enabling neuroscientists to drive and read neural circuits. Recent development of new illumination approaches combined with two-photon (2P) excitation, either sequential or parallel, has opened the route for brain circuits manipulation with single-cell resolution and millisecond temporal precision. However, a deeper understanding of complex brain circuits requires pushing light shaping methods into a new regime: the simultaneous excitation of hundreds of targets, arbitrarily distributed in the three dimensions. To this end we developed a new optical scheme for multiplexed temporally focused light shaping (MTF-LS), based on the spatio-temporal shaping of a pulsed laser beam, to project several tens of spatially confined 2P excitation patterns in a large volume. The compatibility with several different phase shaping strategies allows the system to be optimized towards flexibility, simplicity or multiple independent light manipulations, thus providing new routes for precise three-dimensional optogenetics. By combining MTF-LS with a high-peak power low-repetition rate fiber amplifier we showed *in vivo* optogenetics activation at very low excitation intensity (<1 $mW\mu m^{-2}$). These findings, together with the fact that amplified lasers can deliver several Watts of exit power, indicate that laser power is not the limiting factor for the maximum achievable number of targets using MTF-LS. Yet, establishing the optimal configuration for multi-target *in vivo* optical manipulation, raises questions about the induced heating inside samples. To account for this effect, we present and experimentally validate a theoretical model that enables to simulate both 3D light propagation and heat diffusion in optically scattering samples at unprecedented high spatial and temporal resolution under the illumination configurations most commonly used to perform 2P optogenetics: single- and multi-spot holographic illumination and spiral laser scanning. By investigating the effects of photostimulation repetition rate, spot spacing, and illumination dependence of heat diffusion, we found conditions that enable to design a multi-target 2P optogenetics experiment with minimal sample heating.

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Poster

613. Neuronal Networks Widescale, Multimodal, and Electrophyioslogical

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 613.01/MMM17

Topic: I.07. Data Analysis and Statistics

Support: Simons Collaboration on the Global Brain
Wellcome Trust

Title: The International Brain Laboratory: Reproducing a single decision-making behavior in mice across labs

Authors: V. AGUILLON RODRIGUEZ[1], N. BONACCHI[2], M. CARANDINI[3], F. CAZETTES[2], *A. K. CHURCHLAND[1], I. LARANJEIRA[2], Z. F. MAINEN[2], M. MURAKAMI[2], J. SANDERS[4], A. E. URAI[1], M. J. WELLS[3], L. E. WOOL[3], A. M. ZADOR[1], .. INTERNATIONAL BRAIN LABORATORY[1]
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Champalimaud Ctr. for the Unknown, Lisbon, Portugal; [3]Univ. Col. London, London, United Kingdom; [4]Sanworks LLC, Stony Brook, NY

Abstract: The International Brain laboratory (IBL, Neuron 2017) is a collaboration of 21 labs aiming to understand the neural basis of decision-making. A major goal of the IBL is to establish and implement a mouse behavioral task in multiple institutions, each contributing standardized data to a global repository. In the pilot phase of the project, we implemented the same visually guided task in mice across three institutions (UCL, CCU, and CSHL) in three countries to assess behavioral reproducibility.
We built rigs in accordance with a single assembly protocol and components list, and prepared mice for the headfixed task using similar surgical and animal-handling protocols. To run the task, we developed custom software that automates the progression of training by adaptively adjusting task parameters. A total of 23 mice were trained across the three institutions (11 at UCL, 4 at CCU, 8 at CSHL) on 7 behavioral rigs using this automated training protocol. Behavioral data were saved to a centralized repository using a standard file format (ALF) for integration into a common analysis pipeline.
Mice were considered successfully trained on the spatial contrast detection task (Burgess et al. 2017) if performance on the highest contrasts was > 85% correct and median reaction times were <3 s. 70% of all animals were successful. On average, animals took 11 days of training to reach stable performance, and trained animals did on average 526 trials per day (UCL:454±184; CCU:587±84; CSHL:565±99). As expected, choice accuracy increased and reaction time decreased with visual contrast. Mice were significantly biased towards one choice in the majority (76%) of sessions. Within each mouse, we examined the distribution of biases across sessions; in

16/23 mice, biases were consistently towards the same response side.
These pilot data suggest that reproducible mouse behavior can be achieved in multiple laboratories using a standardized set of materials and methods. We will subsequently implement this behavior in 8 additional experimental laboratories within the collaboration and proceed to neural recordings. Ongoing efforts include documenting experimental procedures and environmental conditions within each lab, creating detailed instructions for rig construction, developing video processing systems to track multiples movements/ physiological indicators, and building a shared data repository and analysis pipeline.
With these considerations, the IBL aims to generate an integrated large-scale dataset for in-depth modeling of neural activity during behavior, and produce standardized and centralized resources for collaborative data acquisition and analysis.

Disclosures: V. Aguillon Rodriguez: None. **N. Bonacchi:** None. **M. Carandini:** None. **F. Cazettes:** None. **A.K. Churchland:** None. **I. Laranjeira:** None. **Z.F. Mainen:** None. **M. Murakami:** None. **J. Sanders:** A. Employment/Salary (full or part-time):; Sanworks LLC. **A.E. Urai:** None. **M.J. Wells:** None. **L.E. Wool:** None. **A.M. Zador:** None. **.. International Brain Laboratory:** None.

Poster

613. Neuronal Networks Widescale, Multimodal, and Electrophyioslogical

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 613.02/MMM18

Topic: I.07. Data Analysis and Statistics

Support: Wellcome Trust
Simons Foundation

Title: The international brain laboratory: Data architecture

Authors: *K. D. HARRIS[1], N. BONACCHI[2], M. L. HUNTER[1], C. REDDY[1], C. ROSSANT[1], N. ROY[3], N. A. STEINMETZ[1], M. J. WELLS[1], O. WINTER[2], .. THE INTERNATIONAL BRAIN LABORATORY[4]
[1]Univ. Col. London, London, United Kingdom; [2]Neurosci., Champalimaud Ctr. for the Unknown, Lisbon, Portugal; [3]Neurosci., Princeton Univ., Princeton, NJ; [4]., ., NY

Abstract: The International Brain laboratory (IBL) is a collaboration of 21 labs aiming to understand the neural basis of decision-making. Ten experimental labs will record from a variety of brain regions, using a variety of modalities, in mice performing a common behavioral task. A primary requirement of the IBL is to establish a common data architecture, seamlessly integrating data from all labs so it can be analyzed together.
Establishing this data architecture presents several challenges. The first challenge is of social

engineering: ensuring that scientists in all labs accurately record metadata concerning their mice and experiments. The second is to integrate and organize this metadata so it is searchable and linked to experimental data files. The third is to organize the large quantities of highly diverse experimental data in a coherent and human-understandable way. The fourth is to establish an analysis pipeline that will automatically run on new data as it arrives.
To solve the first challenge, we have developed a user-friendly, web-based electronic lab notebook system for colony management and metadata entry, known as Alyx (https://github.com/cortex-lab/alyx). Information concerning experimental subjects is entered when they are crossed, born, genotyped, or undergo any procedure, allowing labs to keep an up-to-date record of their animal colony. This information is stored in a relational database.
The second challenge requires linking metadata to files. Because bulk experimental data is too large to store relationally, Alyx stores references to binary files, in a manner that allows copies of the files to be archived and backed up in multiple locations. When an experiment or analysis is performed, the recording or analysis software automatically registers the data files in the database.
For the third challenge of organizing the bulk data files, we have designed a file-naming convention called ALF (github.com/cortex-lab/ALF). ALF provides a principled way to organize diverse data (such as electrophysiology, movies, behavioral traces) in their native formats, with a standard and simple way to represent relationships between them including time alignment. All IBL files are stored on a common central server, and when a contributing lab registers a file with the database, an automatic upload to the central server begins.
Finally, for pipelined automatic analysis, we will make use of DataJoint to compute basic standard analyses and store the results in relational form. This will allow users to browse and download the results using a web interface as well as through protocols such as Neurodata Without Borders.

Disclosures: K.D. Harris: None. **N. Bonacchi:** None. **M.L. Hunter:** None. **C. Reddy:** None. **C. Rossant:** None. **N. Roy:** None. **N.A. Steinmetz:** None. **M.J. Wells:** None. **O. Winter:** None. **.. The International Brain Laboratory:** None.

Poster

613. Neuronal Networks Widescale, Multimodal, and Electrophyioslogical

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 613.03/MMM19

Topic: I.07. Data Analysis and Statistics

Support: KIBM’s Innovative Research Grant

Title: Beyond correlation in zebrafish whole brain activity

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Abstract: Much of data analysis in neuroscience has been dominated by concepts such as Hebbian learning in which correlation of activity patterns lead to learning, sensory, motor or homeostatic activities. In this way, much of the analysis has relied heavily on correlation based methods of brain activity patterns. Since correlation does not imply causation, it is difficult to infer function from correlation. In the present work we used a time delayed embedding approach to identify relationships from whole brain imaging in the hypoxia response of larval Zebrafish. Our analyses reveal that the hypoxic response is complex and state dependent even at the single neuron level. The observation support the view that complexity is low dimensional and it exhibits complex attractor dynamics. Within our data, we identify neurons whose dynamics contain information that allow the prediction of out of sample aggregate whole brain activity. These observations are in vivo evidence for the existence of locally embedded presages of global network bursts as hypothesized by Satohiro Tajima et al. (Tajima et al. PNAS *2017 doi: 10.1073/pnas.1705981114*) from in vitro model random networks. The identified neurons can predict the whole brain activity with >60% accuracy (observed/predicted) although their activities show little correlation (Rho =0.29). These findings suggest a substantial presence of nonlinear responses due to low dimensional attractor dynamics that warrant further investigation. Our results establish a novel approach to map functional connectivity for causal network reconstruction that allows the distinction of correlation from causation and even find causation in the absence of correlation. This was shown in the zebrafish hypoxic response, but should be widely applicable and complement the physical connectome for the understanding of brain maps.

Disclosures: C. Yeh: None. **G. Pao:** None. **A. Groisman:** None. **J.R. Fetcho:** None. **S. Chalasani:** None.

Poster

613. Neuronal Networks Widescale, Multimodal, and Electrophyioslogical

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 613.04/MMM20

Topic: I.07. Data Analysis and Statistics

Title: Loss of inhibitory control causes network-specific functional underconnectivity: A DREADD-fMRI study in C57BL/6J and PV-Cre mice

Authors: *M. MARKICEVIC[1], B. D. FULCHER[2], M. RUDIN[3], N. WENDEROTH[4], V. ZERBI[4]
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Switzerland; [2]Sch. of Physics, Fac. of Sci., Univ. of Sydney, Sydney, Australia; [3]Univ. and ETH Zürich, Zürich, Switzerland; [4]Neural Control of Movement Lab, ETH Zurich, Zurich, Switzerland

Abstract: One popular method for estimating brain-wide functional connectivity patterns is resting-state-fMRI. However, it is unclear how this macroscopic measure reflects local alterations of excitation: inhibition balance (E:I) at the circuit-level. Here we hypothesize that population-wide neuronal synchrony gives rise to BOLD correlated activity under the control of GABAergic interneurons. To address this, we combine resting-state-fMRI with Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), immunohistochemistry and electrophysiological recordings. Specifically, we perturb the E:I in the somatosensory network, a structurally and functionally well-known brain circuit, by i) increasing the overall asynchronous neuronal firing and by (ii) reducing the activity of Parvalbumin interneurons. Right primary somatosensory cortex of C57BL/6J (n=19, 13 controls) and PV-Cre mice (n=28, 14 controls) is unilaterally targeted with hM3Dq and DIO-hM4Di DREADDs, respectively. Four weeks after surgery, cerebral blood flow (CBF) and resting-state-fMRI measurements are acquired with a 7T scanner equipped with a cryogenic coil following well-established pipelines for animal handling, anesthesia, and data acquisition. In both sessions (45 minutes long), 30µg/kg of Clozapine is intravenously injected after 15 minutes to activate the DREADDs. Rs-fMRI data is analyzed to determine changes in connectivity (Zerbi et al., 2018) and classification models are utilized to identify which features of the univariate dynamics of the BOLD signal reflect E:I changes (Fulcher et al., 2013). Increasing asynchronous neuronal firing with hM3Dq results in a significant increase of CBF around the injection site. Rs-fMRI data in both C57BL/6J and PV-Cre mice indicate that increasing neuronal excitability or reducing inhibition via DREADS cause a local disruption of connectivity near the injection site, and long-range interhemispheric connectivity reductions, which are limited to the somatosensory and somatomotor cortices. Feature classification revealed significant decreases in BOLD variance and an increase in the stationarity dynamics of the signal in hM3Dq group compared to controls in both injection site and contralateral regions, but not outside the somatosensory network. In conclusion, we link brain *underconnectivity* (i.e. an output often described in psychiatric disorders) to reduced within-network dynamics due to loss of sufficient local inhibitory control. Our results form the first step towards identifying a causal link between E:I at the cell population level and markers of brain-wide, macroscopic functional connectivity.

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Poster

613. Neuronal Networks Widescale, Multimodal, and Electrophyioslogical

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Program #/Poster #: 613.05/MMM21

Topic: I.07. Data Analysis and Statistics

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Title: The importance of empirical data on the anatomical connectivity of mouse neocortex

Authors: *A. GAMANUT[1,2], K. KNOBLAUCH[1], B. GAMANUT[1,2], A. H. BURKHALTER[3], H. KENNEDY[1,4]
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Abstract: Cortical areas in the mouse are very small and it is difficult to restrict injections of tracers to individual areas. Hence, either connectivity studies are based on empirical data obtained from a relatively small number of successfully area-restricted injections [1], or they use more numerous injections involving multiple areas and then computational methods to indicate the connectivity of individual areas [2, 3]. Cortical networks extracted from empirical data on cortico-cortical connectivity in mouse neocortex show significantly higher density than modeled data. Importantly, the latter fails to accurately capture the connectivity profiles of individual areas; hence errors in the determination of connectivity profiles in modeled data will not reflect the actual specificity of the mouse cortex [1]. Here, we further address the advantages of empirical vs modeled data. Using injections with the retrograde tracer Diamidino Yellow, we analyzed the experiments in which the injection site was restricted to one cortical area. The weight of a given projection is defined by the proportion of labeled neurons (FLN) in a source area divided by the total number of labeled neurons in the cortex. We extend our investigation to the contralateral hemisphere and include the projections from claustrum. The contralateral projections are considerably less numerous than the ipsilateral projections and present a different distribution of weights. We find projections from the ipsilateral claustrum in all experiments, and we provide a quantitative comparison with the contralateral claustrum. Altogether, our results show that there are important differences between the connectivity profiles of ipsi- and contralateral projections, and validate our claim on the need for empirical data. *[1]* Gămănuț, R., et al., The Mouse Cortical Connectome, Characterized by an Ultra-Dense Cortical Graph, Maintains Specificity by Distinct Connectivity Profiles. *Neuron*, 2018. 97(3): p. 698-715.e10. *[2]* Oh, S.W., et al., A mesoscale connectome of the mouse brain. *Nature*, 2014. 508(7495): p. 207-14. *[3]* Knox, J.E., et al., High resolution data-driven model of the mouse connectome. bioRxiv, 2018.

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Poster

613. Neuronal Networks Widescale, Multimodal, and Electrophyioslogical

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

Program #/Poster #: 613.06/MMM22

Topic: I.07. Data Analysis and Statistics

Support: Leona M. and Harry B. Helmsley Charitable Trust grant #2017-PG-MED001
The W.M. Keck Foundation

Title: State dependent large scale integration from whole brain embedology at single neuron resolution

Authors: *G. PAO[1,3], C.-M. YEH[2], S. CHALASANI[4], J. R. FETCHO[5], G. SUGIHARA[3]
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Abstract: The study of neural systems is most frequently approached as following the neural activity that is closely correlated in time with a sensory input or behavioral output. However higher order functions that integrate multiple sensory inputs and more complex processes are slower processes and are more separated in time from both the sensory input and motor output and necessarily become more decorrelated. In many cases the processing and reprocessing with multiple feedbacks will lead to the appearance of complex attractor dynamics in neural systems and is frequently observed as a loss of correlation making understanding difficult when using correlation based methods which dominate most of neural activity data analysis. Here in observations of the larval zebrafish brain, we observe low dimensional dynamics that are consistent with this notion and appear to be ideally suited using the analytical framework of embedology based on the Whitney embedding theorem and the generalized Takens theorem. Our results quantify the changes in dimensionality from the "default state" to a flight response and identifies likely sites of large scale integration in the larval zebrafish brain.

Disclosures: G. Pao: None. **C. Yeh:** None. **S. Chalasani:** None. **J.R. Fetcho:** None. **G. Sugihara:** None.

Poster

613. Neuronal Networks Widescale, Multimodal, and Electrophyioslogical

Location: SDCC Halls B-H

Time: Tuesday, November 6, 2018, 1:00 PM - 5:00 PM

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Topic: I.07. Data Analysis and Statistics

Support: EPSRC Centre for Neurotechnology CDT Studentship (to D. Lucaci)
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Human Frontier Science Program (P.Chadderton)

Title: Combining mGRASP and optogenetics enables high-resolution functional mapping of descending cortical projections

Authors: *J. SONG[1,2], D. LUCACI[3], I. CALANGIU[4,2], J. PARK[1,5], J. KIM[6], S. G. BRICKLEY[3], P. CHADDERTON[2,7]
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Abstract: We have applied optogenetics and mGRASP, a light microscopy technique that labels synaptic contacts, to map the synaptic strength (physiology) and organisation (anatomy) of auditory corticocollicular (CC) connections. Using mGRASP, we show that CC projections form small, medium and large synapses, and both the number and distribution of synapse size varies between different IC regions. Using optogenetics, we show that low-frequency stimulation of CC axons expressing channelrhodopsin produces prolonged elevations of CC miniature EPSC (mEPSC) rate. Remarkably, functional analysis of CC mEPSCs reveals small, medium and large amplitude events, that mirror the synaptic distributions observed with mGRASP. Our results reveal descending ipsilateral projections dominate CC feedback via increased number of large synaptic contacts, especially onto the soma of IC neurons. This study highlights the feasibility of combining microscopy (i.e. mGRASP) and optogenetics to reveal synaptic weighting of defined projections at the level of single neurons, enabling functional connectomic mapping in diverse neural circuits.

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Poster

613. Neuronal Networks Widescale, Multimodal, and Electrophyioslogical

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Title: Wide-field calcium imaging deconvolution methods

Authors: *M. STERN[1], D. WITTEN[2], E. T. SHEA-BROWN[1]
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Abstract: Wide-field Calcium imaging techniques allow recordings of high resolution neuronal activity across one or multiple brain regions. However, since the recordings capture light emission generated by the fluorescence of the calcium indicator, the neural activity that drives the calcium changes is masked by the calcium indicator dynamics. Since we usually wish to explore neural activity dynamics, the recorded signal needs to be de-convolved based on the calcium properties, to reveal the underlying neural spiking rates.
Much effort has been put into de-convolving a calcium trace that originates from a signal neuron. However, the signal recorded in the wide-field method, in contrast with that recorded in two-photon imaging, originates from dozens to thousands of neurons. Hence, different, or modified, de-convolution techniques are required to reveal the spiking rate dynamics from the calcium traces in wide-field recordings. We survey here three different approaches to de-convolution that are standard in distinct disciplines, and their utility for the wide-field recordings. First, we explore the direct convolution by the inverse shape of the calcium response to spiking. This method, while naively correct, magnifies noise at specific frequencies. Second, we explore calculating the positive part of the derivative of the signal. While this method is highly simplified, we show that it relates linearly to the original spiking rate and estimates it well under some conditions. Third, we explore the 'Richardson-Lucy' image recovery method, adopted here to recover temporal dynamics rather than spatial images. The method accounts for signals created by Poisson processes but over smooths and misses some signal fluctuations.
We also develop and test a novel method to de-convolve the calcium traces. This method is based on statistical machine learning, and takes into account both the noise existent in the

recordings and the full shape of the calcium indicator response. The method generates time binning for the original rate signal dynamically, where each bin size depends on the data, and for each bin finds the proper spiking rate.
We compare results for the four methods on both synthetic data where the underlying truth is known, as well as for wide-field calcium recordings where cues from behavior and stimuli are available.

Disclosures: M. Stern: None. **D. Witten:** None. **E.T. Shea-Brown:** None.

Poster

613. Neuronal Networks Widescale, Multimodal, and Electrophyioslogical

Location: SDCC Halls B-H

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Program #/Poster #: 613.09/MMM25

Topic: I.07. Data Analysis and Statistics

Title: An unbiased workflow for isolating and mapping functional dynamics across the developing neocortex

Authors: *B. R. MULLEN, S. C. WEISER, J. E. LAMB, C. P. SANTO TOMAS, J. B. ACKMAN
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Abstract: The neocortex contains a constellation of sensory-motor regions whose functional interactions during development are thought to shape adult brain function. Simultaneously recording neuronal group activity across the cortical hemispheres may provide insight on functional interactions necessary for establishing cerebral networks. To this end, we transcranially image pan-neuronally expressed genetically encoded calcium indicators across the neocortex in unanesthesized mice. Recording from behaving mice produces a unique set of challenges, including optical and blood artifacts associated with movement. In addition, areal patterning of the cortex can vary widely across ages and genotypes- thus an unbiased, flexible workflow for video acquisition and analysis is necessary to map the functional structure of the cortex. To address these challenges, we have developed an eigendecomposition-based workflow that isolates blood and optical artifacts to recover underlying calcium activity patterns, and maps independent regions of the brain to create maps of functional units in the developing cortex. To verify these functionally defined cortical structures, we align our maps to molecular expression patterns that delineate cortical structure. In addition, we quantify the quality of independent source separation, and use the resulting metrics to optimize our recording parameters. These open-source methods are flexible enough to be implemented on different recording rigs, and will become publicly available for use upon publication. Overcoming these hurdles opens the possibility of expanding this technique to address a variety of questions including the exploration of network development by tracing neuronal projections of functionally associated regions,

characterizing intra- or inter-areal connectivity neurodevelopmental disease models, and investigation of plasticity of higher-order cortical regions in real-time feedback experiments.

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Poster

613. Neuronal Networks Widescale, Multimodal, and Electrophyioslogical

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Topic: I.07. Data Analysis and Statistics

Support: Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education (2016R1A6A3A11930410)

Title: MRI marker to predict subcortical vascular cognitive impairment: Comparison among integrity of normal appearing white matter, integrity of white matter hyperintensities, and cortical thickness

Authors: *J.-J. YANG[1], B.-H. KIM[1], G. PARK[1], S. SEO[2], J.-M. LEE[1]
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Abstract: The most common cause of subcortical vascular cognitive impairment is known as white matter hyperintensities (WMHs) and lacunes. However, not all individuals with an apparent identical degree of WMHs experience the same level of cognitive deficits. In this study, we investigated which of cortical thinning, integrity of normal appearing white matter (NAWM), and integrity of WMHs is an appropriate risk-stratification tool to distinguish patients with vascular cognitive impairment from cognitively normal elderly with the same degree distribution of patients' WMHs. We further examined group differences in WMH's spatial distribution over the brain, white matter (WM) integrity, cortical thickness, and the relationships between WM integrity and cortical thickness. We hypothesized that integrity of NAWM can predict for cognitive impairment than those of WMHs because it is likely that the compromised integrity of NAWM affects the overall connective integrity of the brain.
We selected 64 high-risk individuals with the same degree distribution of patient's WMHs but cognitively normal as a beginning in which symptoms are not yet present (wNC). We defined 84 patients with 'pure' subcortical cognitive impairments (pSVCI), who show negative on PiB-PET, as a continuum of disease progression related to only vascular pathology. White matter integrity was estimated by averaged fractional anisotropy (FA) and mean diffusivity (MD) from diffusion tensor imaging in each area of WMHs and NAWM, respectively. Cortical thickness was calculated from structural MRI. Classification accuracy was evaluated from the area under the

curve (AUC) to assess which parameter independently discriminated best between wNC and pSVCI patients. A general linear model was conducted with controlling age, gender, education years to examine local differences of the group in cortical thickness on vertex, FA and MD on skeletonized voxel level.
We found that integrity of NAWM had better contribution on discriminating between wNC and pSVCI with AUC of 85 % FA and 84 % MD than those of WMH (78 % FA and 71 % MD). In contrast, averaged cortical thickness produced 70 % classification. Structural differences were revealed in overall WM integrity with decreasing FA, increasing MD, and cortical thinning in pSVCI when compared to those of wNC. Furthermore, significant relationships between cortical thickness and WM integrity were shown only in pSVCI patients.
Our findings may lead to a better understanding which tissue damage influences with neurological function and clinical status in patients related to vascular cognitive impairment.

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Poster

613. Neuronal Networks Widescale, Multimodal, and Electrophyioslogical

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Program #/Poster #: 613.11/MMM27

Topic: I.07. Data Analysis and Statistics

Support: KIST Grant 2E27850

Title: DBScope: Visualizing DBS effects mapped to standard space

Authors: *W. OH[1], H. JEON[1], Y. LIM[2], S. PAEK[2], J. KIM[1,3]
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Abstract: Deep brain stimulation (DBS) not only provides an effective treatment modality for advanced Parkinson's disease (PD) and other neurological diseases, but also offers a rare opportunity to infer causal relations between neural circuit activity and behavioral outcome in humans. However, clinical data required for such inference is often highly sporadic, heterogeneous, and multi-dimensional, necessitating a systematic approach based on programmatic platforms. Here we fully utilize the open-source software ecology to build a data processing pipeline, named DBScope, that maps clinical benefits and adverse effects of subthalamic DBS in PD patients to volumes of brain tissue electrically activated by DBS. Centered around the R programming environment, the workflow includes 1) a chain of scripts for tidying raw data, 2) a package to interface with a MATLAB toolbox specialized in localizing

DBS electrodes within standard spaces, and 3) an application to interactively visualize the 3-dimensional circuit-behavior mapping. Inputs to the pipeline consist of tabular and imaging data; tabular data include demographics, intervention profiles, and clinical outcomes, while imaging data comprise preoperative magnetic resonance (MR) and postoperative computed tomography (CT) images. Each step is heavily documented under the literate programming paradigm. We provide postoperative profiles of improvement and worsening of various motor and non-motor clinical features. These profiles are mapped to anatomically annotated voxels in the standard Montreal Neurological Institute (MNI) space. We plan to extend this pipeline beyond exploratory analysis to inference and prediction of areas that elicit therapeutic ("hot spots") and adverse ("danger zones") effects following DBS.

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Poster

613. Neuronal Networks Widescale, Multimodal, and Electrophyioslogical

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Program #/Poster #: 613.12/MMM28

Topic: I.07. Data Analysis and Statistics

Support: National Research Foundation of Korea grant funded by the Korea government (2016R1A2B3016609)

Title: Intrinsic connectivity network efficiency for evaluating its contribution to brain network integration

Authors: *Y.-H. PARK, J.-J. YANG, J.-M. LEE
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Abstract: Brain networks are composed of several intrinsic connectivity networks (ICNs), with integration and segregation. Frequency specificity of ICN are revealed by previous studies. However, the previous studies have some deficiency in brain network integration analysis. Sasai, et al. (2014) evaluated the brain network integration using global efficiency in the frequency dimension. But, the global efficiency is only indicators of the overall network. Thompson and Fransson (2015) evaluated the brain network integration through strength contribution in the frequency dimension. But, the strength contribution of ICN considered first degree of each node only. In this study, we used the novel measure called ICN efficiency, which is defined as the difference between the global efficiency and the efficiency of the whole brain network excluding the ICN.
The present study used fMRI data of 352 subjects in Human Connectome Project S900 data released in December 2015. First of all, the connectivity matrices along the frequency were constructed from time-varying frequencies of each region of ICN. Hierarchical clustering

analysis was conducted by considering the difference between connectivity matrices of each frequency. The low-frequency band was divided into two frequency bands with the highest silhouette value. Finally, the ICN efficiency was calculated along the two frequency bands. Paired sample t-test between ICN efficiency of two subbands was performed to confirm that the ICN contribution to brain network integration were different from each other.
We found that ICN efficiency has various tendencies along the frequency. Ascending, descending, or flat tendencies of ICN efficiency were same to the tendencies of number of betweeness centrality (BC) hub in many ICNs including DMN, VN, LN, ECN, SN and PCN. This result supports the suggestion that ICN efficiency is useful for evaluating ICN contributions to brain network integration, because ICN efficiency and the number of BC hubs were used as cross check. We conclude that ICNs have frequency specific contribution to brain network integration.

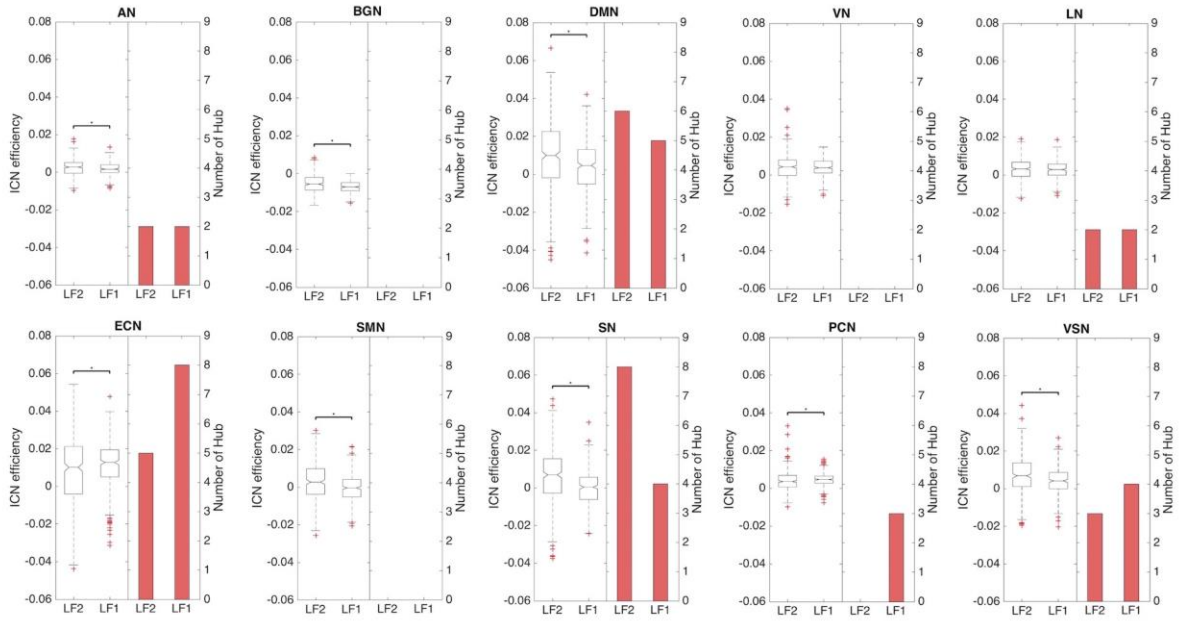

Fig.1 The tendencies of ICN efficiency and number of BC hub along the frequency.
The ICN efficiency of the 10 ICNs compared using paired t-test analysis between the 2 subbands. The results were corrected by Bonferroni correction ($p<0.005$). AN, auditory network; BGN, basal ganglia network; DMN, default mode network; VN, visual network; LN, language network; ECN, executive control network; SMN, sensorimotor network; SN, salience network; PCN, precuneus network; VSN, visuospatial network. LF1= 0.027~0.08 Hz, LF2 = 0.009~0.013 Hz.

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Poster

613. Neuronal Networks Widescale, Multimodal, and Electrophyioslogical

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Support: This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIP) (2016R1A2B3016609)."

Title: Genetic risk factors for cortical thickness in patients with Alzheimer's disease

Authors: *B.-H. KIM, Y.-H. CHOI, J.-M. LEE
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Abstract: In recent GWA studies have been identified risk SNPs for Alzheimer's disease, including APOE, CLU, BIN1. However, these genetic variants only explain the small portion of phenotypic variance of disease. The aim of this study was to identify novel AD susceptible genes through the imaging genetic analysis.
We used 908 (161 AD, 481 MCI, 266 NC) subject's T1-weighted images and genotype information from Alzheimer's Disease Neuroimaging Initiative (ADNI). We downloaded impute genotype information and performed quality control, call rate < 95%, HWE $p < 10^{-6}$, MAF < 5%. We estimated whole brain mean cortical thickness that used as an endophenotype for further analysis using CIVET v2.1. We performed GWAS testing 3,041,429 SNPs, with age, sex, education level, diagnosis, scanner field strength, and APOE4 genotype as covariates using PLINK v1.90. Then, we performed gene-based and protein-protein interaction(PPI) based analysis using KGG v4.0. In gene-based analysis, SNPs that were fell within 5kb of the 3'/5' untranslated regions were considered 'within' gene and summary P value for each gene was calculated by GATES(Gene-based Association Test using Extended Simes Procedure). In PPI based analysis, we used high confidential network (confidence score > 0.7) and combines gene-based P-values for each protein interactions and detects PPI pairs in which two genes are associated with the phenotype using HYST (Hybrid Set-based Test).
In GWAS, no marker reached the genome-wide significant threshold. The SNP rs12320537, which is an intronic SNP of B4GALNT1 (beta-1,4-N-acetyl-galactosaminyltransferase 1) gene on chromosome 12, achieved the strongest evidence for cortical thickness ($P=8.60 \times 10^{-7}$). Six of the 10 most suggestive SNPs were annotated into B4GALNT1 gene and 2 SNPs (rs2619470, rs2640607) were located within 5kb of B4GALNT1 gene. In gene-based analysis, B4GALNT1 (nominal $P=2.71\times10^{-6}$, corrected $P=3.87\times10^{-2}$), LOC1001927583 (nominal $P=4.79\times10^{-6}$, corrected $P=3.87\times10^{-2}$), SLC26A10 (nominal $P=4.79\times10^{-6}$, corrected $P=3.87\times10^{-2}$) genes were significantly associated with cortical thickness. In PPI based analysis, B4GALNT1 and GALNT8 gene pair (1.57×10^{-7}), both on chromosome 12, is significantly associated.
In this study, we identified 4 genes implicated in the pathogenesis of AD using the cortical thickness, among which B4GALNT1 was the most associated genes. Yamaguchi et al. (2016) identified the expression of B4GALNT1 gene enhanced the β-site cleavages of APP protein that produce Aβ which play a central role in AD pathology. We discovered genetic variants on B4GALNT1 underlying the cortical thinning in patients with AD.

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Poster

613. Neuronal Networks Widescale, Multimodal, and Electrophyioslogical

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Topic: I.07. Data Analysis and Statistics

Support: KIST grant 2E27850

Title: Convergent excitatory and inhibitory connectivity in the subthalamic nucleus

Authors: *H. LEE[1,2], W. OH[1], H. JEON[1], J. KIM[1,2], L. FENG[1], J. KIM[1,2]
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Abstract: The subthalamic nucleus (STN) is a network hub of the basal ganglia, receiving extensive inputs from diverse cortical and subcortical areas. Proposed as an integrative regulator of information flow, the STN is considered the primary target of deep brain stimulation (DBS) in various neurological and psychiatric disorders including Parkinson's disease. However, the circuit-level mechanism underlying the effects of STN-DBS remains unknown, and even fundamental characteristics such as functional anatomy and synaptic profile of the STN circuitry are unclear. We thus provide detailed connectome descriptions of excitatory and inhibitory inputs into the STN, i.e. cortico- and external globus pallidus-subthalamic circuits, respectively, both known to be critical in generating and coordinating motor program of the basal ganglia. In particular, we used fluorescent protein-expressing viral tracers and mammalian GFP reconstitution across synaptic partner (mGRASP) for mapping connectivity of the STN at meso- and micro-scale, respectively. We identified complex projection patterns in the STN from cortical regions and external globus pallidus, suggesting STN convergences far more intricate than the conventionally posited discrete tripartite STN division. Our results provide comprehensive axonal projection patterns and input-specific synaptic distributions in the STN area. Such multiscale connectivity landscape will lay the foundations for understanding the cortical and pallidal contributions to the therapeutic mechanism of STN-DBS.

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Poster

613. Neuronal Networks Widescale, Multimodal, and Electrophyioslogical

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Topic: I.07. Data Analysis and Statistics

Support: Australian Research Council Grant DP170102263
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Australian Government Research Training Program Scholarship

Title: Detecting neural assemblies in calcium imaging data

Authors: *J. MÖLTER[1], L. AVITAN[2], G. J. GOODHILL[1]
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Abstract: Activity in populations of neurons often takes the form of assemblies, where specific groups of neurons tend to be co-active. However, in calcium imaging data, reliably identifying these assemblies is a challenging problem, and the relative performance of different assembly-detection algorithms is unknown. Here we show that only some of these algorithms work well in this case. First we generated large surrogate datasets of calcium imaging data and tested the abilities of independent components analysis (ICA), PCA-Promax, frequent item set mining, and a recently proposed graph theory algorithm (SGC) to recover known assemblies. We then applied the same algorithms to evoked activity data from zebrafish tectum. While both SGC and novel variants we propose for ICA and PCA-Promax performed well for the simulated data, on the real data SGC performed best. These findings suggest that SGC is a very reliable algorithm for detecting neural assemblies from calcium imaging data.

Disclosures: J. Mölter: None. **L. Avitan:** None. **G.J. Goodhill:** None.

Poster

613. Neuronal Networks Widescale, Multimodal, and Electrophyioslogical

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Program #/Poster #: 613.16/MMM32

Topic: C.03. Parkinson’s Disease

Support: Parkinson Canada

Title: Neuro-imaging in the common marmoset

Authors: *S. FREY[1], S. G. NUARA[2], A. MATHIEU[4], G. MASSARWEH[5], M. S. KANG[5], P. ROSA-NETO[3], J. C. GOURDON[2], D. BÉDARD[5], A. HAMADJIDA[6], P. HUOT[5]
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Abstract: The common marmoset (*Callithrix jacchus*) is a small primate that is increasingly used in neuroscience and bio-medical research. Its rapid reproduction rate compared to other primates makes it an attractive species to model genetic conditions. A key process in developing new disease models using the marmoset is the ability to characterise, *in vivo*, brain anatomy, and to monitor, longitudinally, neuro-chemical changes that may occur as a result of evolving pathological processes. State-of-the-art neuro-imaging techniques are invaluable tools to achieve such goals. Moreover, obtaining high-quality images is critical to accurately determine the precise co-ordinates of targets, to ensure the precision of stereotaxic surgery, as there is individual variability in the marmoset brain and as most atlases were designed with a limited number of animals. We have developed protocols that enable us to conduct magnetic resonance imaging (MRI), computed tomography (CT) and positron emission tomography (PET) in the common marmoset. Here, we present preliminary data of experiments in which marmosets underwent MRI, CT and PET as part of a pre-surgical characterisation process. We present the image and image juxtaposition data, in addition to target reconstruction, in the striatum, using the Rogue Research Vet Robot Brainsight® neuro-navigation software. Lastly, we present anatomical images obtained in *post-mortem* brain tissue that demonstrate the accuracy of our approach at reaching its target.

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